Abstract# 544

THE SURVIVAL BENEFIT OF LIVER TRANSPLANTATION. Robert M. Merion,[1] Douglas E. Schaubel,[1] Dawn M. Dykstra,[2] Richard B. Freeman,[3] Friedrich K. Port,[2] Robert A. Wolfe.[1] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI; [3]Tufts New England Medical Center, Boston, MA.*

Background: Demand for liver transplantation continues to exceed organ supply. Better understanding of transplant benefit is needed, comparing recipient survival to that of comparable candidates without a transplant.

Methods: Waitlist and posttransplant mortality was studied in a cohort of 13,375 patients placed on the waitlist between 9/01 and 6/03. Time-dependent Cox regression models were fitted to assess relative mortality rates for candidates and recipients.

Results: Crude waitlist death rates (per 1000 patient yrs) ranged over 300-fold from 46 for MELD <10 to 13,500 for MELD 40. Crude posttransplant death rates (per 1000 patient yrs) ranged almost 2-fold from 150 for MELD <10 to 257 for MELD 40. Overall, transplant recipients had a 76% lower adjusted risk of death than candidates (hazard ratio [HR] 0.24; p<0.0001).

Adjusted relative mortality risk for patients after liver transplant compared to patients on the liver transplant waiting list

MELD	HR	95% confidence interval	p value
6-8	3.41	(1.85, 6.28)	<0.0001
9-11	3.10	(1.98, 4.85)	<0.0001
12-14	2.04	(1.35, 2.08)	0.001
15-17	1.03	(0.72, 1.47)	0.87
18-20	0.56	(0.38, 0.82)	<0.003
21-23	0.34	(0.22, 0.51)	<0.0001
24-26	0.20	(0.12, 0.32)	<0.0001
27-29	0.19	(0.11, 0.33)	<0.0001
30-39	0.07	(0.05, 0.10)	<0.0001
40+*	0.03	(0.02, 0.07)	<0.0001
Status 1	0.44	(0.28, 0.79)	0.001
Overall	0.24	(0.21, 0.28)	<0.0001

* includes uncapped MELD>40

At MELD <9, recipient risk of death during the first posttransplant year was more than 3-fold higher than for candidates (p<0.0001). At MELD 9-11, the 3-fold higher risk of death persisted (p<0.0001); a >2-fold difference was observed for MELD 12-14 (p=0.001). Significantly lower risk of death among recipients was observed at MELD >17. Survival benefit increased with higher MELD scores, up to and including the maximum score of 40.

Conclusions: Liver transplant survival benefit is concentrated among patients at higher risk of pretransplant death. On average, transplants among severely ill patients are not futile under current practice. With one-year posttransplant follow-up, patients at lower risk of pretransplant death have no demonstrable survival benefit from liver transplant.

ACUTE REJECTION: BASIC

Abstract# 545 Poster Board #-Session: P1-II

GENE EXPRESSION PROFILES IN A RAT HEART TRANSPLANT MODEL OF ACUTE CELLULAR REJECTION. Katherine J. Deans,[1,3,4] Peter C. Minneci,[1,3] Adrienne E. Hergen,[1] Carol Logun,[1] Kelly J. Sittler,[1] Jennifer J. Barb,[2] Peter J. Munson,[2] Robert L. Danner,[1] Michael A. Solomon.[1,4] *[1]Critical Care Medicine Dept, NIH, Bethesda, MD; [2]Center for Information Technology, NIH, Bethesda, MD; [3]Dept. of Surgery, Massachusetts General Hospital, Boston, MA; [4]Cardiovascular Branch, NHLBI, NIH, Bethesda, MD.*

Introduction:
Gene expression profiling has the potential to identify biomarkers and pathways associated with acute cellular rejection (ACR). Most transplant models examining ACR compare isografts to non-immunosuppressed allografts. A more relevant model to the human condition would incorporate immunosuppression. The goal of this pilot study was to use oligonucleotide microarrays to differentiate between native hearts, cardiac allografts and isografts in an immunosupressed transplant model.

Methods:
Nine heterotopic heart transplants (7 allografts, 2 isografts) were performed in a rat model. Cyclosporine (CSA, 10 mg/kg) was administered in all allografts. In order to establish groups with moderate and mild levels of rejection, CSA was discontinued on post-transplant day (PTD) 6 (n=4) or continued until procurement (n=3). Native and transplanted hearts were harvested on PTD 11. Total RNA from myocardial tissue was isolated, reverse transcribed, labeled and hydridized to RAE230A microarrays (Affymetrix). Using a three group (isograft, allograft, native), one-way ANOVA, differentially expressed genes were selected based on a two-fold change filter and a 1% false discovery rate (FDR) for any group-wise comparison. A separate t-test compared isografts to allografts using a 20% FDR and standardizing transplanted hearts to their paired native heart.

Results:
Allografts exhibited mild to moderate rejection with a mean ISHLT grade of 2. Seven hundred ninety-two genes were differentially expressed among the three groups with the difference between transplanted and native hearts accounting for the majority of the genes. Forty-six genes were differentially expressed between allografts and isografts. Twenty-eight of these genes had at least a two fold change difference in expression. Specific immune genes that were differentially expressed included beta-2 microglobulin, CD 147 receptor, high mobility group box-1, and MHC I (RT1 class Ib gene).

Conclusion:
In a rat transplant model using immunosuppression, we identified differentially regulated genes that may distinguish native hearts from transplanted hearts and moderately rejecting allografts from isografts. These results demonstrate the potential for this technology to accurately diagnose moderate levels of rejection in the presence of immunosuppression.

Abstract# 546 Poster Board #-Session: P2-II

TIME-DEPENDENT CHANGES IN GENE EXPRESSION IN A RAT CARDIAC TRANSPLANT MODEL. Katherine J. Deans,[1,3,4] Peter C. Minneci,[1,3] Adrienne E. Hergen,[1] Carol Logun,[1] Kelly J. Sittler,[1] Jennifer J. Barb,[2] Peter J. Munson,[2] Robert L. Danner,[1] Michael A. Solomon.[1,4] *[1]Critical Care Medicine Dept., NIH, Bethesda, MD; [2]Center for Information Technology, NIH, Bethesda, MD; [3]Dept. of Surgery, Massachusetts General Hospital, Boston, MA; [4]Cardiovascular Branch, NHLBI, NIH, Bethesda, MD.*

Introduction: Most gene expression studies of acute cellular rejection have not reported the effects of surgery on expression levels. Inflammatory changes associated with surgery may confound the identification of expression patterns unique to acute cellular rejection (ACR). We studied the effects of surgery on gene expression profiles by comparing transplanted isografts and native hearts procured on post-operative days 7, 11, 14, 21, and 28 to examine the time course of surgically related inflammatory changes in a rat transplant model.

Methods: Heterotopic cardiac transplantation was performed in 12 syngeneic rat pairs. Native and transplanted hearts were procured on post-operative days 7, 11, 14, 21 and 28 (n = 3, 2, 3, 1, 3 respectively). Total RNA was isolated, reverse transcribed, labeled and hybridized to RAE230A microarrays (Affymetrix). Signal intensities (Affymetrix Microarray Suite 5.0) were transformed and analyzed using scripts written in JMP statistical package (SAS). Relative (transplant/native) gene expression over time was fitted with a linear regression model. A list of genes was selected that were differentially regulated by surgery (mean ≠ 0, false discovery rate (FDR) < 1%). Genes were selected from this list that significantly changed over time back toward native heart expression levels (slope ≠ 0, FDR < 20%).

Results: A significant inverse linear relationship was found between time and the first principal component accounting for 39% of the variation in gene expression. (p = 0.0249, slope = -0.35 ± 0.13; mean ± SE). Three hundred thirty-one genes (165 overexpressed, 166 underexpressed) in isografts compared to native hearts were differentially regulated by surgery and returned toward native heart expression levels over time. Overexpressed genes that could confound the expression profiles of ACR included CD68, IgG Fc receptor, cathepsin B and L, vascular endothelial growth factor, matrix metalloproteinase 2 and 14, and complement, MHC I, and II components,. Underexpressed genes were primarily reflective of alterations in mitochondrial metabolism.

Conclusions: Surgical inflammation affects the gene expression profiles of cardiac isografts over time and may confound expression studies attempting to identify genes associated with acute cellular rejection. Therefore, time-matched controls must be used to better identify expression patterns specific to ACR.

Abstract# 547 Poster Board #-Session: P3-II

TRANSCRIPTIONAL PROFILING SPECIFICALLY DISCRIMINATES BETWEEN CHRONIC AND ACUTE REJECTION IN NON-HUMAN PRIMATE (NHP) RENAL ALLOGRAFT RECIPIENTS. Marc Bigaud,[1] Grazyna Wieczorek,[1] Klaus Menninger,[1] Sabine Riesen,[1] Friedrich Raulf,[1] Jeanne Kehren.[1] *[1]Transplantation and Immunology Research, Novartis Pharma AG, Novartis Institutes for Biochemical Research, Basel, Switzerland.*

Introduction: The detection and treatment of chronic allograft rejection (CAR) remains a major medical need. As new pharmacological targets, markers and therapies could be identified by discriminating CAR-specific transcriptional changes from those linked to acute rejection (AR), we performed a comparative analysis of gene expression in grafts using clinically relevant models of AR and CAR in NHP (cynomolgus monkey) kidney allografts.

Methods:
The AR model consisted in life-supporting kidney allografts (n=5) in non immunosuppressed recipients. AR occured within 6-9 days.

The CAR model consisted in non life-supporting kidney allografts (n=9) in recipients treated with cyclosporine-A (CsA; 10 mg/kg/d, s.c.). Four grafts remained functional up to 100 days, tapered of CsA and terminated within 231-331 days. Five grafts showed signs of vascular AR (transplant endarteritis) within 20-35 days, recieved an anti-rejection therapy combining ATG/methylprednisolone (20/10 mg/kg, i.v. 5 times every 2 days)/Neoral® (150 mg/kg/d, p.o.) and survived up to 44-147 days. All grafts showed various degrees of vascular remodeling similar to those seen in humans.

Kidney samples (including 12 controls) were collected at necropsy. RNA were extracted using RNeasy (Qiagen) and hybridized to U133A Affymetrix Gene Chips. Data were analyzed using GeneSpring 5.0.3 (Silicon Genetics, CA).

Results: Although AR and CAR shared several features related to the loss of kidney function and the cellular infiltrate invading the grafts (over expression of AIF-1, clusterin, versican, osteonectin, osteopontin, MMP-7, MMP-9, Transglutaminase 2), specific changes could be differentiated. AR-specific features included indolamine deoxygenase. CAR-specific features included von Willebrand factor, OB-cadherin, OSF-2, SDF-1, ELC (exodus-3), Matrix GLA protein, Darc, decorin, C3 complement protein and cleavage products, PDGF receptor alpha. These genes are markers of vascular remodeling and of mesenchymal (or stem) cells. Importantly, similar CAR markers were observed in grafts showing early (<150 days) or late (>200 days) CAR development suggesting common underlying mechanisms.
Conclusions: These results demonstrate a clear discrimination between CAR- and AR-specific transcriptional changes in NHP kidney allografts. The fact that some genes appear for the first time as CAR-specific supports the value of the NHP models as tool for target/marker identification and compound testing.

Abstract# 548 Poster Board #-Session: P4-II

REJECTION OF FULLY MHC MISMATCHED ALLOGRAFTS IS INDEPENDENT OF MYD88, A TOLL/IL-1 SIGNAL ADAPTOR.
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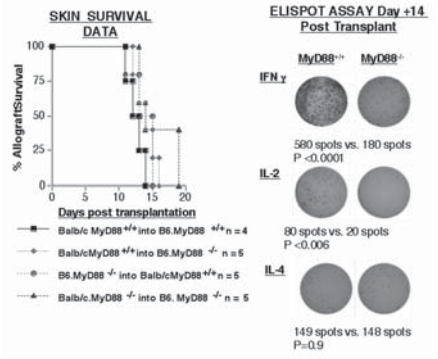
Introduction. Toll Like receptors (TLRs) are important innate immune receptors that are critical for pathogen recognition by augmenting DC maturation and TH1 immunity. However, their role in fully MHC mismatched allograft rejection is unknown. Thus, we planned to examine this question utilizing MyD88 deficient mice (MyD88 = universal TLR signal adaptor).
Methods and Results



Survival data revealed that all groups rejected skin allografts without delay (Balb/c →B6 strain combination see figure, p = NS in all groups) including when Balb/c MyD88$^{-/-}$donors were grafted onto B6. MyD88$^{-/-}$ recipients. Similar results were obtained using a heterotopic cardiac transplant model. Evaluation of the draining lymph nodes of Balb/C skin allografts via flow cytometry did not reveal a difference in the proportion of mature DCs (CD11c+ CD40+CD80+CD86+ 1.6% vs. 1.6%, p = NS) between MyD88$^{+/+}$ and MyD88$^{-/-}$ recipients, respectively. However, ELISPOT analysis at day +14 post transplant demonstrated reduced IFN γ and IL-2 +ve cells within MyD88$^{-/-}$ recipient spleen cells when stimulated by donor SPCs in a 1-way MLR (see fig). IL-4 (TH2 function) was not impaired between the 2 groups (fig). Both groups were equally able to secrete these cytokines in response to Con A. Anti-donor Abs levels (IgG, an assessment of humoral adaptive immunity) measured by flow cytometry were similar between the 2 groups following skin grafting (MyD88$^{+/+}$ 15 vs. MyD88$^{-/-}$ 10 median fluorescence units, p=0.8).
Conclusion. Despite impaired T cell activation and TH1 immune responses, MyD88 is not critical for the rejection of fully MHC mismatched skin and cardiac allografts. This study underscores the importance of MyD88 independent pathways in innate recognition of fully MHC mismatched allografts.

Abstract# 549 Poster Board #-Session: P5-II

MYD88 DEFICIENCY PROLONGS VASCULARIZED SOLID ORGAN GRAFT SURVIVAL.
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Toll-Like receptors recognize pathogen-associated microbial patterns and may bind host stress proteins. TLRs induce the translocation of NF-κB via MyD88 resulting in proinflammatory cytokine expression. In a recent report, the complete absence of MyD88 markedly prolonged skin allograft survival. In this study, our aim is to determine the contribution of MyD88 and TLR2 to graft rejection in a vascularized cardiac transplant model.

Methods: Vascularized heterotopic cardiac transplantation was performed on 8-12 week old male mice. Graft rejection is determined by the absence of palpable graft pulsation and confirmed at harvest. For cell trafficking studies, Balb grafts were transplanted to B6 transgenic mice expressing GFP. Cell suspensions prepared from the graft hearts were sorted via flow cytometry by GFP fluorescence. RNA from GFP$^+$ and GFP$^-$ cell fractions was isolated, treated with DNase, reverse transcribed to cDNA and analyzed by real time PCR. Syngenic transplants harvested at 24 hrs and control untransplanted hearts were also analyzed. Gene expression was normalized to the housekeeping genes GAPDH and β-actin.
Results: Deficiency of MyD88 in either the donor recipient and deficiency of TLR2 in the donor, but not the recipient, produced significantly prolonged graft survival:
Mean Survival Time (in days)
MyD88$^{-/-}$Balb>B6(11.6). B6>MyD88$^{-/-}$Balb(15.3). Complete absence of MyD88(MyD88$^{-/-}$B6>MyD88$^{-/-}$Balb) produces an even greater prolongation of graft survival.
B6TLR2$^{-/-}$>Balb(11). Balb>B6TLR2$^{-/-}$(8).
B6>Balb(8.5). Balb>B6(7.5). Balb>Balb(>100).
At 24 hrs, TLR2 expression is increased in the GFP$^+$ infiltrating cells compared to GFP$^-$ graft cells and untransplanted Balb heart. (14.8, 2.1 and 0.1%GAPDH respectively). Small increases in TLR1, TLR4, and TLR7, but not TLR3, TLR6, or TLR9 were also observed.
Conclusions: MyD88 deficient graft survival (11.6 d) and MyD88 deficient recipient survival (15.3 d) is prolonged compared to allogenic controls. Complete absence of MyD88 results in a greater prolongation of graft survival than deficiency in either the donor or recipient. Also, TLR2 is produced by infiltrating and graft cells at 24 hrs post transplantation. Grafts deficient in TLR2 show a modest delay in allograft rejection compared to allogenic control (11 vs 8.5 d). TLR2 deficient recipients do not show prolongation of rejection. Lack of local expression of TLR2 by the graft is associated with modest prolongation of graft survival. MyD88 is thus important in modulating graft rejection in a vascularized heterotopic cardiac model.

Abstract# 550 Poster Board #-Session: P6-II

T CELL MEDIATED KIDNEY REJECTION ALTERS EPITHELIAL FUNCTION BEFORE TUBULITIS DEVELOPS: EVIDENCE THAT EARLY T CELL EFFECTS ARE INSTRUCTIVE RATHER THAN DESTRUCTIVE.
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We studied relationships of histology to transcriptional changes during rejection of mouse kidney transplants from CBA donors into B6 recipients. Interstitial infiltration and edema developed rapidly by day 5 (D5) and evolved slowly thereafter; tubulitis and arteritis were absent at D5 but extensive by D21. All early changes (D5, D7) were T cell dependent i.e. unchanged in mice lacking immunoglobulins (AJT 3:1501, 2003).
We studied gene expression using Affymetrix MOE430A microarrays. Of 22690 genes on this array, 15% were highly expressed in normal kidneys (signal >1000). By biological function, three main groups accounted for these genes: metabolism, cell maintenance, and cell communication. Subgroups strongly represented in highly expressed genes included mitochondrial transport, energy pathways, and response to oxidative stress.
Following transplantation, there was selective downregulation of certain highly expressed kidney genes, with a subset of 118 genes decreased more than 3 fold at D5. Most were involved in metabolism, energy, and transport. Table 1 shows the 5 genes with the greatest changes. Downregulation occurred early (D5), with gene expression either stabilizing at a low level or continuing to decrease further. No groups of downregulated genes recovered by D21. Some genes mediating epithelial function (chloride/potassium transport, ATPases, water channels) were included in the affected genes, showing early decreased expression followed by continuing slow decline. Mixing kidney RNA with RNA from lymphocytes showed that the decreased gene expression in rejection was not due to dilution by RNA from infiltrating cells.
Thus T cell mediated kidney rejection causes rapid, selective decreases in expression of some renal genes before tubulitis develops, suggesting that early function changes in rejection are instructive rather than destructive.

Table 1: Decrease of renal genes in allograft rejection (fold reduction)

Gene	Normal	D5 Tx	D7 Tx	D21 Tx	Function
Transthyretin	1518	1 (↓388x)	2 (↓512x)	1 (↓955x)	Transport
Betaine-homocysteine metabolism methyltransferase	1356	8 (↓128x)	42 (↓24x)	32 (↓39x)	Amino acid
fatty acid binding protein 1, liver	2290	7 (↓97x)	9 (↓97x)	1 (↓549x)	Transport
4-hydroxyphenylpyruvic acid diogygenase	7414	522 (↓15x)	277 (↓28x)	492 (↓17x)	catabolism
glutathione S-transferase, alpha 2	5741	436 (↓11x)	445 (↓11x)	265 (↓26x)	metabolism

Numbers represent signal strength, numbers in ( ) show the fold changes compared to normal CBA kidneys.

Abstract# 551 Poster Board #-Session: P7-II

SECOND WAVE INFILTRATION OF PMNS INTO CARDIAC ALLOGRAFTS AMPLIFIES T CELL ACTIVITY AND PROMOTES GRAFT REJECTION. Tarek El-Sawy,[1,2] Robert L. Fairchild.[1,2] *[1]Pathology, Case Western Reserve University School of Medicine, Cleveland, OH; [2]Immunology, The Cleveland Clinic Foundation, Cleveland, OH.*

Introduction: Polymorphonuclear cells (PMNs) are the principle cellular mediator of early inflammation following ischemia/reperfusion. In addition, a second wave of PMN infiltration into cardiac allografts was observed near the time of rejection. The goal of the current study was to test the role of this second wave of PMN infiltration on T cell activity and allograft rejection.

Methods: C57BL/6 (H-2b) mice, 129/SVJ (H-2b), and 129/SVJ (H-2b) Cathepsin G-/- mice received A/J (H-2a) cardiac allografts heterotopically. C57BL/6 mice were treated daily with anti-Ly6G (RB6-8C5) starting on day 3 post-transplant until the time of rejection or on days -1, 0, 1 post-transplant.

Results: Treatment with anti-Ly6G around the time of transplant significantly delayed the rejection of full MHC mismatched cardiac allografts to days 15-20. Treatment with anti-Ly6G starting day 3 post-transplant also significantly delayed cardiac allograft rejection to 10-14 days compared to 7-8 days in control antibody treated animals. This delay in rejection was also observed in Cathepsin G-/- recipients (14-19 days). T cell priming of the anti-Ly6G treated or Cathepsin G-/- animals, measured in the spleen by IFN-g ELISPOT at day 7 and at the time of rejection was identical to that measured in wildtype control treated animals. However, the number of allo-reactive IFN-g producing cells isolated from grafts harvested from anti-Ly6G treated recipients was significantly reduced at day 7 as well as at the time of rejection. In addition, treatment with anti-Ly6G prior to transplant or beginning on day 3 post-transplant delayed the infiltration of T cells into the allograft parenchyma.

Conclusions: The early inflammatory response, particularly PMN mediated effects, following transplantation has a strong influence on downstream T cell recruitment. In addition, depletion of PMNs after the early inflammatory response has resolved, can still delay T cell infiltration and graft rejection. A potential PMN mediated mechanism for this observation is the release of factors that amplify T cell effector function. Full MHC mismatched cardiac allograft survival in cathepsin G-/- recipients is significantly prolonged. This suggests that the second wave of PMN infiltration into cardiac allografts can have a significant amplifying effect on the T cell activities that mediate graft rejection.

Abstract# 552 Poster Board #-Session: P8-II

GRAFT SPECIFIC IL-6 DEFICIENCY PROLONGS VASCULARIZED SOLID ORGAN GRAFT SURVIVAL. Yurong Liang,[1] Kenneth Christopher,[1] Rachel DeFina,[1] Thomas F. Mueller,[1] David L. Perkins.[1] *[1]Laboratory of Molecular Immunology, Brigham and Women's Hospital, Boston, MA.*

IL6 influences immune responses. The IL6 cytokine family includes LIF, Oncostatin M (OSM), and IL-11. We previously reported increased IL6 protein in the serum and mRNA in graft tissue following transplantation. In this study, our aim is to determine the contribution of IL6 to graft rejection in a cardiac transplant model.

Methods: Vascularized heterotopic cardiac transplantation was performed on 8-12 week old male mice. Graft survival was determined for IL6 deficient groups (Balb⇒IL6$^{-/-}$B6; IL6$^{-/-}$B6⇒Balb) and control (Balb⇔B6). Serum IL6 was quantitated by ELISA. For cell trafficking studies, GFP transgenic mice were used as recipients (Balb⇒GFPB6). Cell suspensions prepared from grafts were sorted via flow cytometry. RNA was individually isolated from GFP^{+} and GFP^{-} cell fractions, grafts, and lymph nodes. RNA was treated with DNase and reverse transcribed to cDNA. Real Time PCR was used to analyze expression of cellular markers and immune genes including IL6, IL6R, and gp130. Data was analyzed by SAM (Significance Analysis of Microarrays) to determine significant differences in gene expression. RNase Protection Assay was performed to confirm IL6 expression.

Results: Serum IL6 is present in IL6$^{-/-}$ recipients (79-248 pg/ml) following transplantation of wildtype allogeneic grafts at days 1,3,5, and 7. In contrast, serum IL6 is absent in wildtype recipients of IL6$^{-/-}$ grafts (8-34 pg/ml) at days 1,3,5 and 7. Serum IL6 is absent in IL6$^{-/-}$ mice at baseline, but highly expressed in wildtype allogeneic transplants. Interestingly, graft survival was prolonged in the group with IL6$^{-/-}$B6 donors (MST 19.3 days), whereas rejection in the group with IL6$^{-/-}$ recipients and wildtype allogeneic controls was rapid (7.5 and 8.5 days respectively).

Graft IL6 expression peaks at 6 hrs post-transplant (18.0 vs 0.08%GAPDH in the control). Cell trafficking data show that IL6, LIF, IL-11 and gp130 are expressed in graft tissue, but not in recipient infiltrating cells. Infiltrating cells do produce OSM, a stimulus for IL6 production. SAM analysis identified highly upregulated genes in the IL6$^{-/-}$ grafts at days 1 and 7 compared to IL6$^{+/+}$ grafts. IL6 is not upregulated in the recipient LN following IL6$^{-/-}$B6⇒Balbc at days 1 or 7.

Conclusions: IL6 and family members LIF, IL-11 and gp130 are produced by the graft but not by infiltrating cells early post transplant. Importantly, deficiency of local graft production of IL6 prolongs graft survival. Thus, the graft is an important source of serum IL6 production post transplantation.

Abstract# 553 Poster Board #-Session: P9-II

α-PHENYL-*N-tert*-BUTYLNITRONE, A FREE RADICAL SPIN-TRAPPING AGENT, HAS POTENT EFFECTS ON CYTOKINE GENE EXPRESSION IN ACUTE CARDIAC ALLOGRAFT REJECTION. Galen M. Pieper,[1] Vani Nilakantan,[1] Aswhani K. Khanna,[2] Xianghua Zhou,[1] Christopher C. Felix,[3] Mark B. Adams,[1] Allan M. Roza,[1] Christopher P. Johnson.[1] *[1]Surgery (Transplant Surgery); [2]Medicine; [3]Biophysics, Medical College of Wisconsin, Milwaukee, WI.*

Purpose: Nitrones are compounds that have been used in free radical chemistry to stabilize and trap free radicals for detection by advanced biophysical techniques such as electron paramagnetic resonance (EPR) spectroscopy. Since oxidant stress may contribute to or potentiate organ rejection, we examined the protective action of chronic treatment with α-phenyl-*N-tert*-butylnitrone (PBN), a spin-trap agent, on acute cardiac allograft rejection. *Methods:* Graft survival, histological rejections scores and *in situ* graft function (by sonomicrometry) were determined in Wistar-Furth to Lewis rat strain cardiac transplants. Trapping of reactive oxygen by PBN *in vivo* and decreases in nitrosylation of heme protein were determined by EPR spectroscopy. RT-PCR or Western analysis were used to examine expression of inflammatory cytokines and inducible nitric oxide synthase (iNOS). Nuclear binding of transcription factors NF-κB and AP-1 were determined by electrophoretic mobility shift assays. *Results:* Histological rejection scores were significantly decreased by treatment with PBN (4.9 ± 0.3 vs. 3.2 ± 0.4; P<0.01 vs untreated) indicating decreased cell infiltration. PBN improved graft survival time. PBN did not alter the decrease in heart rate and % fractional shortening but did normalize the allotransplant-induced increase in short-axis end-diastolic and end-systolic diameters. A marked decrease in nitrosylation of myocardial heme protein determined by EPR spectroscopy could be explained, in part, by decreased iNOS protein levels. Decreased expression of iNOS was associated with marked decreases (approx. 50%) in nuclear DNA binding activity for both NF-κB and AP-1. RT-PCR analysis revealed significant decreases in gene expression for iNOS, inteferon-γ, interleukin-6 and interleukin-10 normalized to β-actin controls at postoperative day 4 and blockade of increases in expression of these genes by postoperative day 6. *Conclusions*: Nitrone-based spin-traps used traditionally to detect reactive oxygen production by EPR spectroscopy appear also to have significant therapeutic actions when given chronically. The action to limit histological rejection and ablate gene expression of a variety of inflammatory cytokines supports a role of reactive oxygen in the process of acute organ rejection.

Abstract# 554 Poster Board #-Session: P10-II

THE SELECTIVE ESTROGEN-RECEPTOR-BETA AGONIST BIOCHANIN A SHOWS IMMUNOMODULATING EFFECTS IN EXPERIMENTAL TRANSPLANTATION WITHOUT AFFECTING THE REPRODUCTIVE SYSTEM – IN VIVO AND IN VITRO STUDIES. Sonja Schrepfer,[1] Tobias Deuse,[1] Friedrich Koch-Nolte,[2] Hans-Jörg Schäfer,[3] Hermann Reichenspurner.[1] *[1]Cardiovascular Surgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]Immunolgy, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [3]Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*

Objective:

We investigated the effects of biochaninA and ethinylestradiol, selective estrogen receptor beta and alpha agonists, respectively on acute cardiac allograft rejection, uterine effects, adhesion molecule and MHC-II expression in experimental transplantation and verified the observations using in-vitro cell culture.

Methods:

Heterotopic cardiac transplantations were performed in the rat strain combination Lewis to F344. The study groups received biochaninA or ethinylestradiol by oral gavage beginning on day -2, the control group received no treatment. Grafts and uteri were harvested on the fifth postoperative day and blood was taken for drug plasma level quantifications. Purified (MACS) Lewis aortic endothelial cell cultures were pre-treated with biochaninA or ethinylestradiol and stimulated with TNF-alpha or IFN-gamma. Surface protein expression was quantified by immunofluorescence, FACS and western-blotting. Lymphocyte-endothelium adhesion assays and single cell adhesion assays were performed using purified (MACS) F344 lymphocytes.

Results:

Both biochaninA and ethinylestradiol treatment significantly reduced graft mononuclear infiltration of CD8- and ED1-pos. cells and markedly reduced ISHLT grading compared to untreated controls. Either agent significantly inhibited endothelial VCAM-1 upregulation during graft rejection and during TNF-a-stimulation in vitro, whereas no effect was observed for ICAM-1 upregulation. BiochaninA but not ethinylestradiol significantly reduced MHC-II upregulation in vivo and in vitro. Ethinylestradiol treatment strongly affected uterus growth, whereas biochaninA did not.

Conclusions:

Only the treatment with the phytoestrogen biochaninA reduced endothelial MHC-II expression in vivo and in vitro and showed the desired cardioprotective effect without affecting the reproduction system. A supplemental use of phytoestrogens after cardiac transplantation may provide further benefits in the clinical setting and their application to men may also be considered.

Abstract# 555 Poster Board #-Session: P11-II

INCREASED INCIDENCE OF ACUTE REJECTION AMONG CARDIAC TRANSPLANT RECIPIENTS POSSESSING THE GLN12STOP VARIANT OF ADENOSINE MONOPHOSPHATE DEAMINASE – 1. Anne B. Taegtmeyer,[1,2] Jane B. Breen,[1] John D. Smith,[2] Nicholas R. Banner,[1] Margaret M. Burke,[1] Alex D. Bell,[1] Magdi H. Yacoub,[2] Paul J. R. Barton.[2] *[1]Transplant Unit, Harefield Hospital, Harefield, Middlesex, United Kingdom; [2]Heart Science Centre, Imperial College London, Harefield Hospital, Harefield, Middlesex, United Kingdom.*

PURPOSE Adenosine monophosphate deaminase 1 (AMPD-1) catalyses the breakdown of adenosine monophosphate to inosine monophosphate, both of which are important substrates in DNA and RNA synthesis pathways. The C34T (Gln12STOP) T allele of AMPD-1 encodes an inactive enzyme and individuals who possess this variant are likely to have an altered balance of purine metabolism. Inhibition of purine synthesis by azathioprine is used to prevent T cell proliferation and thus acute rejection. We therefore investigated the effect of the possession of Gln12STOP by either the recipient or the donor organ on the development of acute rejection following cardiac transplantation.

METHODS 184 adult cardiac transplant patients transplanted at our institution between 1991 and 1999 and who survived greater than one month were studied retrospectively. Immunosuppression was with cyclosporin, prednisolone and azathioprine. Genotyping was performed using TaqMan technology. 263 Caucasian controls were also genotyped. Rejection was diagnosed histologically and graded according to ISHLT criteria. A total of 4736 right ventricular endomyocardial biopsies were examined. HLA A, B and DR mismatches were also recorded.

RESULTS Recipient and donor genotypes were in Hardy-Weinberg equilibrium and in a similar distribution to controls (CT and TT individuals 20, 20 and 21% respectively). There were a total of 429 rejection episodes ranging from grade 1B to grade 4, 98% of which occurred during the first year after transplantation. Examined as a whole, recipients carrying the T allele (CT and TT, n= 36) experienced a greater total number of rejection episodes with a mean of 2.8 rejection episodes per recipient compared to 2.1 rejection episodes per CC recipient (n= 148) ($p < 0.01$). Donor genotype was not associated with rejection (2.2 and 2.3 episodes per patient for donor organs of CC and CT or TT genotype respectively). HLA A, B and DR mismatches were similar for CC and CT or TT recipients (1.36 *vs* 1.35 HLA A, 1.6 *vs* 1.5 HLA B and 1.3 *vs* 1.26 HLA DR mismatches, p = ns).

CONCLUSIONS Cardiac transplant recipients who possess the C34T T allele of AMPD-1 had a higher incidence of biopsy-proven acute rejection. The underlying mechanisms remain unclear but may involve imbalance of nucleotide metabolic pathways.

Abstract# 556 Poster Board #-Session: P12-II

GROWTH FACTOR INDUCED INHIBITION OF ALLOSENSITIZATION IN SKINTRANSPLANTS. Bart M. Stubenitsky,[1] Lauren Brasile,[1] Moshe Kon.[1] *[1]Reconstructive Surgery, UMCU University Hospital, Utrecht, Netherlands.*

Introduction- Allogenic skin transplantation is a rigorous test of any intervention designed to prevent rejection. We demonstrated that creating an interface between skin allograft and wound surface consisting of a bioengineered acellular matrix membrane resulted in statistically significant prolongation of skin survival. However, humoral response eventually was detected following rejection of the skin graft. In the present study incorporating fibroblast growth factor 1 (FGF-1; 10ug) into the matrix membrane for further prolongation of skin graft survival and prevention of a humoral reaction was evaluated.

Methods- Full thickness skin grafts were cross-transplanted between BALBC and C57BL/6 mice:

Group 1 (n=15) - skin allografts transplanted without treatment

Group 2 (n=15) - skin allografts transplantated with matrix membrane treatment alone

Group 3 (n=15) - skin allografts transplantated with matrix membrane + FGF-1

Animals were followed by visual inspection, biopsy, lymphocyte evaluation by flow cytometry and alloantibody determination. Rejection was determined on the basis of erythema, hair loss, flakiness, and/or scabs.

Results- Rejection in the Group 1 occurred with a mean of 6.8 days (+/- 1.5). Group 2 and 3 showed a prolonged allograft survival, with a mean of 25 days (+/- 3.8 days) and 28 days (+/-5.1 days) respectively. Immunologic screenings indicated that untreated skin grafts stimulated a shift in the number of CD4 positive cells and the development of donor-specific antibody by 14 days posttransplant. Allosensitization was reduced in Group 2 mice that received skin grafts treated with the matrix membrane alone. A humoral response to their donors skin cells was observed in 42% (7/15), although treatment lead to a substantially reduced shift in CD4 positive cells. When FGF-1 was combined with the solubilized membrane prior to administration and polymerization, a statistically significant inhibition of allosensitization was observed. FGF-1 administration prevented the development of humoral responses to the donor skin cells (0/15) as determined by flow cytometry ($p<0.005$). **Conclusions-** Physical interruption of the recipient/donor interface mediated by a bioengineered matrix membrane, results in prolongation of skin allograft survival. The addition of growth factor further potentiates this prolongation effect and appears to prevent the development of donor-specific antibody. The mechanism(s) for the observed modulatory effects is currently under study. Possible protective mechanisms under evaluation are the effect of FGF-1 on inflammation and wound repair.

Abstract# 557 Poster Board #-Session: P13-II

PIOGLITAZONE SUPPRESSES ACUTE AND CHRONIC ALLOGRAFT REJECTION. Hisanori Kosuge,[1] Jun-ichi Suzuki,[1] Go Haraguchi,[1] Noritaka Koga,[1] Ryo Gotoh,[1] Mitsuaki Isobe.[1] *[1]Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Peroxisome proliferator-activated receptor-γ (PPARγ) plays an important role in regulating inflammatory and proliferative action. Although cardiac transplantation is an established therapy for patients with end-stage heart disease, allograft rejection is a major problem of limitation for their long-term survival. We investigated the role of PPARγ agonist in acute and chronic allograft rejection. **Methods and Results:** We performed heterotopic murine cardiac transplantation. Recipient mice were orally administrated with either control chow or chow containing pioglitazone (30 mg/kg/day) from 1 day before cardiac transplantation. In total allomismatch combinations (C3H/He recipients and BALB/c donors), treatment with pioglitazone significantly prolonged cardiac allograft survival in comparison to that in mice receiving control chow (mean survival time (MST): 10.6 ± 0.7 days vs. 8.4 ± 0.4 days; $p<0.03$; n=8). To investigate the effect of pioglitazone for chronic rejection (graft arterial disease (GAD)), hearts from Bm12 mice were transplanted into C57BL/6 (B/6) mice (class II mismatch) and allografts were harvested at 8 weeks after cardiac transplantation. Although severe GAD developed in cardiac allografts from mice receiving control chow (luminal occlusion: 61.8 ± 6.7 %), GAD was significantly attenuated in cardiac allografts from mice treated with pioglitazone (17.9 ± 6.6 %, $p<0.001$). In total allomismatch and class II mismatch combinations, expression of IFN-γ in cardiac allogarfts was significantly suppressed in pioglitazone-treatment group compared with control group. We performed proliferation assays of smooth muscle cells (SMCs) in vitro. SMCs were obtained from thoracic aorta of bm12 mice. After SMCs were arrested for 24 hours, co-culture with SMCs and inactivated splenocytes was performed. SMCs were significantly proliferated by co-culture with SMCs and inactivated splenocytes. This proliferation was significantly inhibited by addition of pioglitazon (1 μM). **Conclusions:** Pioglitazone prolonged allograft survival and attenuated GAD through suppression of SMCs proliferation. PPARγ agonist has promise in suppressing acute and chronic allograft rejection.

Abstract# 558 Poster Board #-Session: P14-II

HLA CLASS I LIGATION ACTIVATES FAK AND MEDIATES ASSOCIATION OF ADAPTER PROTEINS WITH FAK VIA PHOSPHORYLATION OF FAK AT TYROSINE 925 IN ENDOTHELIAL CELLS. Yi-ping Jin,[1] Michael S. Bennett, Elaine F. Reed.[1] *[1]Department of Pathology and Laboratory Medicine, University of California at Los Angeles, Los Angeles, CA.*

There is substantial evidence to suggest that the development of anti-donor HLA class I antibodies (Ab) is involved in the process of transplant arteriosclerosis, the hallmark of chronic rejection. In previous studies we have shown that anti-HLA Ab may contribute to the process of chronic rejection by binding to endothelial cells (EC) and smooth muscle cells and stimulating fibroblast growth factor expression, increased ligand binding and cell proliferation. Investigation of the intracellular signaling cascade showed that ligation of class I molecules stimulated Src phosphorylation and complex formation between Src, and the focal adhesion proteins FAK and paxillin. In the present study, we have characterized the role of Src kinase activity on the regulation of FAK activation. and triggering of molecular complexes with other downstream signaling proteins. For these studies, EC were treated with anti-HLA class I monoclonal Ab (mAb) W6/32 for various time points and cell lysates were immunoprecipited and immunoblotted with anti-phosphotyrosine Ab. Treatment of EC with anti-HLA class I Ab stimulated a time dependent increase in phosphorylation of FAK at residues Tyr-577, Tyr-576 and Tyr-925. Treatment with the pharmacological inhibitor PP2 completely inhibited class I mediated FAK phosphorylation at these residues indicating a critical role for Src kinase activity in this process. Ligation of class I molecules also triggered a Src dependent assembly of molecular complexs of FAK/Shc/Grb2 and FAK/Grb2/Sos providing a link between class I signaling and the MAP kinase pathway. Elucidation of the molecular basis of antibody-mediated alterations in graft cells may permit the development of immunotherapuetics designed to regulate the response to injury.

Abstract# 559 Poster Board #-Session: P15-II

LIGATION OF HLA CLASS I MOLECULES TRIGGERS ACTIN REORGANIZATION IN A RHOA DEPENDENT MANNER. Eric J. Lepin,[1] Yi-ping Jin,[1] Elaine F. Reed.[1] *[1]Department of Pathology & Laboratory Medicine, University of California at Los Angeles, Los Angeles, CA.*

The binding of anti-HLA antibodies on endothelial cells transduces activation signals and initiates cell proliferation in a model relevant to the development of transplantation associated vasculopathies.

Recently, we demonstrated that inhibition of actin function by cytochalasin D prevents increased FAK and paxillin tyrosine phosphorylation in response to HLA class I stimulation underscoring an important role of the actin cytoskeleton in this process. We hypothesize that actin reorganization controlled by Rho GTPases could be an early event regulating the HLA class I signaling cascade. Detection of F-actin with fluorochrome conjugated phalloidin in cells treated with W6/32 showed a rapid increase in stress fiber formation, compared to both mouse IgG treated or untreated cells. In accord with HLA class I induced stress fiber formation and the established role of RhoA in regulating stress fiber formation, we observed a rapid and transient increase of RhoA activity upon HLA class I crosslinking. However, ligation of HLA did not result in any significant Rac or Cdc42 activation. Finally, the most important finding of this study is that, inhibition of either Rho GTPase or ROK in endothelial cells abrogates HLA class I induced FAK and paxillin phosphorylation, two central elements of the focal adhesion signaling complex activated by HLA class I molecule ligation. Taken together these results suggest that MHC class I signaling through the focal adhesion complex in endothelial cells is dependant on the regulation of actin reorganization by Rho GTPase; with RhoA having a predominant role. Elucidation of the class I signal transduction cascade has the potential to identify novel therapeutic strategies to prevent development of transplant vasculopathies.

Abstract# 560 Poster Board #-Session: P16-II

CELL TRAFFICKING IN MURINE CARDIAC TRANSPLANTATION. Kenneth Christopher,[1] Thomas F. Mueller,[1] Yurong Liang,[1] Rachel DeFina,[1] David L. Perkins.[1] *[1]Laboratory of Molecular Immunology, Brigham and Women's Hospital, Boston, MA.*

The trauma and ischemia/reperfusion injury sustained by the graft induces early chemokines that direct neutrophils and macrophages to grafts. Using GFP transgenic mice we sought to differentiate recipient infiltrating cells versus donor graft cells *in vivo.* In this study, early infiltrating cell types are identified and differential gene expression of infiltrating cells versus graft cells is demonstrated.

Methods: Vascularized heterotopic cardiac transplantation was performed on 8-12 week old male mice (Balb⇒B6). Grafts were harvested at 3, 6, 12 hrs and days 1-7. RNA from grafts is isolated, treated with DNase, reverse transcribed to cDNA and analyzed by Real Time PCR for a large panel of immune related genes.

For cell trafficking studies, B6GFP mice are used as recipients (Balb⇒B6GFP) and grafts are harvested at 12, 24 and 36 hrs. B6GFP mice constitutively express the enhanced green fluorescent protein transgene driven by a Class I promoter. Single cell suspensions prepared from the graft heart are incubated with PE conjugated Anti-Gr1 or Anti-F4/80. Cell suspensions are sorted via flow cytometry. RNA from cell fractions are isolated, treated with DNase, reverse transcribed to cDNA and analyzed by Real Time PCR for expression of genes identified as upregulated in the Balb⇒B6 data.

Results: Graft expression of IL6, IL1β, GCSFR, C5aR, properdin, C1qb and calreticulin are all significantly upregulated post-transplant relative to control heart. GCSFR peaks at 3 hours, IL6 peaks at 6 hours while calreticulin, properdin, C1qb, C5aR, and IL1β all gradually increase expression and peak at day 6-7. Infiltrating GFP+ cells post transplant are detectable at 3 hours and increase dramatically after 3 days and peak at 6 days ($4.3X10^4$,$6.9X10^5$,$5.5X10^6$ cells/graft respectively). Cell trafficking studies at 12-36 hours show IL1β and GCSFR are produced only by neutrophils (GFP^+Gr1^+), properdin is produced by non-neutrophils (GFP^+GR1^-), C5aR is produced by both neutrophils and macrophages ($GFP^+F4/80^{hi}$, GFP^+Gr1^+), Calreticulin and C1q are produced by macrophages ($GFP+F4/80^{hi}$, $GFP+GR1^-$), and Calreticulin and IL6 are produced by the graft ($GFP\text{-}F4/80^{low}$,GFP-Gr1-).

Conclusions: The use of transgenic GFP mice as graft recipients allows for cell trafficking to be studied *in vivo* post-transplantation. Cell trafficking identifies macrophages and neutrophils as early infiltrating cell types. Distinct patterns of highly expressed genes (IL6, IL1β, GCSFR, C5aR, properdin, C1qb and calreticulin) are observed in the graft, infiltrating macrophages and neutrophils.

Abstract# 561 Poster Board #-Session: P17-II

ANTIGEN LOCATION INDEPENDENTLY DETERMINES THE PATHOLOGIC FEATURES OF A TRANSPLANTED ORGAN. Yifa Chen,[1] Yilmaz Demir,[1] Anna Valujskikh,[1] Peter S. Heeger.[1] *[1]Immunology and The Glickman Urologic Institute, The Cleveland Clinic Foundation, Cleveland, OH.*

Organ-specific injury following transplantation presents with a variety of clinical and pathologic phenotypes, yet the factors influencing development of each outcome are poorly understood. As primed T lymphocytes must re-encounter their antigen within the target organ to engage effector functions, we postulated that the cellular location of antigen within that organ could significantly impact the induced pathology. To assess this issue, we used female Mar CD4 T cell receptor transgenic mice (n= 4-6 per group), in which all T cells are specific for the male minor transplantation antigen plus $I\text{-}A^b$, as recipients of heterotopic heart transplants. Notably, these recipients were bred onto a RAG -/- background and therefore have no other T cells and no B cells. The Mar recipients were either given male heart grafts expressing the relevant peptide: MHC complex on graft parenchymal/vascular cells or, alternatively, only on graft-infiltrating mononuclear cells. Transplantation of the two different graft types led to equivalent activation of recipient T cells as assessed by frequency, cell surface marker expression by flow cytometry, cytokine production by ELISPOT, and the ability to traffic to the graft as demonstrated by histologic and immunohistochemical examination of graft tissue. Nonetheless, at the effector stage, if the target antigen was expressed on graft vascular and/or parenchymal cells, the outcome was acute graft destruction. In contrast, at the effector stage, if the antigen was expressed only on graft-infiltrating mononuclear cells, the same primed T cell repertoire caused chronic rejection and vasculopathy. The data demonstrate for the first time that target antigen location in the graft, independent of T cell specificity, frequency and effector function can markedly influence pathologic outcome. The results have significant implications for understanding the pathogenesis of acute and chronic transplant rejection in humans.

Abstract# 562 Poster Board #-Session: P18-II

TUBULAR CHIMERISM OCCURS REGULARLY IN RENAL ALLOGRAFTS AND IS NOT RELATED TO THE OUTCOME. Michael Mengel,[1] Danny Jonigk,[1] Wolfram Kleeberger,[1] Wilfried Gwinner,[2] Hermann Haller,[2] Hans Kreipe.[1] *[1]Institute of Pathology, Medizinische Hochschule Hannover, Hannover, Germany; [2]Department of Nephrology, Medizinische Hochschule Hannover, Hannover, Germany.*

Aims: Recent studies demonstrated an integration of recipient derived cells into solid allografts, leading to intragraft microchimerism. Whether or to which extent this finding is influencing function and survival of the allograft has still to be elucidated.

Methods: To detect epithelial chimerism in renal transplants from 36 patients tubular cells were harvested from paraffin embedded sequential allograft biopsies (n=72) by laser-microdissection. To prevent contamination by recipient lymphocytes a preceding immunohistochemical stain for CD45 was done. DNA was isolated following standard protocols and PCR assay was performed for analysis of highly polymorphic short tandem repeat marker (SE33, heterozygosity 93%). In transplants with sex-mismatch (female donor to male recipient) chimerism was detected by in situ hybridization for the Y-chromosome, too. Findings were correlated to morphological diagnosis and clinical course. Additionally we investigated six neoplasms arising in renal allografts for their derivation.

Results: Epithelial chimerism was detectable as early as 8 days after transplantation and lasted for nearly 8 years in our cohort. 88% (32/36) of our patients showed an epithelial chimerism. 26 of 36 (72 %) patients had a stable chimerism in sequential biopsies (time between biopsies: 3-96 months; mean 16,6 months). 4 patients had no epithelial chimerism in two sequential biopsies. 3 patients showed a late onset of chimerism. In one patient a loss of chimerical epithelial cells was found. 4 pre-implant biopsies showed no chimerism. Y-chromosome in situ-hybridization (n=8) revealed low percentages of chimeric tubular epithelial cells (2.4-6.6%). No correlation to morphological findings in allograft biopsies was detected. No correlation was found to outcome of graft function. Neoplasms arising in renal allografts are mostly donor derived, except metanephric adenomas demonstrating a chimeric derivation.

Conclusion: Epithelial microchimerism is an early and persistent phenomenon after renal transplantation. There is no correlation to morphological or functional outcome. Obviously recipient derived cells contribute in a minor fashion to tissue homeostasis, and cell turn over in renal allografts is predominantly enabled by donor cell renewal.

Abstract# 563 Poster Board #-Session: P19-II

LOW ACCUMULATION OF FOXP3 TRANSCRIPTS IN BLOOD CD4+ T CELLS OF PATIENTS WITH CHRONIC REJECTION. Cecile Braudeau,[1] Stephanie Louis,[1] Alexandre Dupont,[1] Magali Giral,[1] Jean-Paul Soulillou,[1] Sophie Brouard.[1] *[1]INSERM U437-ITERT, CHU Hotel Dieu, Nantes, France.*

FOXP3 is a forkhead transcription factor encoded on the X chromosome. Recently it has been described as the most specific marker for T regulatory cells (T_R). FOXP3 seems to control both development and at least some aspects of suppressor functions of T_R cells. We report analyses of FOXP3 in the blood of several informative groups of patients with renal transplantation to determine implication of this transcriptional factor on graft outcome.

Methods: Four groups of individuals have been studied: a) 4 kidney recipients spontaneously tolerating their graft 8±3 years after complete interruption of immunosuppressive drug (2 PTLD and 2 incompliance), b) recipients with similarly stable long term kidney graft function but under classical immunosuppression, c) patients with histologically documented chronic rejection and graft function degradation and d) normal healthy individuals. We used real time PCR to quantify FOXP3 in PBL in separated subsets CD4+ and CD8+ cells purified from patients blood. Data were normalized by HPRT transcripts.

Results: Spontaneously tolerant patients presented more circulating CD4+CD25+ cells (20.5±7.3% versus 10.6±5.2%; $p<0.05$) than patients with chronic rejection. Then, we analysed the level of FOXP3 transcripts in PBL, and showed no difference between

patients tolerating their graft and patients with chronic rejection. However, a decrease of accumulation of FOXP3 transcripts was observed in CD4+ cells in patients with chronic rejection compared both to drug-free tolerant patients and stable patients. In contrast, FOXP3 transcripts were almost undetectable in CD8+ cells, whatever total purified CD8+ cells or CD8+ cells from specific Vβ families, harboring a highly selected Vβ species (CDR3 length distribution) were tested. FOXP3 transcripts are now analysed on CD4+CD25+ and CD25- subsets. Our data suggest a decrease of FOXP3+ regulatory cells in patients with chronic rejection rather than an increase in tolerant and stable patients.

Abstract# 564 Poster Board #-Session: P20-II

NON-INVASIVE IMMUNE MONITORING OF PERFORIN/ GRANZYME B IN PERIPHERAL BLOOD PREDICTS RENAL ALLOGRAFT REJECTION. Leonard W. Liang,[1] Qiuheng Zhang,[1] David Gjertson,[1] Elizabeth Kendrick,[2] Phuong-Thu T. Pham,[2] Alan H. Wilkinson,[2] Gabriel M. Danovitch,[2] Elaine F. Reed,[1] H. Albin Gritsch.[2] *[1]Immunogenetics Center, University of California Los Angeles, Los Angeles, CA; [2]Renal Transplantation, University of California Los Angeles, Los Angeles, CA.*

Despite improvements in immunosuppression, allograft rejection remains a problem in renal transplantation. Monitoring serum creatinine is of poor prognostic value in predicting acute rejection. Renal allograft biopsy is invasive and predisposes the patient to serious complications. Immune monitoring may provide an early and non-invasive means for identifying patients at risk of acute cellular rejection (ACR). Forty-seven renal allograft recipients were monitored following transplantation for expression of T cell activation markers in the peripheral blood. Samples were collected weekly during the first 8 weeks and then at weeks 13, 18, 26, 39 and 52. Gene expression of perforin and granzyme B was quantitated by Real-Time PCR and results reported as normalized values to the endogenous control. Assay results were correlated with histologic changes in the renal biopsy and clinical data. Levels of Perforin and Granzyme B correlated closely to one another indicating that these markers are concomitantly expressed. Six patients had biopsy confirmed ACR. Elevated perforin levels correlated with ACR. The mean perforin value around the time of rejection was 160 units. The mean perforin level when there was not rejection was 94. (P=0.03) Similarly, the mean level of Granzyme B was 142 units around the time of rejection and 80 units when there was not rejection (p=0.01). It was found in 3 patients with ACR, elevations in perforin and granzyme B gene expression preceded increases in serum creatinine. The data indicate that a program of immune monitoring may serve as a noninvasive aid in the diagnosis of acute rejection.

Abstract# 565 Poster Board #-Session: P21-II

UP-REGULATION OF AIF-1 EXPRESSION IN KUPFFER CELLS BY Th1 CYTOKINE IN LIVER ALLOGRAFTS AFTER TRANSPLANTATION. Yuichi Nagakawa, Zhaoli Sun, Shuji Nomoto, Yukihiko Kato, Andrew Klein. *Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.*

Allograft inflammatory factor-1 (AIF-1) transcript levels are significantly decreased in allografted animals that receive immunosuppressive and immunomodulatory regimens, suggesting an association of AIF-1 with the inflammatory process during allograft rejection. Kupffer cells (KC), which reside in the hepatic sinusoids, can directly interact with circulating and infiltrating T lymphocytes. The specific aims of this study were to determine whether: (1) KC recovered from liver allografts express AIF-1, (2) AIF-1 expression in KC correlates with liver allograft rejection, (3) AIF-1 expression is regulated by T cell-derived cytokines. **Methods**: Orthotopic liver transplantation was performed in two allogeneic rat combinations: Lewis into DA (chronic acceptance model) and DA into Lewis (acute rejection model). Liver samples were collected on days 1, 5, 7 and 10 post-transplantation. *In vitro* studies: KC and T lymphocytes were isolated, and KC were co-cultured with Con-A activated T cells, IFN-γ, IL-4 or IL-10. AIF-1 expression in liver allografts and KC were quantified by immunohistochemistry staining and RT-PCR analysis. **Results**: KC AIF-1 expression can be detected at low levels in non-transplanted control livers. Following liver transplantation AIF-1 expression increases in a time dependent fashion during the initial week post-transplant. The increased mRNA expression of AIF-1 is associated with increased mRNA levels of INF-γ in liver allografts. Both AIF-1 and IFN-γ mRNA expression peaked at 7 days after transplantation. Interestingly, AIF-1 and IFN-γ expression were increased two-fold in KC recovered from acutely rejecting liver allografts compared with KC recovered from the chronically accepted livers on day 5, 7 and 10 after transplantation. Peak AIF-1 expression paralleled peak CD3-positive T cell infiltration in the hepatic sinusoids, which was also significantly greater in the rejection model compared to the acceptance model. Co-culture of KC with either activated T cells or IFN-γ (Th1 cytokine) significantly increased AIF-1 expression in KC. However, AIF-1 expression in KC was not affected by addition of IL-4 or IL-10 (Th2 cytokines). **Conclusion**: Increased AIF-1 expression in liver allografts is associated with increased CD3-positive T cell infiltration and increased mRNA expression of IFN-γ. The over-expression of AIF-1 correlates with liver allograft rejection. AIF-1 expression in KC may represent an important biomarker for Th1 cell mediated immune response in transplanted liver allografts.

Abstract# 566 Poster Board #-Session: P22-II

TRANSIENT TELOMERASE EXPRESSION IN NORMAL SOMATIC CELLS ALTERS TELOMERE LENGTH. Annie T. L. Young,[1] Jonathan R. T. Lakey,[1] Shaheed Merani,[1] Erik Johnson,[1] John C. Mullen,[1] Ronald B. Moore.[1] *[1]Surgery, Surgical Medical Research Institute, University of Alberta, Edmonton, AB, Canada.*

Rationale: Progressive loss of telomeres through cell division leads to cell cycle arrest and apoptosis rendering cells incapable of unlimited tissue repair. This reduces allograft survival as a result of injury from acute and chronic rejection. We hypothesized that transient telomerase (hTERT) expression in normal somatic cells imparts a growth advantage to these cells via telomere maintenance and reversal of replicative senescence.
Methods: Telomerase activity in hTERT-transfected normal human umbilical vein endothelial cells (HUVECs) was assessed using the telomeric repeat amplification protocol (TRAP). Telomere length in HUVECs was examined before and after telomerase transfection with Effectene. A flow-FISH procedure was used with a FITC-labelled telomere-specific PNA probe containing sequence complementary to telomeric DNA. The signal intensity of fluorescence was standardized into molecules of equivalent soluble fluorochrome (MESF) unit (M) that directly correlates to telomere length.
Results: Telomerase activity was detectable on the first, second and third day post-transfection and decreased to near baseline level (untransfected HUVECs) on the fourth and fifth day. Heat-denatured control samples of hTERT-transfected HUVECs also demonstrated baseline activity level.
A decline in telomere length, as a function of culture passage, was detected in normal aging HUVECs. HUVECs, at escalating passage numbers (p), showed mean MESF values of 53±16.0 kM (n=4):p5-7, 40±7.8 kM (n=6):p10-13, 28±5.3 kM (n=5):p14-17, 24±3.5 kM (n=4):p18-20 and 20±3.2 kM (n=3):p21-23. Following hTERT transfection, variable telomere extension was found in all cultures. The net increase in telomere length ranged from 2-154 kM, 7-199kM, 5-214 kM and 17-222 kM for HUVECs of p5-7 (n=3), p11-14 (n=3), p17-19 (n=3) and p22-23 (n=2) respectively. The *in-vitro* age (p) of cells did not seem to dictate the degree of length extension. No significant difference (p>0.05) in mean transfection efficiency was detected between senescent (12±1.55%, n=5, p20-23) and young (17±2.52%, n=5, p5-8) HUVECs. Proliferation index in terms of %S-phase cycling cells in hTERT-transfected cultures displayed a net increase of up to 7% despite *in-vitro* aging.
Conclusions: Transient telomerase expression leads to telomere lengthening in normal somatic cells, which can potentially increase cellular lifespan.

Allorecognition/Antigen Presentation

Abstract# 567 Poster Board #-Session: P23-II

DIFFERENTIAL ROLES OF RENAL CELLS IN REGULATION OF EOSINOPHIL RECRUITMENT IN ALLOGRAFT REJECTION. Charles R. Nolan,[1] James D. Hernandez,[2] Hanna E. Abboud.[2] *[1]Surgery - Organ Transplant, University of Texas Health Sciences Center, San Antonio, TX; [2]Medicine - Nephrology, University of Texas Health Sciences Center, San Antonio, TX.*

Eosinophil infiltration of arterial walls and tubulointerstitium of renal allografts occurs in both acute rejection and chronic vascular rejection. Production of fibrogenic cytokines such as TGF-β by eosinophils may be involved in pathogensis of tissue injury in renal allograft rejection. The mechanisms of eosinophil recruitment and accumulation in the kidney have not been fully elucidated. Infiltration of the lung by activated eosinophils is controlled by various cytokines including interleukin (IL)-1, IL-4, tumor necrosis factor-α (TNF-α) and granulocyte-monocyte colony stimulating factor (GM-CSF); chemokines such as eotaxin; and the adhesion molecule VCAM-1. Expression of VCAM-1 on vascular endothelium promotes eosinophil adhesion, eotaxin promotes eosinophil chemotaxis, while GM-CSF prevents eosinophil apoptosis and prolongs eosinophl survival in the tissue. This study was designed to evaluate *in vitro* the involvement of various renal cell types (renal cortical interstitial fibroblasts [RCIF], renal arteriole microvascular smooth muscle cells [MVSMC], and proximal tubular epithelial cells [PTEC]) in the process of eosinophil infiltration and accumulation in renal allografts. The effects of IL-1, IL-4 and TNF-α on expression of eotaxin, VCAM-1 and GM-CSF by the various cells types was investigated. Northern analysis showed that IL-1, IL-4 and TNF-α upregulated eotaxin mRNA expression in RCIF and MVSMC but not PTEC. IL-4 and TNF-α had a synergistic effect on eotaxin production by RCIF and MVSMC. Moreover, eosinophils migrated toward conditioned medium from cytokine-stimulated RCIF and MVSMC but not that derived from PTEC. This eosinophil chemotaxis was inhibited by pretreatment with eotaxin neutralizing antibody. In addition, coculture of RCIF or MVSMC with eosinophils lead to increased expression of VCAM-1 by RT-PCR analysis. Coculture with PTEC did not effect VCAM-1 expression. IL-4 and TNF-α also had a synergistic effect on VCAM-1 expression by RCIF and MVSMC but not PTEC. In contrast, IL-1, IL-4 and TNF-α increased GM-CSF mRNA expression by PTEC but have no effect on GM-CSF production by RCIF or MVSMC.
Conclusions: Our data suggest that different renal cell types may play distinct roles in eosinophil recruitment and accumulation in rejecting renal allografts. RCIF and MVSMC by producing VCAM-1 and eotaxin may participate in eosinophil recruitment by promoting eosinophil adhesion and chemotaxis. Production of GM-CSF by PTEC may prevent eosinophil apoptosis and promote eosinophil accumulation in the renal allograft.

Abstract# 568 Poster Board #-Session: P24-II

COMBINED TERATMENT OF PRAVASTATIN AND LOSARTAN HAS SYNERGISTIC EFFECT ON CHRONIC CYCLOSPORINE NEPHROPATHY. Chul Woo Yang,[1] Can Li,[1] Bum Soon Choi,[1] Sun Woo Lim,[1] Bo Kyoung Sun,[1] Yong Soo Kim,[1] Byung Kee Bang.[1] *[1]Internal Medicine, Kangnam St.Mary's Hospital, Seoul, Republic of Korea.*

This study was performed to evaluate whether combined treatment of statin (pravastatin; PRVT) and angiotensin II receptor antagonist (losartan; LSRT) affords superior protection compared with the respective monotherapies in chronic cyclosporine (CsA) nephropathy. Rats on a low salt diet were given vehicle (VH group, olive oil, 1 mg/kg per day), CsA (15 mg/kg per day), CsA and LSRT (CsA + LSRT group, 100 mg/L per day), CsA and (CsA + PRVT group; 6 mg/kg per day), or CsA and LSRT and PRVT (CsA + LSRT + MMF group). Control groups received each drug without CsA treatment. Renal function, histological parameters (arteriolopathy, tubulointerstitial fibrosis and inflammatory cell infiltration), systemic and intrarenal CRP, mediators of CsA-induced nephrotoxicity (angiotensin-II, osteopontin and transforming growth factor [TGF]-b1 and b-ig) and NF-kB and AP-1 were evaluated, The CsA-treated rats showed decreased renal function and increased histological parameters compared with the VH-treated rats. The CsA + LSRT treatment significantly improved histopathological parameters compared with the CsA group, and combined treatment of PRVT and LSRT further improved those parameters compared with each drug alone. Increased expression of intrarenal CRP, osteopontin and TGF-b1 mRNAs in the CsA group were significantly decreased with PRVT or LSRT, and further decrease was observed with the combined treatment of PRVT and LSRT. Intrarenal angiotensin II expression was significantly increased with LSRT treatment and further decrease of angiotensin II expression was observed with combined treatment of both drugs. Activation of NK-kB and AP-1 in CsA treated rat kidneys was decreased with LSRT or PRVT treatment and further decreased was observed with combined treatment of both drugs. Combined treatment with PRVT and LSRT has a synergistic effect on preventing interstitial inflammation and fibrosis in chronic CsA nephrotoxicity. This finding provides a therapeutic rationale for these drugs in decreasing CsA-induced nephropathy.

Abstract# 569 Poster Board #-Session: P25-II

CYTOMEGALOVIRUS MODULATES SOLUBLE HLA-RELEASE FROM ACTIVATED ENDOTHELIAL CELLS. Yuri Bushkin,[1] Jaroslava Lieskovska,[1] William J. Burlingham,[2] Sergey Smirnov,[3] Sergei V. Kotenko.[3] *[1]Molecular Immunology, Public Health Research Institute, Newark, NJ; [2]Surgery, University of Wisconsin, Madison, WI; [3]Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, Newark, NJ.*

We studied the release of soluble HLA (sHLA) proteins via the metalloproteinase (MPase) pathway from endothelial cells (EC) activated in vitro by allogeneic T cells, and the effects of cytomegalovirus (CMV)-infection on this release.

Primary human umbilical vein EC and the microvascular EC line HMEC-1 were activated by co-culture with allogeneic peripheral blood mononuclear cells (PBMC) or with EC/PBMC conditioned medium. Surface expression of the HLA-releasing MPase ADAM17, β2-microglobulin (β2m)-free heavy chains (HC) and native β2m/HC complexes, and VCAM-1 on cultured and activated EC was analyzed by immunostaining with specific mAb and flow cytometry. The sHLA-release was measured by immunoprecipitation of supernatants from uninfected or CMV-infected biotin-labeled EC with mAb followed by SDS-PAGE and Western blotting.

We showed previously that interferon-γ produced by activated T cells drives the sHLA-release by the MPase from primary bronchial epithelial cells. We now demonstrate that EC activation resulted in a dramatically increased expression of surface HLA and VCAM-1. The expression of ADAM17 was modestly but reproducibly increased as well. Up-regulation of these molecules on activated EC was accompanied by vigorous release of both soluble β2m-free HC and β2m/HC proteins by the MPase pathway likely involving ADAM17. However, CMV-infection of EC abrogated the release of soluble β2m/HC which did not resume even under activation conditions. These sHLA proteins have been shown to contain high affinity peptides. In contrast, the release of soluble β2m-free HC from CMV-infected EC was not diminished. Surprisingly, the release of the latter sHLA was only marginally inhibited by the MPase inhibitor BB-2116 (hydroxamate derivative), thus suggesting that an alternative MPase-independent pathway is operating in newly infected cells.

sHLA proteins that are released by ADAM17 from activated graft cells may present alloantigens to the host immune system. In this way, the transplantation outcome may be compromised by CMV-infection. Our data suggest that CMV can alter the sHLA-release from activated EC in at least two distinct modes. Thus, the release of sHLA capable of cross-presenting peptides is abrogated, although the consequences of this blockade are yet to be defined. However, the release of sHLA without peptides is sustained by yet unknown MPase-independent mechanism, and this may have an impact on response of CD4+ T cells to HC-derived allopeptides.

Abstract# 570 Poster Board #-Session: P26-II

POLYCLONAL CD8 T CELLS CAN REJECT MAJOR HISTOCOMPATILIBILITY COMPLEX CLASS I DEFICIENT SKIN ALLOGRAFTS VIA AN INDIRECT EFFECTOR MECHANISM. Chunshui He,[1] Peter S. Heeger.[1] *[1]Immunology and The Glickman Urologic Institute, The Cleveland Clinic Foundation, Cleveland, OH.*

Following transplantation, recipient T cells can recognize and respond to donor antigens expressed directly on donor cells and can respond to donor-derived peptides that have been processed and presented in the context of recipient MHC through the indirect pathway. Indirectly primed CD4 T cells have been well studied in transplantation, but little information is available regarding whether indirectly primed CD8 T cells participate in rejection at the effector stage. To address this issue, we placed MHC class I deficient D^bK^b double knockout (KO) skin grafts onto allogeneic H-2^k SCID recipients followed by adoptive transfer of purified H-2^k CD8 T cells. In this situation, the responding CD8 T cells cannot interact with any antigen on the graft cells because the graft cells lack MHC class I molecules. As a negative control, skin grafts were placed without adoptive transfers. D^bK^b KO skin grafts placed onto wild type H-2^k recipients were included as positive controls. In this latter situation recipient CD4 T cells were anticipated to reject the grafts via direct interactions with donor MHC class II. The MHC class I deficient grafts were rejected by day 35 in the SCID recipients (n=5) only if given adoptive transfers of purified CD8 T cells. CD8 T cells, but not CD4 T cells, were detectable in the recipient lymphoid organs. The CD8 T cells in these mice did not respond to any antigen directly expressed on D^bK^b KO stimulator cells as assessed by IFNg ELISPOT assays, but responded to control mitogen stimulation. Immunohistochemical analysis showed that only CD8 T cells were found in the D^bK^b KO grafts undergoing rejection and furthermore were detected in close proximity to vascular endothelial cells and to recipient infiltrating macrophages, suggesting specific interactions. Control K^bD^b KO grafts placed onto WT recipients were rejected by day 14 (n=4). Immune cells from these control animals directly responded to D^bK^b KO stimulator cells in recall assays, and the grafts were diffusely infiltrated with CD4 T cells. These data definitively demonstrate that cross-primed polyclonal CD8 T cells can function as active participants in the effector phase of rejection and suggest that they mediate graft injury via specific interactions with recipient endothelial cells feeding the grafts. The findings confirm and extend previous studies using monoclonal TCR transgenic T cells and shed light on mechanisms of acute and chronic graft injury that are potentially relevant to human transplant recipients.

Abstract# 571 Poster Board #-Session: P27-II

CD103 EXPRESSION DURING DIFFERENTIATION OF ALLOREACTIVE CELLS *IN VITRO* AND *IN VIVO*. Elena Uss,[1] Natalia Nikolaeva,[1] Rene A. W. van Lier,[1] Ineke J. M. ten Berge.[2] *[1]Laboratory for Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands; [2]Department of Internal Medicine; Div. of Nephrology and Clinical Immunology & Rheumatology, Academic Medical Center, Amsterdam, Netherlands.*

Objective: Recent studies revealed that renal allograft infiltrating CD8+ cells express CD103, an αEβ7 integrin that has specificity for the epithelial ligand E-cadherin, suggesting a role for these cells in allograft rejection. We studied the expression and regulation of the CD103 molecule on alloreactive lymphocytes in MLR *in vitro*.

Methods: Peripheral blood mononuclear cells from 8 healthy individuals were labelled with CFSE to monitor subsequent cell divisions. Cells were cocultured with allogeneic irradiated cells for 5 days. After staining for several surface markers related to cell differentiation, flowcytometric analysis was performed. Furthermore, cells were stained intracellularly for INF-γ, TNF-α, perforin and granzyme B. *In vitro* regulation of CD103 expression on CD4+ and CD8+ cells was also examined.

Results: Alloreactive CD8+ cells upregulated CD103 to a much higher extent than alloreactive CD4+ cells. Nearly 60 % of alloreactive CD8+ cells were found to express CD103, whereas only 10% of alloreactive CD4+ expressed this integrin. Phenotypically, CD103+ cells appeared to be $CD27^{bright}CD45RA^{-}CCR7^{-}$ in both subsets. Moreover, differentiation at later stages of proliferation was characterized by production of the cytolytic effector molecules granzyme B and perforin and by expression of INF-γ and TNF-α by CD103+ cells. Other integrin molecules (VLA-4, LFA-1) were upregulated during allodifferentiation as well. Interestingly, cyclosporine A enhanced expression of CD103 on alloreactive CD8+ cells *in vitro*. Addition of interleukin-4 to the MLR upregulated CD103 expression on T cells, whereas interleukin-12 downregulated this. Neither addition of other Th1 and Th2 cytokines, nor blockage with CD25 mAb affected CD103-expression. In contrast to *in vitro* studies, no CD103+ cells were found in the peripheral blood compartment of kidney-transplanted patients with acute rejection.

Conclusion: Our data show that CD8+ T cells clearly express CD103 after allostimulation *in vitro*, which is further enhanced by cyclosporine and by interleukin-4 and downregulated by interleukin-12.

Abstract# 572 Poster Board #-Session: P28-II

TYPE-1 POLARIZATION OF CD8+ T CELL RESPONSES AGAINST EBV ANTIGENS IS PRESERVED IN STABLE SOLID ORGAN TRANSPLANT RECIPIENTS. Iulia Popescu,[1] Camila Macedo,[1] Adriana Zeevi,[1] Ron Shapiro,[1] Kareem Abu-Elmagd,[1] Walter Storkus,[1] John Fung,[1] Diana Metes.[1] *[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Introduction: It is well accepted that a type-1 (IFNγ) predominance of T cell immunity is critical for efficient control of EBV replication and latency establishment. In this study we analyzed the immune polarization of anti-EBV CD8$^+$ T cell responses (IFN-γ/IL-5 ratio) in immunosuppressed (IS) stable stable solid organ transplant (SOTx) patients. Our goal is to understand what impact IS may have on the increased susceptibility and progression towards posttransplant lymphoproliferative disorder (PTLD) observed with SOTx patients, and to determine if *ex vivo* anti-EBV T cell generation for prevention or treatment of PTLD is feasible. **Methods:** 8 EBV+/HLA-A02+ IS stable SOTx patients and 8 healthy controls were recruited for this study. ELISPOT assays for IFN-γ and IL-5 production were performed on freshly isolated lymphocytes or on *ex vivo* generated EBV specific CTLs. CTLs were generated by co-culturing for 10 days autologous T cells with DC loaded with a mixture of three HLA-A02 restricted peptides derived from latent (EBNA3A, LMP2) and lytic (BMLF1) EBV Ags. PMA/Ionomycin or EBV-derived peptides were used for ELISPOT screening. **Results:** When resting lymphocytes were screened with PMA/Ionomycin, patients exhibited a type-1 polarization of immune responses (ratio=6.4) that was comparable to healthy individuals (ratio=4.5). Although the frequency of CD8+ T cells from IS SOTx patients releasing IFN-γ in response to EBV-peptide stimulation had a tendency to be lower than in healthy controls, resting lymphocytes from patients still showed comparable IFN-γ/IL5 ratios for (EBNA3A=3.9, LMP2=5.1, and BMLF1=4.2) to healthy individuals (EBNA3A=6.7,LMP2=4.8, and BMLF1=5.5). After 10 days of *ex vivo* cultures, anti-EBV specific CTLs were successfully generated from patients and normal controls. The EBV-specific effectors displayed a mixed IFN-γ/IL-5 cytokine reactivation upon *ex vivo* stimulation. However a significant predominance of type-1 polarization of CD8$^+$ T cell responses was detected in patients (EBNA3A=4.2, LMP2=4.6, and BMLF1=4.6) as well as in healthy controls (EBNA3A=4.9, LMP2=4.4, and BMLF1=4.2). **Conclusion:** Our results suggest that IS stable SOTx patients display an overall predominance of type-1 immune responses to EBV Ags, comparable with controls. These data imply that *ex vivo* DC-based protocols can be successful in eliciting EBV-specific CD8+ T cells from IS stable SOTx patients for immunotherapy and vaccination.

Abstract# 573 Poster Board #-Session: P29-II

IDENTIFICATION OF HLA-DR4 RESTRICTED BMLF1-DERIVED PEPTIDES RECOGNIZED BY EBV-SPECIFIC CD4$^+$ T CELLS. Iulia Popescu,[1] Camila Macedo,[1] Ron Shapiro,[1] John Fung,[1] Walter Storkus,[1] Diana Metes.[1] *[1]Thomas E. Starzl Trnsplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Introduction: Posttransplant lymphoproliferative disorders (PTLD) are complications occurring after solid organ transplantation (SOTx), and are triggered by EBV infection in the context of chronic immunosuppression (IS). The seminal role of CD8+ T lymphocytes in EBV load control and tumor elimination is well characterized and understood, while EBV-derived epitope recognition by CD4+T cells has been less well investigated. Recent evidence has emerged showing that development and maintenance of effective CD8+ cytotoxic T lymphocytes is dependent on CD4+ T cell help. In this study we sought to identify novel MHC Class II-restricted EBV- derived peptides from BMLF1 Ag recognized by CD4+ T cells that could be used for clinical vaccination and adoptive immunotherapy of EBV-associated lymphomas. **Methods:** A peptide-binding algorithm was used with the BMLF1 sequence in order to select HLA-DR4-binding peptides. Five high-scoring candidate 15-mer peptides were synthesized. Three of the peptides, i.e. P1(324-339), P2(180-194) and P5(32-47) were predicted to have higher HLA-DR4 binding scores than the other two peptides P4(385-396) and P3(425-438). All peptides were assessed for their ability to be recognized by EBV-reactive bulk CTLs derived from EBV+/HLA-DR4+ donors (8 healthy subjects and 3 IS stable SOTx recipients) after 7 days *in vitro* stimulation culture. Autologous monocyte-derived dendritic cells pulsed with individual and/or pooled synthetic BMLF1 peptides were used as stimulators. IFN-g ELISPOT assays were then used as a read-out of specific Th1-type CD4+functional responses. **Results:** Four out of eight normal donors and one out of three patients displayed Th1-type responses against the P1 (324-339) peptide. In contrast, only two normal donors reacted against the P2 (180-194) peptide and only a single patient recognized the P5 (32-47) peptide. Interestingly none of the subjects responded by IFNg secretion when tested against peptide P3 (425-438), whereas CD4+ T cell responses to the P4 (385-396) peptide appeared rather promiscuous. We are currently screening additional subjects to further characterize these three potentially clinically- relevant BMLF1-derived peptide epitopes. **Conclusions:** The BMLF1 protein encodes at least three distinct HLA-DR4-restricted Th1 epitopes that elicit specific CD4+ T cell responses *in vitro*. These peptides may be implemented in prospective adoptive immunotherapy protocols designed to prevent and/or treat PTLD in SOTx patients.

Abstract# 574 Poster Board #-Session: P30-II

SELF-LIGAND DEPRIVATION LEADS TO A DEFECT IN THE ABILITY OF CD4 T CELLS TO INTERACT WITH DENDRITIC CELLS. Ursula Fischer,[1] Alexander Khoruts,[1] Elizabeth Ingulli.[1] *[1]Center for Immunology, University of Minnesota, Minneapolis, MN.*

The importance of self-peptide/MHC molecule ligands (self-ligands) for the selection of the T-cell repertoire in the thymus is well established. However, the effects of long-term deprivation of self-ligand stimulation on T cell behavior and function have not been studied in detail. In order to test the role of self-ligand stimulation on T cell responses to cognate antigens in vivo we used a TCR transgenic CD4 T cell adoptive transfer system with MHC class II deficient and sufficient recipients. We demonstrate that naïve CD4 OT-II RAG deficient T cells (OT-II cells) developed a progressive defect in their ability to proliferate following injection of antigen-bearing dendritic cells (DCs). The CFSE content of OT-II cells co-injected with antigen-bearing DCs at the same time was the same in MHC class II deficient and sufficient recipients. However, a defect in proliferation was noted in OT-II cells deprived of self-ligand contact for as little as 24 hrs, and the defect became increasingly profound with additional duration of deprivation. A similar progressive defect was noted in CD69 expression. Further in vivo studies revealed that OT-II cells had a marked defect in their ability to physically interact with cognate antigen/MHC-bearing DCs, which progressively worsens over time. These results suggest a new mechanism of how self-ligand signals are critical for normal function of CD4 T cells. Our results should also be taken into account in interpretation of transplantation experiments that rely on MHC class II-deficient hosts. The conventional interpretation of these data suggests a critical role of indirect antigen presentation in transplant rejection. However, it is noteworthy that the direct pathway is dominant in our system. Indeed, we find no rejection of Act-mOVA full thickness skin grafts in MHC class II-deficient recipients of OT-II cells as compared with rejection in CD4-deficient, MHC class II-sufficient hosts, and wild-type B6 hosts. In fact, injection of OVA peptide-pulsed DCs at the time of transplantation also failed to induce graft rejection. Defective initiation of naïve T cell responses and effector function following self-ligand deprivation offer a potentially important alternative explanation for rejection failure. These considerations offer alternative mechanisms of how major immunosuppressant therapies that block TCR signaling (e.g., calcinurin inhibitors, anti-CD3 blocking antibodies) may work, and help develop future therapeutic strategies that may selectively augment these effects.

Abstract# 575 Poster Board #-Session: P31-II

HLAMATCHMAKER AS A PREDICTOR OF POST RENAL TRANSPLANTATION SENSITISATION. Judith E. Worthington,[1] Susan Martin.[1] *[1]Transplantation Laboratory, Manchester Institute of Nephrology and Transplantation, Manchester, United Kingdom.*

Introduction: Recently published data have demonstrated a significant association between the production of donor HLA specific antibodies (dspAbs) and subsequent renal transplant (tpx) failure. However not all patients whose grafts failed produced dspAbs and antibodies were not produced to all tpx HLA mismatches. HLA Matchmaker is a computer algorithm where donor-recipient HLA compatibility is assessed at the structural level. Intralocus and interlocus comparisions are made of polymorphic amino acid triplet sequences, the software then determines which triplets on mismatched HLA molecules are different or shared between donor and recipient. This study aimed to use the HLA Matchmaker to see if it was predictive of post-tpx sensitization. **Methods:** The study group comprised 35 adult recipients of primary renal tpx (transplanted between 1981 and 1998) whose grafts had failed. Sera taken pre-tpx, 1,3,6 months post-tpx and annually thereafter until graft failure were tested by ELISA for the presence of HLA class I and class II specific antibodies. Antibody specificity was defined by a combination of cytotoxicity, ELISA and flow cytometry techniques. Antigen mismatches (AgMM) were analysed for each locus individually for immunogenic triplets according to the HLA Matchmaker software. **Results:** All recipients were negative for dspAbs pre-tpx; post-tpx 20 were positive and 15 were negative. The table below summarizes the correlation between triplet mismatches (TrpMM) and dspAbs production. **Summary:** This study has illustrated that donor-recipient matching by conventional criteria can be further differentiated by analysing at the structural level, as a conventional single AgMM corresponds to a wide range of TrpMM. We have also shown that with an increasing number of TrpMM there is a corresponding increase in the percentage of patients who are antibody positive. This correlation is most evident in the HLA-DR and -DQ loci. **Conclusion:** For each locus when there is a single AgMM knowledge of the level of triplet mismatching indicates the likelihood of post-tpx sensitisation. As post-tpx sensitisation is associated with graft failure this study suggests that HLA Matchmaker might therefore be used for donor selection.

HLA Locus	No. AgMM	No. Trp MM	No. Patients	Patients Antibody Positive (%)
HLA-A	0	0	12	0
	1	2-4	8	12.5
	1	5-12	14	35
	2	17	1	100
HLA-B	0	0	8	0
	1	2-4	8	12.5
	1	5-9	14	28.5
	2	10-15	5	60
HLA-DR	0	0	18	0
	1	2-4	6	33
	1	5-9	10	80
	2	12-15	2	100
HLA-DQ	0	0	24	0
	1	3-18	10	90
	2	20	1	100

Abstract# 576 Poster Board #-Session: P32-II

A LACK OF INDIRECT ALLOREACTIVITY IS ASSOCIATED WITH RENAL ALLOGRAFT TOLERANCE IN A NONHUMAN PRIMATE MODEL. Ognjenka Nadazdin,[1] Siewlin Wee,[1] Tatsuo Kawai,[1] Svetlan Boskovic,[1] Ichiro Koyama,[1] Henry Winn,[1] David Sachs,[2] A. Benedict Cosimi.[1] *[1]Surgery, Transplantation Unit, Massachusetts General Hospital, Boston, MA; [2]Surgery, Transplantation Biology Research Center, Massachusetts General Hospital, Charlestown, MA.*

Donor reactive T cells activated via both the direct and indirect allorecognition pathways have been implicated in mediating allograft rejection. This study seeks to determine if alloresponses to either or both pathways can distinguish between the immunological states of stable graft function / tolerance and rejection. **METHODS**: Fifteen renal allograft recipients treated with combined mixed chimerism and co-stimulatory blockade protocols were studied. At the time of testing, 6 had rejection (POD 173 –582) and 9 were stable/tolerant (POD 145 – 643). An ELISPOT assay was used to measure the frequencies of donor reactive cells producing either IFNg (Th1) or IL4 (Th2) in recipients' PBMC following 48hr of co-culture with either irradiated donor PBMC (for direct pathway) or with donor PBMC sonicates (for indirect pathway). Third party and autologous cells cultures were similarly set up. Both cross-sectional and longitudinal studies were performed. **RESULTS**: Based on IFNg analyses, all rejecters (n=6) responded positively in the direct pathway (mean 290 +/- 89 spots/million) but so did 33% of the tolerant animals (3 of 9; 343 +/- 149 spots/million) although the rest (6 of 9) were nonresponsive (<50 spots/million). In contrast, all tolerant animals (n=9) failed to respond in the indirect pathway (<10 spots/million) while 67% of the rejecters did (4 of 6; mean 109 +/- 27 spots/million). The nonresponsiveness in both activation pathways was donor specific and stable over time. One tolerant recipient who later succumbed to chronic rejection showed a corresponding shift in its indirect alloreactivity from negative (<10 spots/million) to positive (29.7 +/-1 spots/million). Among the rejecters, similar shifts were observed although seldom occurred earlier than POD200 (e.g the 2 rejecters who were negative were both tested <POD201). Significant correlation was observed between alloantibody production and positive indirect, but not with positive direct, alloreactivity. Results of IL4 analyses suggest no TH1/Th2 shift had occurred. **CONCLUSION**: These observations suggest that alloreactivity in the indirect, rather than the direct, pathway is most relevant in assessing the clinical outcome of the allografts following attempted tolerance induction in our monkey model.

Abstract# 577 Poster Board #-Session: P33-II

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) REGULATES THE IMMUNE RESPONSE THROUGH INHIBITION OF DENDRITIC CELL FUNCTION. Stuart W. Robertson,[1] Sreenivas Laxmanan,[2] Debarata Mukhopadhyay,[2,3] David M. Briscoe.[1] *[1]Transplantation Research Center, Childrens Hospital & Harvard Medical School, Boston, MA; [2]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [3]Mayo Clinical Cancer Center, Mayo Clinic, Rochester, MN.*

VEGF has proinflammatory properties in acute allograft rejection via effects on endothelial-dependent mechanisms of lymphocyte trafficking. However, in tumors VEGF is expressed in abundance without T cell infiltrates. Moreover, patients with metastatic cancer have antigen-specific hyporesponsiveness. This paradox has led us to speculate that the action of VEGF on different cell types (endothelial cells vs. monocytes) may have different effects on the immune response. Recent studies suggest that the VEGF receptors KDR and Flt-1 provide for positive and negative signals respectively. Flt-1 and neuropilin, but not KDR, are expressed by monocytes. Only VEGF-Flt-1 interactions are reported to activate these cells. Here, we have evaluated the effect of VEGF on the maturation and function of myeloid-derived dendritic cells (DC). DC were generated from human monocytes using GM-CSF and IL-4 in the absence or presence of high concentrations (20ng/ml) of VEGF (VEGF-DC). After 9 days in culture, we found no difference in the expression of the mature DC markers CD1a, CD83, MHC class II, CD80 and CD86 on DC vs. VEGF-DC. Interestingly, in the MLR, VEGF-DC were markedly less stimulatory than normal DC - T cell proliferation was reduced by approx. 40% (n=5), while IL-2 and IFNγ production was reduced by approx. 80%. To address antigen-specificity, we also generated murine bone marrow derived VEGF-DCs and cultured them with an ova peptide-specific DO.11 T cell clone. Again, antigen-dependent production of IL-2 was significantly reduced vs. normal DC. To define this mechanism, we first assessed the expression of VEGF receptors on DC and found that Flt-1 was consistently downregulated and neuropilin upregulated during DC maturation in the absence or presence of VEGF. Thus, VEGF-neuropilin interactions may be of importance in DC. We are currently examining the effects of VEGF on the known negative costimulatory molecules PD-L1 and PD-L2. We find that PD-L1 and PD-L2 are not expressed on human monocytes but are markedly upregulated during DC maturation on all DC in our model. VEGF, therefore, inhibits DC function via a novel mechanism, perhaps involving the induction of a novel negative costimulatory molecule. It is possible that VEGF may function in chronic rejection by limiting indirect (APC) responses.

Abstract# 578 Poster Board #-Session: P34-II

LFA-1 INHIBITORS BLOCK NATURAL KILLER CELLS IN HUMAN BLOOD, AND MAY HAVE A POTENTIAL FOR A NEW CLASS OF THERAPEUTIC AGENTS IN SOLID ORGAN TRANSPLANTATION. Gabriele Weitz-Schmidt,[1] Simone Schmutz,[1] Orane Guillaume-Gentil.[1] *[1]Transplantation & Immunology, Novartis Institutes of Biomedical Research, Novartis Pharma AG, Basel, Switzerland.*

Introduction. Recent findings in animal models of solid organ transplantation established the importance of natural killer (NK) cells during acute rejection. In patients, NK cells infiltrate renal or cardiac allografts and are activated despite immunosuppressive therapy. These findings suggest that immunomodulatory agents inhibiting NK cell function could be beneficial in solid organ transplantation. The goal of this study is to compare the effects of allosteric low molecular weight (LMW) LFA-1 inhibitors on NK cell function, to immunosuppressants currently used in clinical practice.

Methods. The compounds were characterized in a flow cytometry-based NK cell cytotoxicity assay measuring NK-92 cell mediated K-562 target cell lysis, in NK-92 cell/ ICAM-1 adhesion and NK-92 cell homotypic aggregation assays. The effects of the compound in undiluted human whole blood were analyzed by quantifying K-562 target cell induced CD69 expression on NK cells.

Results. Both LMW LFA-1 inhibitors and an anti-LFA-1 mAb blocked NK cell cytotoxicity in a dose-dependent manner with IC_{50}s < 1 μM and 1 μg/ml, respectively. Further both classes of LFA-1 inhibitors were shown to interfere with IL-2 induced adhesion of NK cells to immobilized ICAM-1 and homotypic NK cell adhesion. In contrast, known immunosuppressants including basiliximab, cyclosporin A and everolimus did not affect NK cell function *in vitro* at therapeutic concentrations. Under more physiological conditions, in undiluted human whole blood, the LMW LFA-1 inhibitors were able to interfere with NK cell activation as assessed by CD69 expression.

Conclusions. Our results demonstrate that the LFA-1/ICAM-1 interaction plays an important role in NK cell activation, adhesion and killing. Here we demonstrate for the first time that LMW inhibitors of LFA-1 with an allosteric mode of action block NK cell function *in vitro* which is in contrast to immunosuppressive drugs currently in clinical use. LFA-1 inhibitors may become important tools to further elucidate the role of NK cells in solid organ transplantation.

Abstract# 579 Poster Board #-Session: P35-II

INDIRECT ALLORECOGNITION OF MHC CLASS I PEPTIDES ACCELERATES PULMONARY ALLOGRAFT REJECTION IN MINIATURE SWINE. Tsuyoshi Shoji,[1] Douglas R. Johnston,[1] Ruediger Hoerbelt,[1] John C. Wain,[2] Stuart L. Houser,[1] Louis C. Benjamin,[1] Richard S. Lee,[1] Dax A. Guenther,[1] Ashok Muniappan,[1] Rebecca S. Hasse,[1] Levi G. Ledgerwood,[1] Marjory A. Bravard,[1] Mohamed H. Sayegh,[3] Joren C. Madsen,[1,4] James S. Allan.[1,2] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [2]Division of Thoracic Surgery, Massachusetts General Hospital, Boston, MA; [3]Laboratory of Immunogenetics and Transplantation, Brigham and Women's Hospital, Boston, MA; [4]Division of Cardiac Surgery, Massachusetts General Hospital, Boston, MA.*

Background. Indirect allorecognition is hypothesized to play an important role in the rejection of solid-organ allografts. We examined the role of indirect allorecognition in a pre-clinical model of lung transplantation using MHC-inbred miniature swine. **Methods.** Orthotopic left lung allografts were performed using MHC class I-disparate donors. All recipients received a 12-day post-operative course of cyclosporine as their only immunosuppression. The experimental group was immunized 21 days prior to transplantation with synthetic peptides derived from the hypervariable region of the alpha-1 domain of the donors´ class I MHC (n = 6). The control group consisted of un-immunized recipients (n = 6). **Results.** Pigs immunized with class I allopeptides showed *in vitro* proliferative responses and *in vivo* delayed-type hypersensitivity responses to the immunizing peptides. Lung grafts in recipients immunized with the donor-derived allopeptides showed a marked acceleration in the development of acute rejection, in comparison to the control group. In particular, the experimental swine lost their grafts between PODs 11-158, while the control grafts exhibited survivals ranging from 67 to >605 days ($p < 0.05$ [log-rank test]). There was no evidence of anti-donor antibody formation following immunization with the synthetic allopeptides, and rejection responses appeared to be T-cell mediated. **Conclusions.** This study demonstrates that indirect allorecognition of MHC class I peptides accelerates pulmonary allograft rejection. These data also imply that indirect allorecognition is a significant mechanism of allograft rejection, and that the induction of tolerance to this pathway of allorecognition may be necessary for long-term graft acceptance.

Abstract# 580 Poster Board #-Session: P36-II

PEPTIDES CORRESPONDING TO HYPERVARIABLE REGION OF CLASS I MHC IN CONJUNCTION WITH SELF EPITOPS INDUCE DONOR-SPECIFIC TOLERANCE IN TWO DISTINCT RAT STRAINS. Xiu-Da Shen,[1] N. V. Semiletova,[1] F. Gao,[1] Q. Jiao,[1] S. J. Slomowitz,[1] R. W. Bussuttil,[1] J. W. Kupiec-Weglinski,[1] R. M. Ghobrial.[1] *[1]Surgery, UCLA Transplant Center, Los Angeles, CA.*

Background: Peri- or post-operative administration of class I MHC allochimeric moloecules, that contains donor-type hypervariable epitops in the α_1-helical (h) region flanked by recipient-type self sequences in α_1-β-sheet, and in α_2 domain, induced donor-specific tolerance. This study examined the immunogenicity of α_{1h} epitopes and their interaction with recipient self-sequences to induce transplantation tolerance. **Methods:** The α_{1h} sequence of RT1.A^l, which shares α_{1h} RT1.A^u epitopes, was substituted in the RT1.A^a molecule to produce the composite [$\alpha_{1h}^{l/u}$]-RT1.A^a MHC class I allochimeric protein. Peptides (P) P1 and P3 corresponded to recipient self-sequences of RT1. A^a α_1-βsheet (a.a. 10-49) and α2-βsheet (a.a. 91-120) and P2 originated from donor-type hypervariable of α1h region of [$\alpha_{1h}^{l/u}$]-RT1.A^a (a.a. 50-90) was used. **Results:** In BUF (RT1^b) hosts, pre-transplant s.c. injection (day -7) of 20x10^6 WT-RT1.A^a bearing cells caused accelerated rejection of ACI (RT1A^a) hearts (MST3.7±0.5 days), compared to non-transfected cells (5.7±0.5 days, P <0.001). On the one hand, transfectants bearing [$\alpha_{1h}^{l/u}$]-RT1.A^a, which lacks α1h^a determinants, failed to immunize recipients against ACI (RT1A^a, 5.7±0.0 vs 5.7±0.5 days in control) hearts, indicating the presence of immunogenic epitopes in the α1h^a region. On the other hand, [$\alpha_{1h}^{l/u}$]-RT1.A^a, which exhibits α1h^l and α1h^u determinants, immunized hosts toward LEW (RT1^l, 4.5±0.5 vs 6.75±0.5 days; P <0.01) and WF (RT1^u, 4.2±0.5 vs 6.0±0.0 days; P <0.001) grafts. Thus, α_{1h} region defines immunogenic epitopes for RT1.A^u and RT1.A^l. The ability of these epitopes to prolong allograft survival was tested by p.v. administration in the presence of subtherapeutic CsA. Individual peptide administration (0.125-0.25mg) did not prolong WF allograft survival (MST 14.3±4.0, 13±4.4 and 17±1 days). In contrast, a single p.v. administration of P1+P2+P3, in conjunction with subtherapeutic CsA, induced indefinite survival (>100 days) of WF and LEW grafts in ACI hosts (n=12). Tolerance was verified by acceptance of a second donor-type (WF and LEW), but rejection of third-party (BN) allografts. **Conclusion:** Immunodominant epitopes that caused accelerated allograft rejection is present within the hypervariable region of the α_1 domain of RT1.A^a, RT1.A^l, and R1.A^u. Interaction between immunogenic and recipient-self sequences may be required to orchestrate tolerance by the indirect pathway.

Abstract# 581 Poster Board #-Session: P37-II

TCR DIVERSITY AND THE MOUNTING OF ALLOIMMUNE RESPONSES. Cristina Joao,[1] Brenda M. Ogle,[1,2] Jeffrey L. Platt,[1,3,4,5] Marilia Cascalho.[1,3,5] *[1]Transplantation Biology Program, Mayo Clinic, Rochester, MN; [2]Department of Physiology, Mayo Clinic, Rochester, MN; [3]Department of Immunology, Mayo Clinic, Rochester, MN; [4]Department of Surgery, Mayo Clinic, Rochester, MN; [5]Department of Pediatrics, Mayo Clinic, Rochester, MN.*

Immune competence is generally thought to be a function of TCR diversity but the immune response to transplantation may not be so. Because of the high frequency of responder cells MHC-incompatible grafts in non-immunosuppressed individuals are rapidly rejected regardless of diversity; however, with immunosuppression and in adults (with a large memory population), indirect responses to minor antigens might instead determine graft outcome. In this setting, repertoire contractions may impair responses to minor antigens but also cause expansion of broader cross-reactive memory cells. To which extent repertoire contractions, per se, modify immune responses to allotransplantation is not known. To answer this question we analyzed survival of H-Y incompatible skin transplants in mice with contracted T cell repertoires (see table). We used skin transplants to avoid the impact of humoral rejection. JH-/- mice lack B cells and have a 1000-fold contracted TCR Vβ diversity, while μMT mice that produce few B cells have a 10-fold contracted TCRVβ diversity compared to the wild type. Our results indicate that the 10-fold contraction of TCR diversity in the μMT mice did not impair H-Y incompatible skin graft rejection. On the other hand, slower rejection by JH-/- mice could be owed to other factors in addition to the contracted TCR repertoire. For example, while JH-/- mice lack follicular dendritic cell (FDC) network in the lymph nodes, our studies revealed that μMT mice have a FDC network like wild type mice. To determine whether decreased TCR diversity or lack of wild type FDC network in the lymph nodes delays skin graft rejection in JH-/- mice, we increased TCR diversity in these mice to levels comparable to μMT mice by administering polyclonal immunoglobulin (Ig), which did not reconstitute the FDC network. The Ig reconstituted mice still showed delayed rejection of H-Y incompatible skin compared with μMT mice. These results show that rejection of grafts across minor antigen barriers is driven by FDC network and not by TCR diversity.

TCR Vβ diversity and survival of H-Y incompatible skin grafts in JH-/-, μMT and C57BL/6 mice

Strains	TCR Vβ Diversity	Time for rejection (days)	p value (time for rejection)
C57BL/6	6 x 10^5 ± 3.3 x 10^5	15 ± 1.7	
μMT	3.5 x 10^4 ± 4.7 x 10^4	17 ± 3.3	p>0.05
JH-/-	4.8 x 10^2± 7.4 x 10^2	26 ± 5.4	p = 0.0015
JH-/- + Ig	8.8 x 10^4± 1.5 x 10^5	34 ± 18	p = 0.016

Abstract# 582 Poster Board #-Session: P38-II

MOLECULAR IDENTIFICATION OF A NOVEL GENE PRODUCT INVOLVED IN SOLUBLE HLA-RELEASE BY THE METALLOPROTEINASE PATHWAY. Yuri Bushkin,[1] Hidehiro Watanabe,[1] Jaroslava Lieskovska,[1] Peter Tolias,[1] Sandra Demaria.[2] *[1]Molecular Immunology, Public Health Research Institute, Newark, NJ; [2]Pathology, New York University School of Medicine, New York, NY.*

We described previously the metalloproteinase (MPase) pathway of soluble HLA (sHLA)-release which may have a role in indirect allorecognition and cross-presentation of antigens. The mechanism involved in processing of surface HLA to soluble forms is poorly understood. We identified a novel protein apparently involved in this mechanism which is recognized by our sHLA-release blocking mAb 7F11.

7F11 was obtained by immunizing mice with human T-cell membranes. Expression of 7F11 was determined in various cultured cell lines and cells from different normal tissues. Since the epitope is not accessible on intact cells, acetone-fixed cells were immunostained with 7F11 and FITC-conjugated goat anti-mouse IgM. Cell lysates were resolved on reducing SDS-PAGE followed by probing with 7F11 in Western blots (WB). Blocking activity of 7F11 was assayed in biotin-labeled and digitonin-permeabilized cells expressing MHC class I and tumor necrosis factor receptor 2 (TNFR2). Labeled cell supernatants were tested in ELISA for the presence of soluble β2-microglobulin (β2m)-free heavy chains (HC) and TNFR2 captured by mAb HC10 and M180R, respectively. The gene encoding the protein recognized by 7F11 was identified by screening an expression library with this mAb and cDNA cloning.

7F11 inhibited the release of soluble β2m-free HC as effectively as the MPase inhibitor BB-2116 (hydroxamate derivative). The blocking of sHLA-release was specific, since release of TNFR2 by ADAM17 from the same cells was not affected. WB and immunostaining with 7F11 revealed that lymphoid, endothelial, epithelial cells and fibroblasts express the 65 kilodalton (kD) protein with a predominant membrane localization. The screen yielded three similar cDNA clones identified by database analysis as BC036469. This gene was originally found in hippocampus, but its protein product was not characterized. In brain, hippocampus expressed the 43 kD protein while cortex was negative in WB. Thus, alternatively spliced and/or differentially glycosylated forms may exist in some tissues.

The BC036469 protein identified here is not a MPase. This protein contains a ring Zn-finger motif in its C-terminus which suggests involvement in protein turnover, chaperoning and intracellular trafficking and secretion functions. Thus, BC036469 may mediate interactions between the HLA-releasing MPase and its substrate. Its precise role in the MPase pathway of sHLA-release is currently under investigation.

Abstract# 583 Poster Board #-Session: P39-II

ALLOANTIGEN-SPECIFIC CD8+ T CELL PRIMING IS MORE DEPENDENT ON ICAM-1 INTERACTIONS THAN CD4+ T CELL PRIMING. John C. Rabets,[1] Qi-Wei Zhang,[1] Tarek El-Sawy,[1] Robert L. Fairchild.[1] *[1]Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, OH.*

INTRODUCTION: Priming of alloantigen-specific T cells requires recognition of allogeneic MHC complexes and /or allogeneic peptide/self MHC complexes as well as the delivery of co-stimulatory signals to the T-cell. Optimal activation of T-cells may require the interaction of T-cell expressed LFA-1 with ICAM-1 or ICAM-2 molecules on the APC surface. We have previously demonstrated that completely MHC-mismatched cardiac allografts from ICAM-1 deficient donors had prolonged survival and attenuated T-cell priming compared to wild-type mismatched donors. We now wish to determine whether ICAM-1 is more important in alloreactive CD4+ or CD8+ T cell priming.

METHODS: Full-thickness skin allografts from wild-type C57BL/6 (H-2d) or B6.ICAM-1 deficient mice were placed onto MHC-mismatched A/J (H-2a) recipients. Alloantigen-specific T-cell priming was assayed by IFN-g ELISPOT assay after CD4+ and CD8+ T cell isolation using magnetic bead separation of graft draining lymph node cells or using cells from recipients treated with depleting CD4 or CD8 antibodies.

RESULTS: Skin allografts from both wild-type and ICAM-1 deficient mice rejected between days 12 and 14 post-transplant. On day 8 post-transplant, the level of alloantigen-specific T-cell priming from ICAM-1 deficient donors was less than half that observed in wild-type donors. Depleting recipient CD8 T cells prior to transplantation resulted in a diminution of alloantigen priming in the wild-type compared to the ICAM-1 deficient donors by 40%. Likewise, there was 14% less interferon-gamma production from isolated CD4+ T cells from recipients of wild-type grafts than from ICAM-1 deficient grafts.

CONCLUSION: These observations indicate that ICAM-1 interactions are more important for alloantigen-specific CD8+ T cell priming to mediate allograft rejection.

Abstract# 584 Poster Board #-Session: P40-II

NOVEL, HIGHLY POTENT, ORALLY BIOAVAILABLE CCR5 ANTAGONISTS ARE POTENTIAL IMMUNOMODULATORY AGENTS FOR PRIMATE TRANSPLANTATION MODELS. Gebhard Thoma, Francois Nuninger, Marc Schaefer, Kayhan Akyel, Rainer Albert, Christian Beerli, Eric Francotte, Marcel Luyten, Duncan McKenzie, Lukas Oberer, Markus B. Streiff, Trixie Wagner, Hans-Rudolf Walter, Gisbert Weckbecker, Hans-Guenter Zerwes. *Transplantation and Immunology Research, Novartis Institutes for BioMedical Research, Basel, Switzerland.*

Introduction. Both epidemiological studies and experiments using CCR5 KO mice indicate that the chemokine receptor CCR5 plays an important role in transplant rejection by affecting the trafficking of effector T cells and monocytes. Thus, antagonists of CCR5 are believed to be of therapeutic value to interfere with transplant rejection. Here we describe the preparation and the structure activity relationship of a series of novel, highly potent and selective competitive CCR5 antagonists which, contrary to published compounds, cross react with cynomolgus monkey CCR5, thus, allowing for further testing in non-human primate transplantation models.

Methods. These compounds were profiled in a number of *in vitro* assays to gain information on different molecular events (i) at the extracellular side of the receptor (ligand binding assay), (ii) at intracellular sites i.e. G-protein association with the intracellular domains of CCR5 following receptor engagement; (GTPgS assay), (iii) downstream in the signal transduction pathway (Ca^{2+} mobilization assay) and (iv) cell migration as the result of ligand / receptor interaction (chemotaxis assays).

Results. The most promising CCR5 antagonist, NIBR-1182, was equally potent on human and cynomolgus monkey CCR5 showing IC_{50} values in the low nanomolar range in all assays. NIBR-1182 is highly selective for CCR5 and showed no activity up to >1000 nM when tested against a panel of >40 G-protein coupled receptors and ion channels. The oral bioavailability in cynomolgus monkeys was determined to be 58%.

Conclusion. We identified novel, small molecule CCR5 antagonists that are potent and highly selective agents interfering with the migration of lymphocytes. These compounds can be tested for immunomodulatory efficacy in non human primate transplantation models to demonstrate their potential for use in humans.

Abstract# 585 Poster Board #-Session: P41-II

DONOR TREATMENT FOR THE INDUCTION OF HO-1 IS ASSOCIATED WITH REDUCED INTRAGRAFT AND PERIPHERAL DONOR-SPECIFIC DC. Paulo N. A. Martins,[1] Henriette Kessler,[1] Anke Jurisch,[1] Anja Reutzel-Selke,[1] Andreas Pascher,[1] Johann Pratschke,[1] Peter Neuhaus,[1] Hans-Dieter Volk,[2] Stefan G. Tullius.[1] *[1]Dept. of Surgery, Charité, Virchow-Clinic, Berlin, Germany; [2]Dept. of Med. Immunology, Charité, Mitte, Berlin, Germany.*

Perioperative unspecific inflammatory alterations have a strong impact on graft survival. Donor treatment for the induction of HO-1 prevented both, acute rejections and chronic graft deterioration in previous experiments. We wondered whether HO-1 mediated graft-protective effects are associated with decreased allograft immunogenicity.

Kidneys from DA rats were transplanted into untreated LEW. Donor animals were treated with CoPP (5mg/kg) 24h prior to organ harvesting to induce HO-1. Controls remained untreated or received ZnPP (20 mg/kg) to block HO-1 induction. Grafts, spleens, lymph nodes (LN) and blood analysis of LEW recipients were performed by days 1 and 3, respectively. Donor-specific MHC-class II+ APC were determined by haplotype-specific mAb and flowcytometry ($RT1A^{ab}$, OX62+). Graft sections were used for immunohistochemical staining of relevant surface markers (CD4/CD8+ T cells, ED1+ monocytes, MHC class II+/CD86+ APC). T-cell alloreactivity of recipient splenocytes was measured by ELISPOT.

Untreated control grafts demonstrated high numbers of intragraft donor-specific DC by day 1 and 3. Induction of HO-1 significantly reduced the number of donor-specific DC while blocking of HO-1 by ZnPP reversed those effects (untreated controls/ZnPP- vs. CoPP- treated donors: $p<0.01$/day 1 and $p<0.05$/day 3). Although the total number of DA-DC in blood, spleen and LN of the recipients was lower vs. those in allografts, extrarenal DC were significantly reduced following CoPP treatment of the donor ($p<0.01$). Loss of donor-derived DC in the CoPP group was associated with reduced frequencies of donor-specific T cells as measured by ELISPOT analysis by day 1 (IFN-γ+ cells/CD4+ T cells: untreated controls and ZnPP vs. CoPP: $p<0.01$). Those changes demonstrated comparable trends by day 3 while not reaching statistical differences. Infiltration of CD8+ T cells by day 3 was significantly reduced in CoPP treated grafts (cells/field of view: untreated controls: 115±7 vs. CoPP: 67±4, $p<0.05$). Intragraft OX-62+ DC were significantly reduced on day 1 following HO-1 induction ($p<0.01$).

A single donor treatment for the induction of HO-1 shortly prior to organ harvesting reduces the immunogenicity of the graft. Those events are reflected by reduced frequencies of donor-reactive T cells. Reduced amounts of donor derived DC may explain improved short- and long-term allograft survival.

Abstract# 586 Poster Board #-Session: P42-II

RELATIVE CONTRIBUTION OF DIRECT AND INDIRECT PATHWAY OF ALLORECOGNITION AFTER DIFFERENT ALLOGENIC CELLS OR ORGAN TRANSPLANTATION. Hideyoshi Toyokawa,[1] Robert J. Bailey,[1] Atsunori Nakao,[1] Kei Kimizuka,[1] Jerome L. Lemoine,[2] Glenn D. Papworth,[3] Leaf Huang,[2] Noriko Murase.[1] *[1]Thomas E. Starzl Transplantation Institute; [2]Center for Pharmacogenetics; [3]Center for Biologic Imaging, University of Pittsburgh, Pittsburgh, PA.*

Allorecognition via the direct and indirect pathways (IDP) initiate immune reactions after transplantation; however, the relative contribution of each pathway remains unclear. We analyzed the degree of host T cell activation after stimulation with different types of alloantigens in recipients deprived of IDP with liposome clodronate (LC). **Methods:** CFSE labeled LEW rat unfractionated, purified T, or non-T cells (2×10^8 cells) were infused into unmodified or LC (200 mg/kg) treated BN rats. The real-time migration pattern of infused cells was studied by optiscan confocal endoscope (OCE). Host T cell activation was studied by Th1 cytokine mRNA levels and expansion of T cell area. LEW to BN heart transplant (HTx) was performed with or without LC to determine the ultimate roles of IDP. **Results:** LC eliminated phagocytic cells (macrophages and immature DC), resulting in <25% $ED1^+$ and <5% $ED2^+$ cells in the spleen. Real-time tracing of $CFSE^+$ cells in the spleen with OCE revealed that allogenic cells localized at permanent positions in 24 hrs without relocalization. Infused T cells were found in T cell area, while non-T population in B cell follicles and red pulps, regardless of LC treatment. Splenic INFγ mRNA was significantly more upregulated with allogenic non-T than T cells. Recipients deprived of IDP had minimum INFγ upregulation after both T and non-T cell injection. Similarly, expansion of splenic T cell areas was less prominent with non-T than T cell infusion with LC. Heart graft survival without immunosuppression in LC-treated recipients was slightly prolonged vs unmodified recipients. Under short course FK506 (0.5 mg/kg, d0-6), lack of IDP resulted in early lower Th1 cytokine mRNA upregulation, higher CD4/CD8 ratios, and splenic CD25 (IL-2R) reduction after HTx. Long follow-up is underway. **Conclusion:** Infused allogeneic cells migrate into the predetermined location of host spleen in 24 hrs without reposition. Non-T cells in B cell follicles and red pulps are more efficient for T cell activation probably via the IDP. Altogether, IDP of allorecognition appears to play an important role in host T cell activation.

Group	Allogenic cell type	Indirect pathway	INFγmRNA (24hr)	T cell area (24hr)
1	None	NA	1.0±0.7	6.7±11.6
2	T cells	Yes	90.5±10.8	7.2±8.7
3	Non T cells	Yes	146.6±12.6*	9.7±17.2
4	T cells	No	3.6±1.7	6.1±11.6
5	Non T cells	No	8.2±2.5**	5.6±8.1**

*p<0.05 vs Group2, **p<0.05 vs Group3, mRNA levels; fold-increase vs normal

Abstract# 587 Poster Board #-Session: P43-II

VASCULAR ENDOTHELIAL CELLS ESCAPE FROM APOPTOSIS TRIGGERED BY HLA-DR LIGATION MEDIATED BY ALLOSPECIFIC ANTIBODIES. Stéphanie Le Bas-Bernardet,[1] Stéphanie Coupel,[1] Jean-Paul Soulillou,[1] Béatrice Charreau.[1] *[1]Institut de Transplantation et de Recherche en Transplantation, Institut National de la Santé Et de la Recherche Médicale Unité 437 " Immunointervention en Allo et Xénotransplantation ", Nantes, France.*

By expressing donor HLA class I and class II antigens, activated graft endothelial cells (ECs) can be the target of anti-HLA alloantibodies. HLA-DR ligation mediates cell death of APCs such as mature B and dendritic cells. This study investigates the apoptotic effect of HLA class II ligation on human vascular graft ECs. Apoptotic effect of anti-HLA-DR mAbs was investigated *in vitro* on, HLA-typed, primary cultures of vascular ECs isolated from cadaveric kidney transplant donors. Expression of HLA-DR, DQ and DP was selectively induced by IFNγ and reach a maximal level at 72h. The induced expression of HLA-DQ was lower than that of HLA-DP while the induced expression of HLA-DP was lower than that of HLA-DR. Furthermore, expression of HLA-DR was significantly reduced in comparison to the frequency of these antigens on B cells. Apoptosis was measured by DNA content and annexinV labeling of ECs in comparison with B lymphocytes. DNA content analysis shows that incubation of B cells with monomorphic anti-HLA-DR mAb induce 51% of apoptosis. In the same conditions, no significant apoptosis of resting (DR negative) or IFNγ-activated (DR positive) ECs was observed, while incubation with TNFα and cycloheximide efficiently induced apoptosis. AnnexinV-FITC labeling further indicates that, mAbs specific for monomorphic or polymorphic determinants, (DR11 or DR16), were not able to induce ECs apoptosis. In conclusion, these data suggest that in contrast to professional APC, graft ECs escape from apoptosis mediated by HLA-DR ligation due to low expression of HLA-DR and costimulatory molecules or specific protective pathway or both.

Abstract# 588 Poster Board #-Session: P44-II

HLA-E IS AN ENDOTHELIAL-SPECIFIC NON CLASSICAL CLASS I MOLECULE EXPRESSED IN VASCULARIZED ORGANS: UPREGULATION AND SHEDDING UPON INFLAMMATION. Stéphanie Coupel,[1] Anne Moreau,[2] Vaclav Horesji,[3] Béatrice Charreau.[1] *[1]INSERMU437, ITERT, Nantes, France; [2]Service d'Anatomo-Pathologie, C.H.U. Hôtel-Dieu, Nantes, France; [3]Institute of Molecular Genetics, Praha, Czech Republic.*

In contrast to classical MHC class I molecules (HLA-A, -B and -C), non classical MHC class I molecules (HLA-E, -F and -G) are broadly defined by a limited polymorphism and a restricted pattern of cellular expression. HLA-E interacts with the inhibitory receptor CD94/NKG2A on natural killer (NK) cells and regulates NK cell functions. HLA-E can present allogeneic peptides and interact with T-cell receptors leading to alloreactive CTL. Cell surface expression of HLA-E requires the signal peptide from other HLA class I molecules or viral peptides by a TAP-dependent mechanism. Although HLA-E transcripts have been detected in almost all cell types, only few data report on protein expression *in vivo*. In the present study, we show that HLA-E expression in human tissues is mainly restricted to endothelial cells (EC). Histology using MEM/E2 anti-HLA-E mAbs shows HLA-E expression on all EC types including both venous and arterial ECs, glomerular ECs, microvascular ECs and HEV. Our data indicate that HLA-E is also expressed by B and T lymphocytes in lymphoid organs and by macrophages. *In vitro,* we demonstrate that HLA-E transcripts are present in cultured ECs (HUVEC and human arterial EC, HAEC) and are strongly up-regulated upon activation with the cytokines TNFα, IFNγ and IL1α. Increase in mRNA level for HLA-E correlates with enhanced protein expression on endothelial cell surface, assessed by flow cytometry and western blotting, and production of soluble HLA-E (sHLA-E) molecules by activated ECs. Western blots revealed the presence, in supernatants of activated ECs, of two bands of 37kDa and 42kDa corresponding respectively to β_2m-free and β_2m-complexed soluble HLA-E. Inhibition of mRNA or protein synthesis (with actinomycin D or cyclohexamide, respectively) and blockade of exocytosis with brefeldin A prevent shedding of sHLA-E. In contrast, treatment of ECs with protease inhibitors increases cell surface expression while abrogates shedding of HLA-E. To conclude, our data indicate that, in vascularized organs, expression of HLA-E is restricted to ECs and that EC activation increases HLA-E expression and promotes release of sHLA-E. These findings suggest that HLA-E expressed by graft ECs as well as release of sHLA-E may play a role in allograft immunoregulation. The effect of sHLA-E on NKG2A-bearing NK cells is currently under study and attempts are made to determine whether the levels of sHLA-E in recipient sera correlate to transplant outcome.

Abstract# 589 Poster Board #-Session: P45-II

IN VIVO GENERATION AND CHARACTERIZATION OF TWO DISTINCT DENDRITIC CELL SUBPOPULATIONS IN THE LIVER. Yalan Wang,[1] Zhengbin Lu,[2] Lianfu Wang,[1] Xiaoyan Liang,[1] John J. Fung,[1] Lina Lu,[1] Shiguang Qian.[1] *[1]Thomas E. Starzl Transplantation Institute, Surgery, University of Pittsburgh, Pittsburgh, PA; [2]Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Discovery of liver transplant tolerance has led to an enthusiasm of investigating liver dendritic cells (DC) which play a key role in determining immunity or tolerance. However, most of the experiments have been performed in DC propagated *in vitro* with cytokines and stimuli, such as GM-CSF for myeloid DC, and IL-3 and CD40L for lymphoid DC. In this study, we adopted an established approach to overexpression of interested genes in the liver by tail vein rapid injection of naked plasmid carrying genes encoding GM-CSF, IL-3 or CD40L (GFP as reporter) into B6 ($H2^b$) mice. This approach enabled significant levels of transgene expression in the liver in 8h, reached the peak in 24 hr, decreased thereafter, and lasted for 5-7 days, as demonstrated either by detection of reporter gene expression with fluorescent microscopy or by serum levels of GM-CSF or IL-3 with ELISA. Histochemistry showed that overexpression of GM-CSF expanded $CD11b^+CD11c^+$ mononuclear cells mixed with neutrophils, morphologically resembling macrophages and myeloid DC, and mostly located in portal and periportal areas. In contrast, transfect of IL-3/CD40L resulted in marked expansion of $B220^+CD205^+$ mononuclear cells, which were often clustered together forming many big aggregates within both portal tracts and expanded sinusoid spaces in the lobules. FACS analysis of isolated liver NPC confirmed that two distinct DC subsets were expanded. GM-CSF transfection expanded $B220^-CD205^-CD11b^+CD11c^+$ (17.7% vs 2.0% in controls), and IL-3/CD40L expanded $B220^+$ $CD205^+CD11b^-CD11c^-$ DC (16.0% vs. 1.1% in controls). Both subsets of DC expressed low MHC class II, CD80/CD86/CD40, and developed typical DC morphology. Further maturation occurred following overnight culture. In addition to unique phenotype, each subset of DC displayed distinguished allostimulation activity in MLR; $CD11b^+CD11c^+$ DC, but not $B220^+CD205^+$ DC, induced profound allogeneic T cell (C3H, $H2^k$) proliferation. Pretreatment of allergenic recipients 7 days before heart allograft transplantation with 2×10^6 $CD11b^+CD11c^+$ DC accelerated allograft (B6) rejection in C3H recipients. The effect of administration of $B220^+$ $CD205^+$ DC on heart allograft survival is currently under observation. In conclusion, two distinct DC populations in the liver were identified *in vivo*, whose phenotype and function are similar to the myeloid and lymphoid DC propagated *in vitro*. This provides a more physiological approach for study of DC biology in the liver.

Abstract# 590 Poster Board #-Session: P46-II

LONG-TERM RESULTS (OVER 15 YEARS) OF KIDNEY TRANSPLANTATION FROM NON-HEART-BEATING DONORS. A SINGLE CENTER EXPERIENCE. Hiroaki Shimmura,[1] Kazunari Tanabe,[1] Hideki Ishida,[1] Tadahiko Tokumoto,[1] Nobuo Ishikawa,[1] Naoshi Miyamoto,[1] Kiyoshi Setoguchi,[1] Satoshi Teraoka,[2] Hiroshi Toma.[1] *[1]Department of Urology, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan; [2]Kidney Surgery, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.*

Introduction: Despite great efforts to promote the donation of cadaveric organs, the number of organ transplantation from brain-dead donor in Japan is not increasing, and a serious shortage of cadaveric organs exists. Therefore, kidney transplantation from non-heart-beating donors (NHBDs) has been carried out. We have been using new immunosuppressants, such as tacrolimus and mycophenolate mofetil, for renal transplantation and better results have been obtained. In this study, we reviewed the outcomes of transplantation from NHBDs which was performed between 1983 and 2002 at Tokyo Women's Medical University.

Materials and Methods: Between 1983 and 2002, 202 patients underwent cadaveric renal transplantation from NHBDs at Tokyo Women's Medical University. Seventy recipients were females and 132 were males with a mean age of 39.5 years (range 4 to 66 years). A mean donor age was 45.2 years (range 1 to 73 years). A mean HLA-AB and -DR mismatch numbers were 1.29 and 0.58. Total ischemic time (TIT) and warm ischemic time (WIT) were 704±335 min (242-2077 min) and 6.7±11.0 min (0-100 min). The median pretransplant dialysis interval was 97.3 months (range 0 to 336 months). All our NHBDs were categorized as III or IV of the Maastricht criteria.

Results: Patient survival rates were 92.0% at 5 year, 50.4% at 10 years and 40.6% at 15%. Graft survival rates were 72.1% at 5 year, 50.4% at 10 years and 40.6% at 15 years. The incidence of delayed graft function (DGF) was 79.7% (161 of 202 patients). The DGF lasted 15.8 ±15.1 days (range 2-110). The lowest levels of serum creatinine after transplantation were 1.6±8.1 mg/dl (0.7-6.0 mg/dl). Among the 202 patients, 12 (5.9%) showed primary non-function. Acute rejection occurred in 82 (42.6%) of the 202 patients.

Conclusion: Our results demonstrated that the transplantation of kidneys from NHBDs led to acceptable long-term graft survival despite the high incidence of DGF.

Abstract# 591 Poster Board #-Session: P47-II

AN ASSESSMENT OF THE EFFECTIVENESS OF A COMMUNITY BASED TRANSPLANT EDUCATION PROGRAM FOR HIGH SCHOOL YOUTH. Clive O. Callender,[1] Margruetta B. Hall,[1] Patrice V. Miles,[1] Kay L. Butler,[1] Phyllis Daen,[1] Phyllis Ermann,[1] Rhonda Gaines,[1] Rhonda DeLaremore.[1] *[1]National MOTTEP, Howard University, Washington, DC.*

Purpose:

This study evaluated the effectiveness of a community-based high school youth health education program. The purpose was to increase the number of 15-18 year olds who held family discussions and the number of youth that "Say Yes" to donation on their driver's licenses.

Methods:

The study targeted minority high school students in Baltimore County, Maryland and Arlington, Virginia who were African-Americans, Latinos, or other minorities. White student participants in the classes were also included. A quasi-experimental design pre-intervention and post-intervention data were collected from 300 youth (Baltimore County, Maryland driver's education classes and Arlington, Virginia health education classes) between September, 2000 and April, 2002.

Results:

T-Tests were used to determine if there were any treatment effects of the program on youth in drivers' education classes and youth in health education classes. For all youth participants, there were significant increases in trust in the medical system, and future plans to become donors. In Virginia, there was a highly significant increase (P=.000) in knowledge in both the study and comparison groups.

Expected Outcomes	Balt. Co. Study Group	Balt. Co. Comp. Group	Arlington Co. Study Group	Arlington Co. Comp. Group
Knowledge	No Significant Change	Significant Change (p<.06)	Significant Change (p<.000)	No Significant Change
Future Plans	Signficant Change (p<.006)	Highly Significant Change (p<.000)	Significant Change (p<.06)	Significant Change (p<.000)
Trust	Marginally Significant	No Significant Change	Highly Significant Change (p<.015)	No Significant Change

Conclusions:

Trust in the medical system and future plans for donation were significantly affected by culturally appropriate health education programs designed for multi-cultural high-school youth. Education programs focusing specifically on behavior change and increasing trust for the medical system were sufficient to significantly increase the number of youth willing to become donors and hold family discussions.

Abstract# 592 Poster Board #-Session: P48-II

HISTOLOGICAL CRITERIA FOR MARGINAL KIDNEY ALLOCATION. IS THERE ROOM TO FURTHER EXPAND THE DONORS POOL? B. Infante,[1] G. Stallone,[1] A. Schena,[1] G. Grandaliano,[1] S. Di Paolo,[1] M. Battaglia,[2] P. Ditonno,[2] F. P. Selvaggi,[2] L. Gesualdo,[3] F. P. Schena.[1] *[1]Div. of Nephrology; [2]Div. of Urology, DETO, Univ. of Bari; [3]Chair of Nephrology, Univ. of Foggia, Italy.*

Introduction. The allocation criteria of kidneys from "marginal" cadaveric donors to single (SKT) or double transplant (DKT) are still debated. The aim of this study was to compare graft function and survival in three groups of recipients from marginal cadaveric donors in the attempt to determine through pre-transplant renal biopsy a demarcation line for SKT or DKT.

Patients. 211 patients underwent kidney transplant from 125 cadaveric donors. Recipients were divided in four groups on the basis of donor clinical evaluation and pre-transplant global histological score (0-3 for each renal compartment: glomerular, vascular, tubular and interstitial): SKT from ideal donors (Control), SKT from marginal donors with score 0-3 (group I), SKT from marginal donors with score 4 (group II), and DKT from marginal donors with score 5-6 (group III).

Results. Control-group presented a significantly lower level of serum creatinine compared to recipients of group I, II and III both at discharge (Control 1.6±0.5, group I 2.1±0.9 mg/dl, p=0.0001, group II 2.1±0.8 mg/dl, p=0.0001, group III 2.0 ± 0.6 mg/dl; p=0.005) and two years post-transplant (Control 1.4±0.5, group I 1.7±0.4 mg/dl, p=0.003, group II 1.8±0.4 mg/dl, p=0.0001, group III 1.9±0.7 mg/dl, p=0.003), while no significant difference in serum creatinine at the same time points was present between group I, II and III. Actuarial allograft survival at 24 months after engraftment showed no statistically significant difference in the experimental groups. Finally, the incidence of delayed graft function (DGF) was significantly higher in the three experimental groups compared to Control (p=0.02), but did not differ among groups I, II and III. Length of DGF was comparable in all groups.

Conclusion. Our approach in term of definition of "marginal" donor and allocation their kidneys shows a satisfactory recipients renal function and an excellent short-term graft survival. These findings might offer positive perspectives to exceed the actual organ shortage expanding donor criteria.

Abstract# 593 Poster Board #-Session: P49-II

BENEFITS OF USING PULSATILE PERFUSION PUMP FOR CADAVERIC KIDNEYS. Hamid Shidban,[1] Jim Locke,[2] Tom Mone,[2] Robert Mendez,[1] Shirley Mirador,[1] Ramaiah Indudhara,[1] Sali Aswad.[1] *[1]National Institute of Transplantation, Los Angeles, CA; [2]One Legacy, Los Angeles, CA.*

Introduction: Immediate renal allograft function following kidney transplantation is most desirable. Cold storage is associated with a significant amount of early post-transplant renal dysfunction. While pulsatile perfusion is more costly and technically demanding for preservation of renal allografts, a significant reduction in the need for post-transplant dialysis with concomitant shorter hospital stays for transplanted patients, offsets the moderately increased costs of preservation.

Aim: To evlauate the benefits and outcome of perfusing a kidney for transplant.

Methods: There were 112 harvested kidneys that were pumped from June 2001 to Aug. 2003., of which 59 were transplanted at our Hospital, 30 kidneys discarded, and the remainder distributed to other transplant centers. No kidneys in this study were discarded due to lack of availability of a suitable recipient. UW perfusate solution was used. The data for the pumped group was provided by One Legacy, an organ procurement organization in Southern California. The control group consisted of 261 cadaveric non-pumped, transplanted kidneys transplanted during the same period.

Results: Statistically there was no significant difference between the two groups in donor age, gender, mismatch, and donor cause of death. Patient & graft outcome are shown below.

Demographic Data	Pumped Group N=59	Control Group N=261	P
Donor Age>55yrs	19(32.2%)	72(19.6%)	0.24
CIT>36hrs	7(11.9%)	21(8.0%)	0.76
Induction	58(98.3%)	164(62.8%)	0.0001
Pre-Op Recipient S. Cr.	8.6(±3.3)	7.2(±3.4)	

Outcome Data	Pumped Group N=59	Control Group N=261	P
Immediate Functioning	41(69.5%)	127(48.7%)	0.02
DGF	9(15.3%)	114(43.7%)	0.09
Primary Non-Functioning	7(11.9%)	11(4.1%)	0.03
Length of Stay	9.7±9.6	9.4±3.0	0.69
Graft Survival			
6mos	88.1%	90.0%	0.63
1yr	81.3%	85.0%	0.44
Patient Survival			
6mos	96.6%	95.7%	0.74
1yr	90.0%	93.1%	0.43

Conclusion: Cadaver renal preservation using pulsatile perfusion with UW has lowered the incidence of post-transplant DGF due to preservation injury from 29.1% to 15.3%. The rate of primary non-functioning in the pumped group was 11.9% vs. 2.7% in the control group. Although use of pump preservation was primarily for marginal donor kidneys, the outcome was comparable to the control group. Pulsatile preservation helped to identify high risk kidney for primary non functioning kidneys by using pump pressure and resistance idex criteria.

Abstract# 594 Poster Board #-Session: P50-II

CLINICAL RESULTS OF A REGION-WIDE PROGRAM TO INCREASE UTILIZATION OF DONORS AFTER CARDIAC DEATH (DCD). James F. Whiting,[1] Francis Delmonico,[2] Paul Morrissey,[3] Giacomo Basadonna,[4] Scott Johnson,[5] W. David Lewis,[6] Richard Rohrer,[7] Kevin O'Connor,[8] James Bradley,[8] Tammy D. Lovewell,[4] George Lipkowitz.[9] *[1]Surgery, Maine Medical Center, Portland, ME; [2]Surgery, Massachusetts General Hospital, Boston, MA; [3]Surgery, Rhode Island Hospital, Providence, RI; [4]Surgery, UMass Medical Center, Worcester, MA; [5]Surgery, Beth Israel Deaconess Medical Center, Boston, MA; [6]Surgery, Lahey Clinic, Burlington, MA; [7]Surgery, New England Medical Center, Boston, MA; [8]New England Organ Bank, Newton, MA; [9]Surgery, Baystate Medical Center, Springfield, MA.*

Background. In an effort to stimulate organ donation, a region-wide effort was undertaken to initiate or reinvigorate DCD programs over a 3 1/2 year period. Components of the effort included commonality of protocols, pulsatile perfusion of virtually all kidneys, centralization of data and a regional ALU designed to encourage short cold ischemia times. **Results.** In one OPO, six centers initiated DCD programs in 9 hospitals, while one center reinvigorated an established program. DCD donors increased steadily over the study period from 4 donors in 1999 to19 in the first 10 months of 2003. Mean age of the donors was 35.9 ± 14 years. Nine donors had care withdrawn in the operating room, while 40 had care withdrawn in the ICU. 85 patients received renal transplants from these 49 donors (12 kidneys discarded, one double transplant) while 4 patients received liver transplants. Mean time from withdrawal of care to asystole was 25.3 ± 45 minutes(range 2-181). Mean time from asystole to either femoral or aortic flush was 14.6 ± 7 minutes(range 5-43). All four liver grafts functioned. One, two and three year kidney graft survival rates were 90%, 90% and 82% respectively. 64% of patients needed at least one session of hemodialysis postoperatively. Mean recipient hospital length of stay was 8.8 ± 6 days. Mean creatinines at 3, 6,12 and 24 mos were 1.65, 1.45, 1.48 and 1.54 respectively. Uni- and multivariate analysis of the effect of donor and pulsatile perfusion variables on outcome (discard rate, delayed function rate and graft function) demonstrated only a relatively weak correlation between final perfusate flow and discard rate ($p<0.05$). **Conclusion.** DCD donors can be an important source of donor organs and provide excellent overall outcomes. Regional cooperation and a prospectively considered allocation and distribution system are important considerations in stimulating DCD programs.

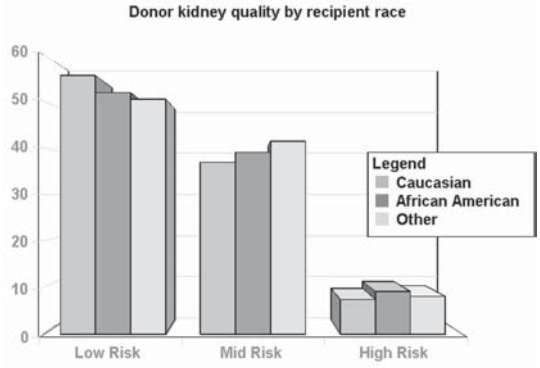
Abstract# 595 Poster Board #-Session: P51-II

DISPARITIES IN THE QUALITY OF DECEASED DONOR KIDNEYS BY RECIPIENT RACE. Jesse D. Schold, Herwig-Ulf Meier-Kriesche, Titte R. Srinivas, Michael Bucci, Bruce Kaplan. *University of Florida, Gainesville, FL.*

Allocation of deceased donor kidneys in kidney transplantation (tx) encompasses innumerable practical, ethical, and medical issues. With the knowledge that the quality of the donated organ is an important factor in patient outcomes, we undertook an analysis of registry data to examine the distribution of the quality of donated kidneys by recipient ethnicity and gender.

All first solitary kidney txs (n=57,182) from 1995-2002 were utilized in our analysis. A donor risk score was generated from the parameter estimates of significant risk factors for graft loss including donor age, race, BMI, CMV status, gender, history of hypertension, and cause of death in a multivariate Cox model. This score was then delineated into groupings via cluster analysis and distributions examined by recipient race and gender.

Results indicated significant differences in risk groupings (Chi-square p-value <.0001) and for overall risk score tested with an ANOVA model for recipient race. Caucasian recipients received a low risk donation in 55.5% of txs, African American recipients in 51.8% of txs, and other race recipients 50.4%. The relative frequency of high risk donations by recipient ethnicity was African Americans 9.2%, other 8.2%, and Caucasian 7.5%. Recipient gender was not significantly different among the risk levels. Overall graft survival was significantly associated with risk group (p<.0001), adjusted for recipient characteristics; relative risks attributed to a mid risk level donation and high risk level donation for graft loss were 1.5 (1.4, 1.5) and 2.2 (2.0, 2.3) respectively. There exists a significant disparity in the quality of deceased donor kidneys by recipient ethnicity. Observed decreased graft survival in African American recipients may in part be attributed to receiving lower quality donations. Whether this disparity is systemic, a function of recipient geography, other recipient characteristics, or more subtle causes requires further diligent scrutiny.



Abstract# 596 Poster Board #-Session: P52-II

RENAL TRANSPLANTATION FROM NON- HEART-BEATING DONORS (NHBD): A SINGLE CENTRE 10 – YEAR EXPERIENCE. Angel Alonso,[1] Constantino Fernandez,[1] Pedro Villaverde,[1] Rafael Garcia,[1] Juan Oliver,[1] Francisco Valdes.[1] *[1]Nephrology, Hospital Juan Canalejo, A Coruna, Spain.*

The acceptance of NHBD kidneys is limited by effects of prolonged warm ischemia on short and long term renal function.

The aim of present study was to analyze patient and allograft survival, and renal function of patients NHBD in our hospital in the last decade.

Methods. 1667 kidneys allograft were performed since 1981.We studied 97 patients (62 male), median age 47±14 years old, that received NHBD kidneys (77 male), median age 38 ±14 years old. Primary immunosuppressive agents were: 84% cyclosporine, 22% azathioprine and 4% tacrolimus. Secondary agents were 73% azathioprine and 27% mycophenolate mofetil. In 12 patients mono or polyclonal drugs were used. Factors analysed: warm and cold ischemia time, primary non function, delayed graft function, acute rejection in three first months, time of duration of delayed graft function, allograft and patient survival and renal function at 1º, 5º and 10º year.

Results: Follow-up time was 51±51 months (1 -170). Warm ischemia time was 16± 12 minutes and cold ischemia time 23±5 hours. 80 patients (82%) showed delayed graft function and 18 (18,6 %) primary non function. Time of duration of delayed graft function: 23±17 days and the time of hospitalization was 39±24days. Acute rejection occurred in 14 patients (14.5%). 16 patients death mainly of infectious causes. Patient survival was 89%, 80% and 79% at 1º, 5º and 10º year. Allograft survival was 74%, 62% and 62% al 1º, 5º and 10º year. Causes of graft loss were 13 thrombosis, 9 acute rejections, 5 chronic nephropathy of allograft and 7 others. Creatinine at 1 year was 2.1±0.9 mg/ dl, at 5º year: 1.7±0.7 mg/ dl and 10º year: 2±1.2 mg/dl.

Conclusions. Although the incidence of primary non function and delayed graft function in NHBD was higher, and allograft survival at 1º year was lower, that in heart beating donors, allograft survival at 5º and 10º year and renal function were similar in both groups.

Abstract# 597 Poster Board #-Session: P53-II

THE CELL POPULATION IN PRESERVATION SOLUTION AND IT'S INFLUENCE ON DELAYED KIDNEY ALLOGRAFT FUNCTION. P. Malanowski,[1] G. Korczak-Kowalska,[1] P. Wierzbicki,[1] M. Kosieradzki,[1] A. Kwiatkowski,[1] M. Wszola,[1] J. Pliszczynski,[1] R. Danielewicz,[1] L. Adadynski,[1] L. Paczek,[1] M. Durlik,[1] W. Rowinski.[1] *[1]Transplantation Institute, Medical University of Warsaw, Warsaw, Poland.*

The function of the kidney harvested from the cadaveric donor depends on large group factors: donor dependent, accompanying brain death, ischemia, preservation and reperfusion. The aim of the study was to examine cell quantity, viability and phenotype flushed from the organ during preservation and kidney function after transplantation. The preservation solution was flushed from the kidneys after preservation in continuous hypothermic pulsatile perfusion (CHPP) or after static perfusion at the end of preservation in simple hypothermia(SH/SP). 40 samples of the preservation solution were examined, 23 from CHPP, 17 from SH/SP. Number of cells, their viability and type (CD3, CD4, CD8, CD14, CD16, CD15, CD69) were analyzed. Mean cell count in the preservation solution for SH/SP was $10{,}84x10^6$, for CHPP $31{,}52x10^6$. Viability of the cells 98%-99%. The cells flushed from the kidney proved to be lymphocytes and monocytes. There were more of these cells in the solution than in the peripheral blood. In the CD3 population, mean percentage of CD4 cells was 40.1% (standard peripheral blood 31%) and mean of CD8 was 60.1% (peripheral blood 41%). We then examined activated T and NK cells measuring the expression of CD69. As well as in the T cells as in NK cells percentage of the CD69+ was higher than in peripheral blood. That suggests that both lymphocytes and NK cells could become activated in the kidney. Percentage of T cells $CD69^+$ was similar in the solution after CHPP and in SH/SP, however NK $CD69^+$ percentage was higher in the SH/SP solution. T cell subpopulation examination proved that there were more $CD8^+CD69^+$ cells in the solution. Furthermore it seems that $CD69^+$ cell concentration is higher in the solution obtained from the kidneys which present delayed graft function after transplantation. Analysis of the cells flushed from the kidney shows that in the organ (in the donor or during preservation) activation of lymphocytes and NK cells starts. In 10 cases of kidneys being stored in CHPP delayed graft function (DGF) after transplantation was observed (43.4%), among kidneys stored by SH 7 cases of DGF was observed (41.1%). No significant statistical influence between delayed graft function for both preservation methods was observed. Analysis of the cells flushed from the kidney shows that in the organ (in the donor or during preservation) activation of lymphocytes and NK cells was observed.

Abstract# 598 Poster Board #-Session: P54-II

OUTCOME OF SOLITARY PEDIATRIC (13 MONTH-5 YEAR) DONOR KIDNEY TRANSPLANT IN ADULT RECIPIENTS. Shafiq Cheema,[1] Rafik El-Sabrout,[1] Kahlid Butt,[1] Patricia Hanson,[1] Veronica Delaney.[1] *[1]Nephrology and Transplant Surgery, Westchester Med Ctr, NY Med College, Valhalla, NY.*

The transplantation of kidneys from cadaveric donors ≤5 years of age into adult recipients is controversial. The large disparity between donor renal mass and recipient body mass is feared to be problematic. The UNOS database showed that en bloc kidney survival was better than a single kidney from donors 0-5 years.

Methods:

Retrospective analysis of thirty consecutive solitary pediatric kidney transplants into adults. Groups (gp) based on donor age:≤2 years (gp I, n=12) and 2-5 years (gp II, n=18).All recipients received steroids, tacrolimus, rapamune(n=20) or MMF(n=10) and induction with Thymoglobulin(n=28) or daclizumab(n=2). Follow-up period was 3 to 84 months (mean=14.6m) and included serial sonograms.

Results:

1-Mean age of donor=34.8 months (range[r]=13-60), mean serum creatinine of donor =0.35 mg/dl (r =0.2-0.8), mean age of recipient =42.6 year (r =19-73), mean weight of recipient =62.2 kg (r =51-78) and mean cold ischemia time was 23hrs (r =15-29).

2-At the end of follow-up, 24/30(80%) allografts were functioning. Mean creatinine(Crt) at 3 months and end of follow-up (mean=16m, range=3-78m) was 1.9 and 1.8 mg/dl respectively

3-Rate of acute rejection = 3/30 (10%). Five (17%) patients developed DGF and 5 (17%) had renal artery stenosis (RAS). Only two patients with RAS required intervention, angioplasty and reimplantation of RA.

4-Mean increase in kidney size at 3 month was 1.26 cm (7.8 to 9.15,16%).

5-Outcome of two groups is shown below:

	Gp I (n=12)	Gp II (n=18)	P value
1 Y graft survival	10/12, 83%	16/18, 88%	ns
Crt @ 3 M	2.0	1.8	ns
Crt @ last follow-up	2.0	1.7	ns
Kidney Size increase	1.27cm	1.25 cm	ns

6-Causes of allograft loss: Patient death with functioning graft(1), primary non-function secondary to renal vein thrombosis(1), chronic allograft nephropathy(2), Severe, untreated rejection(1) and Immunosuppression withdrawl (1)

Conclusion:

1-Solitary kidneys from cadaveric donors of age **one year and older** are suitable for transplantation into adults and provide excellent graft function.

2-Significant renal mass increase occurs in the absence of acute rejection or severe renal hypoperfusion.

3- Given the current shortage of donors and availability of more potent immunosuppressive drugs, larger trials are indicated to confirm our findings and prevent underutilization of small solitary pediatric kidney transplantation into adults.

4-We recommend selecting recipients of small body mass index, induction with thymoglobulin and use of rapamune for transplantation of small solitary pediatric kidneys into adults.

Abstract# 599 Poster Board #-Session: P55-II

PROSPECTIVE ANALYSIS OF A DONOR SCORING SYSTEM FOR DECEASED DONOR RENAL TRANSPLANTATION. Edwina Baskin-Bey,[1] John Franco,[1] Meg Rogers,[2] Mark Stegall,[1] Scott Nyberg.[1] *[1]Division of Transplant Surgery, Mayo Clinic, Rochester, MN; [2]LifeSource, Minneapolis, MN.*

A donor scoring system was developed as a quantitative approach to identify kidneys prior to transplantation that are at increased risk for delayed graft function and or failure (Am J Tx 2003:715-21). The scoring system was developed from retrospective data, but had not previously been validated in a prospective trial. **PURPOSE:** To ascertain through a prospective analysis if a donor scoring system was useful in predicting outcomes after deceased donor renal transplantation. **METHODS:** 188 recipients of a kidney transplant from a deceased donor were prospectively followed for 30 days after transplantation from 2001-2003. Donor score was determined at the time of procurement from the following five donor variables: age, 0-25 points, history of hypertension, 0-4 points; final creatinine clearance before procurement, 0-4 points; cause of death, 0-3 points; and HLA mismatch, 0-3. Donor kidneys were stratified by cumulative score: grade A, 0-9 points; grade B, 10-19; grade C, 20-29; and grade D, 30-39. Primary study endpoints included graft loss, utilization of hemodialysis, creatinine clearance and serum creatinine 30 days post renal transplant. **RESULTS:** The majority of kidneys were grade A; 47% with grades B, C, and D respectively, 31%, 20% and 2%. Graft loss was 29.2-fold greater for grade D (1 of 3) vs. grade A (1 of 88) kidneys. The need for hemodialysis was 100% for grade D kidneys compared to Grade A, B and C kidneys respectively 19%, 35%, and 39%. Creatinine clearance measured 30 days posttransplant was 2-fold greater for grade A kidneys (72 mL/min) compared to grade D kidneys (38 mL/min) (p= 0.038). Similarly, serum creatinine assessed 30 days posttransplant was higher with grade D kidneys (3.2 mg/dL) compared to grade A kidneys (1.5 mg/dL) (p=0.053). **CONCLUSION:** The donor score of a kidney from a deceased donor showed a close correlation with its likelihood for posttransplantation hemodialysis, renal function, and graft failure. The donor score appears to be a useful tool for the allocation of deceased donor kidneys for transplantation.

Abstract# 600 Poster Board #-Session: P56-II

CORRECTION OF BASE DEFICIT IN DECEASED DONORS FACILITATES FLUID MANAGEMENT AND IMPROVES IMMEDIATE RENAL ALLOGRAFT FUNCTION. Gary K. Shen,[1] John F. Recicar,[2] Jeffrey A. Salisbury,[1] Timothy D. Browder,[1] Patricia A. Niles.[2] *[1]Department of Surgery, University of Nevada School of Medicine, Las Vegas, NV; [2]Nevada Donor Network, Las Vegas, NV.*

Background: Effective resuscitation of deceased donors is critical to the success of organ recovery. Accurate determination of fluid status in brain dead donors is usually confounded by iatrogenic dehydration, hemorrhage, use of vasopressors, and coexisting diabetes insipidus. Maintenance of blood pressure and urine output alone as resuscitation endpoint is inadequate. We hypothesize that correcting base deficit, a measure of tissue perfusion, will facilitate fluid management in these donors and thereby improve renal allograft function. **Methods**: Consecutive donors over a 12-month period were prospectively studied. Group I consisted of 12 donors whose resuscitation was based on maintaining SBP greater than 100 mm/Hg and urine output greater than 1 cc/kg/hr. Group II consisted of 15 donors whose resuscitation was based in addition on correcting their base deficits to the normal range (< 2 mmole). Ongoing monitoring was achieved by q2h measurements of base deficit obtained from ABG's. Immediate outcome of kidney allografts was determined by the presence of delayed graft function (DGF, dialysis requirement during week 1) and calculated creatinine clearance at day 7. **Results**: Age and cause of death in the two donor groups were similar. Base deficits were corrected to the normal range in all donors in Group II by the time of surgical recovery. Outcome data were available from 21 patients who received renal allografts from Group I and 27 from Group II. Recipient demographics and cold ischemic times were similar in the two groups. DGF occurred in 10/21 in Group I (48%), and 5/27 in Group II (19%, p< 0.05, Fisher's exact test). Creatinine clearance at day 7 calculated by the Cockroft-Gault formula was 29 ± 6 ml/min in Group I and 41 ± 8 ml/min in Group II (p< 0.05, T-test). **Discussions**: Brain dead donors have unique physiology where blood pressure and urine output alone do not adequately reflect organ perfusion. Base deficit is an easily obtained measurement to guide fluid resuscitation in this situation. Moreover, in our group of donors, this approach improves immediate renal allograft function. We recommend using base deficit as a routine to expedite organ recovery and potentially improve function of transplanted organs.

Abstract# 601 Poster Board #-Session: P57-II

TRANSPLANTATION OF ADULT RECIPIENTS WITH SINGLE CADAVERIC KIDNEYS FROM PEDIATRIC DONORS WEIGHING ≤25 KG IS A RELIABLE OPTION. Douglas P. Slakey,[1] A. K. Sharma,[1] Scott Meier,[1] Sander Florman,[1] Sunil Geevarghese,[1] Philippe Gauthier.[1] *[1]Center for Abdominal Transplantation, Tulane University, New Orleans, LA.*

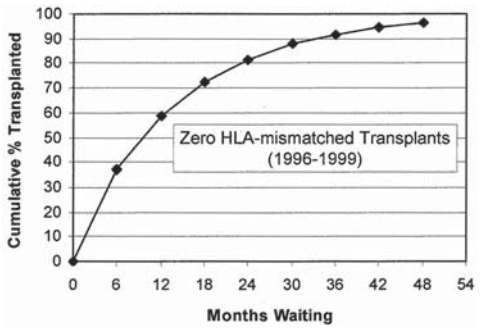
Background: The transplantation of single kidneys from cadaveric pediatric donors into adult recipients is not routine, most being used enbloc. We reviewed our experience with the transplantation of single kidneys from pediatric donors weighing ≤25 kg.

Methods: 32 adults were transplanted with single renal allografts from pediatric donors weighing ≤25 kg (study group) between April 1994 and October 2003. They were compared with 30 matched adult recipients of kidneys from adult donors (control group).
Results: There was no difference between both the groups in regards to sex, age, recipient weight, HLA mismatch, PRA, type of immunosuppression and duration of follow up. In the study group the recipients were 37.6±14.3 years old with a sex ratio of 15:14 (Male:Female) and had median A-B-DR mismatch of 2-1-1, and a median follow-up of 23 months(range 3 to 96). Mean age of donors in study group was 4.4±2.1 years weighing 15.9± 5.3kg with a donor/ recipient weight ratio of 0.22±0.09. Arterial anastomosis was done with a patch, in all except one, with main arterial lumen size being 4.8±2.3 mm. Ureteral stent was used in 62.5% in study group versus 25.0% of controls, P<0.02. In the study group, surgical complications occurred in 4/24 patients (hydronephrosis 1, hematoma 1 and ureteric stenosis 2) needing surgical intervention in two of them. In the control group, 4/29 developed surgical complications (fluid collection 1, hydronephrosis 1, uretic stenosis 1, uretero-vesical leak 1) needing surgical intervention in two. Serum creatinine reached nadir in 51 days in study group versus 30 days in controls (p <0.01). Serum creatinine at 1 and 3 years were comparable in both groups. In study group 38.9% had proteinuria at 1 year compared to 22.7% in controls (p=0.36). 1-year graft survival was 91.7% versus 92.8% for controls.Conclusions: Though the incidence of proteinuria is more frequent, the surgical complications, 1 and 3 year serum creatinine, and graft survival in adult recipients of single renal grafts transplanted from small paediatric donors are comparable to that of controls. Transplantation of single rather than en-bloc pediatric donor kidneys yields comparable results and has the additional benefit of expanding the donor pool.

Abstract# 602 Poster Board #-Session: P58-II

MOST ZERO HLA-MISMATCHED KIDNEY TRANSPLANTS OCCUR SOON AFTER LISTING LEAVING A WAITING LIST OF PATIENTS WITH INCREASINGLY UNCOMMON HLA PHENOTYPES. Michael Cecka,[1] David Gjertson,[1] Gabriel Danovitch,[2] Erick Edwards.[3] *[1]UCLA Immunogenetics Center, University of California, Los Angeles, CA; [2]Medicine, David Geffen School of Medicine UCLA, Los Angeles, CA; [3]United Network for Organ Sharing, Richmond, VA.*

Background: Approximately 16% of deceased donor kidneys each year are transplanted to zero HLA-mismatched recipients in the US. However, the offer of a zero HLA-mismatched kidney for any waiting patient is unpredictable and represents an important challenge in managing the growing lists of waiting patients at many transplant centers. Our goal was to examine the dynamics of zero HLA-mismatched kidney transplantation with regard to time after listing.
Methods: We analyzed waiting times for recipients of zero HLA-mismatched deceased donor kidneys shared under the OPTN/UNOS national sharing program between 1996-1999. We also assessed the changing distribution of HLA phenotypes over time among patients who entered the waiting list in 1997 by determining the number of patients who had zero HLA mismatches with at least one donor during 1997, then repeating the analysis for those patients listed in 1997 who were still waiting in 1998 against 1998 donors and so on for up to 5 years.
Results: Nearly 75% of the HLA-matched transplants performed between 1996-1999 occurred within 18 months of listing as shown in the figure. Among 18,847 patients first listed in 1997, 37% had no HLA mismatches with at least one donor in1997. Of the 2,852 who still remained on the list in 2002, only 19% had no HLA mismatches with at least one donor in 2002. Thus, over a period of 5 years, common donor HLA phenotypes were depleted from the waiting pool by approximately 50%.
Conclusions: We demonstrated that nearly 75% of zero HLA-mismatched transplants occur within 18 months after listing and that patients with common HLA phenotypes are rapidly depleted from the waiting pool leaving patients who are unlikely to be offered a zero HLA-mismatched graft. This information may facilitate ensuring patients are medically ready when a zero HLA-mismatched kidney is offered.



Abstract# 603 Poster Board #-Session: P59-II

NON-HEART-BEATING DONOR KIDNEYS IN THE NETHERLANDS, ALLOCATION AND OUTCOME OF TRANSPLANTATION. Karin M. Keizer,[1] Hans W. de Fijter,[2] Bernadette J. J .M. Haase-Kromwijk.[1] *[1]Dutch Transplant Foundation, Leiden, Netherlands; [2]on Behalf of the Dutch Kidney Transplant Centers.*

Introduction: Since the early eighties kidneys from non-heart-beating (NHB) donors have successfully been transplanted in the Netherlands. At this moment one out of three of all renal transplantations in the Netherlands are done with NHB donor kidneys, compared to 3% in the United States.

These NHB kidneys are allocated through the regular renal allocation system, hence all renal transplant candidates on the waiting list are potential NHB kidney recipients.

Aim: Three and 12 months outcome data for kidneys from NHB donors are compared to the outcomes for kidneys from heart-beating (HB) donors.

Methods: This study investigated the serum creatinin and graft survival of all (non-heart-beating and heart-beating) cadaveric kidneys transplanted in the Netherlands between 1-2-2001 and 1-03-2002. Risk factors for graft failure were evaluated in a Cox proportional hazard model.

Results: In this period 222 patients received a kidney from a HB donor and 111 from a NHB donor. Seventeen (8%) HB and 17 (15%) NHB kidneys failed within three months. The three months graft survival rate was 92.3% and 84.3%, respectively (p=0.04). Three months after transplantation the renal function measured as serum creatinin, was similar in the two groups: 152 mmol/l and 158 mmol/l in HB and NHB, respectively (p=0.4).

Multi variate analysis identified NHB donor (RR 2.2 (p=0.02) and a previous kidney transplantation (RR 2.2 (p=0.04)) as independent risk factors for transplant failure within three months.

Within the subset of NHB kidneys, multi variate analysis showed that a warm ischemic period of thirty minutes or more was a predictor for graft failure (RR 4.9 (p=0.002)).

Conclusion: Kidneys from NHB donors are an important addition to the organ pool. The incidence of graft failure within three months is significantly higher in recipients of kidneys from NHB donors. A subset analysis shows that graft survival in the NHB group is jeopardized by a warm ischemic period of 30 minutes or more. Shortening of this period should improve the survival rate of the kidneys of NHB donors.

Abstract# 604 Poster Board #-Session: P60-II

UPDATE ON ORGAN DONATION AFTER CARDIOPULMONARY DEATH USING EXTRACORPOREAL MEMBRANE OXYGENATION SUPPORT. Juan D. Arenas, Mark T. Gravel, Richard S. Chenault, John C. Magee, Randy S. Sung, Shawn J. Pelletier, Meelie A. Debroy, Jeffrey D. Punch. *Department of General Surgery, Division of Transplantation, University of Michigan, Ann Arbor, MI.*

BACKGROUND: Organ donation following cardiac death (DCD), continues to grow as a an alternative method to bridge the gap between organ supply and demand. However, most centers are still reluctant to use such organs, due to the high incidence of delayed or primary non-function (PNF) inherent to organ ischemia. We report on the use of extracorporeal membrane oxygenation (ECMO) to limit prolonged circulatory arrest prior to cold preservation.

METHODS: Since 1999 we have utilized (ECMO) in controlled DCD, to support organ perfusion in the absence of cardiac activity after declaration of death. ECMO flow rates are maintained at = 2 lts/min. PH at =7.1 = 7.4, arterial saturation > 80% and ACT > 500 sec.

RESULTS: Between October 2000 and August 2003 a total of 17 patients underwent pre-mortem cannulation and withdrawal of ventilatory support. Mean donor age was 29 years (9-53). Cardiopulmonary death occurred at a mean of 17.9 mins (5-50) after withdrawal and ECMO support was used an average 103 mins (73-121). Six of 26 kidneys were not subsequently transplanted due to serology results and poor perfusion parameters. One of 5 livers procured was not used due to recipient complications. Kidney recipient's age averaged 43.7 years (6-67), delayed graft function (DGF) occurred in 11% (2/19), 1 kidney was lost early due to technical complications, with all remaining kidneys functioning normally. Liver recipients age averaged 52.7 years (41-59), all grafts function immediately, one patient died 125 days post-op due to sepsis. All 3 remaining patients are alive and well, 2 with normal liver functions and 1 with resolving cholestasis at mean follow-up 14 months (2-24).

CONCLUSIONS: ECMO supported DCD permits controlled withdrawal of support in the intensive care unit without compromising organ viability. The low rate of DGF and the absence of PNF, suggest that ECMO prevents organ damage prior to cold preservation. The positive experience has prompted us to use ECMO in other areas of organ donation including the support of unstable donors that are brain dead. In addition, we are developing protocols to initiate ECMO supported DCD programs at other institutions in our region. Although the size of the study is limited, these data suggest the use of ECMO will significantly decrease the rates of DGF/PNF when compared to other results reported in the literature.

Abstract# 605 Poster Board #-Session: P61-II

ROLE OF RECOMBINANT PLASMINOGEN ACTIVATOR IN KIDNEYS WITH DISSEMINATED INTRA VASCULAR COAGULATION. Dai D. Nghiem,[1] Peter R. Olson,[2] Kalathil K. Sureshkumar,[3] Richard J. Marcus.[3] *[1]Dept of Surgery, Transplantation Services, Allegheny General Hospital, Pittsburgh, PA; [2]Dept of Pathology, Allegheny General Hospital, Pittsburgh, PA; [3]Division of Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

As the number of patients far exceeds the number of organs, it becomes imperative to use kidneys with DIC.

Methods: Since January 2000 four cadaver donors with DIC gave 8 kidneys. They averaged 31.5 years of age, with admission serum creatinine 1.0 mg, last 24 hours urine output 1050cc. The cause of death was CVA (3) and GSW to the head (1). Wedge biopsy performed on all kidneys showed extensive glomerular thrombi, but no cortical necrosis. Kidneys were then flushed with 20mg of Activase®, a recombinant plasminogen activator (tPA) and stored in slushed ice for 40 minutes. They were reflushed with Viaspan®. All but one were rebiopsied prior to transplantation. CIT averaged 26.5 hours. Simultaneous CSA, MMF and Prednisone therapy was used. Methylprednisolone was given for rejection.

Results: All repeated biopsies showed complete resolution of fibrin thrombi. No recipient developed post operative bleeding or primary non-function. One kidney, which did not undergo post tPA biopsy showed no fibrin thrombi on biopsy performed 20 days later for rejection. One kidney clotted on the 10th day to rejection. Three patients had ATN, for an incidence of 37.5%. Current serum creatinine averages 1.8 mg after 14-38 months of follow up. GS is 87.5%

Conclusion: Back table intra arterial tPA flush lyses rapidly microthrombi in kidneys with DIC and makes them successfully transplantable. Pre treatment with tPA should be added to the therapeutic armamentarium of kidneys with DIC.

Abstract# 606 Poster Board #-Session: P62-II

CADAVERIC RENAL TRANSPLANTATION AND EXPANDED CRITERIA DONORS: SHOULD WE REASSESS THEIR USE? Jean Tchervenkov,[1] Marcelo Cantarovich,[2] Steven Paraskevas,[1] Myriam Fernandez,[1] Dana Baran,[2] Roman Mangel,[2] Jeffrey Schiff,[2] Marc Lipman,[2] Peter Metrakos.[1] *[1]Surgery, McGill University Health Center, Montreal, QC, Canada; [2]Medicine, Mcgill University Health Center, Montreal, QC, Canada.*

Purpose: Renal Transplant waiting lists continue to grow. The use of expanded criteria cadaveric donors may increase the donor pool. Since 1997 we have liberalized our cadaveric donor criteria and assessed our results.

Methods: Between Jan 1,1997 to Sept 30, 2003 we performed 172 cadaveric renal transplants using induction with Thymoglobulin (Thy), Mycophenolate Mofetil (MMF) and Prednisone. Tacrolimus (TAC) (80%), or Cyclosporine Neoral (CsA) (20%) were introduced only when serum creatinine was < 3.0mg/dl. We divided the patients into 2 groups according to the scoring system described by Nyberg et al. (Am J Transplant 2003;2;3:715) which was donor age, history of hypertension, donor creatinine clearance, cause of death, and HLA mismatch. Group 1 (optimal donors) had a score of 0-19 and group 2 (expanded criteria donors) had a score of 20-39. We compared the 2 groups for graft survival, acute rejection rate (ACR), creatinine at 1 yr, delayed graft function (DGF, hemodialysis or creatinine>3.0 mg/dl on day 5).

Results:

Overall, graft survival for all 172 patients was 92.2 and 81.8% at 1 & 5 yrs. Patients in Group 2 had a higher rate of DGF and a higher creatinine at 1 yr, however graft survival at 5 yrs was still relatively good (78 vs 83% p=ns). Patients with DGF (n=61) had a lower 1 and 5 yr graft survival compared to patients without DGF (86 vs 98 % and 72 vs 88% p<0.004) irrespective of the group they belong to. DGF had an equally bad impact on "ideal" donors and "extended criteria" donors. All patients with DGF had a renal biopsy in the 1st 4 wks after transplant and none had rejection.

Conclusion: A protocol with Thymoglobulin, MMF, Predinisone and TAC or CsA results in very low acute rejection rate and excellent graft survival. Expanded criteria donors had a higher risk for DGF. DGF resulted in poorer 5 yr graft survival in both groups. Expanded criteria donors without DGF had an excellent 5 yr graft survival. Expanded criteria donors should be used, and strategies to minimize DGF should result in excellent graft survival.

Results

	%Graft Survival 1 yr / 5 yr	Median (mg/dl) Creatinine at 1yr (range)	ACR (%)	Median Donor age	DGF (%)	No DGF graft survival % 1 yr / 5 yr	DGF graft survival % 1 yr / 5 yr
Gr 1 n=80	92 / 83.4	1.2 (.6-5.7)	4 (5.6)	33.5 (5-58)	25 (31)	96 / 89	86 / 71
Gr 2 n=92	91.8 / 78	1.67 (.9-8.2)	5 (6.1)	59 (41-77)	46 (50)	94 / 81	87 / 72
P. Value	NS	.03	NS	.02	.013	.07	NS

Abstract# 607 Poster Board #-Session: P63-II

OUTCOME OF RENAL TRANSPLANTS FROM PEDIATRIC DONORS <5 YEARS OF AGE. Rafik A. El-Sabrout,[1] Veronica A. Delaney,[1] Linda E. Bonini,[1] Pat E. Hanson,[1] Shafiq Cheema,[1] Khalid M. H. Butt.[1] *[1]Transplantation/Vascular Surgery, New York Medical College, Valhalla, NY.*

Aim: Outcome of renal transplants from donors <5 years old has traditionally been inferior to that from older donors. Inadequate renal mass, vascular catastrophes, and acute rejection have been the main obstacles to wider application. We retrospectively studied our overall experience with patients who received renal transplants from such young donors to determine the utility of these organs.

Patients: 105 patients received transplants from donors <5 years of age between September 1991, and July, 2003, and were followed-up for a mean of 35 months (range, 5-145). Patients were divided into 4 groups based on wether they received single or en-bloc kidneys, and wether donors were </= 2 years or 2-5 years (table I). Patients in groups I and II were transplanted subsequent to 1996, and were maintained on either tacrolimus and sirolimus combination with antibody induction (group I), or neoral and cellcept combination with antibody induction (group II).

Results: Patient demographics (age, race, primary vs subsequent transplants, cold ischemia time, number of HLA mismatches, and panel reactive antibody percentage) were not significantly different between the various groups. On the other hand, recipients of single kidneys had smaller body mass index (BMI), were more commonly femlaes, and tended to be older, than recipients of en-bloc kidneys. However, the differences were not significant.

Conclusions: Pediatric donor kidneys are an invaluable source for transplantation. Single use of such kidneys is feasible, and can expand the donor pool. Careful attention to surgical technique is crucial to minimize graft loss. Newer immunosuppressive agents including tacrolimus and sirolimus, in combination with antibody induction is indicated to reduce the detrimental effect of acute rejection on graft growth and function.

	group I single <2 y n=24	group II single 2-5 y n=23	group III en-bloc <2 y n=36	group IV en-bloc 2-5 y n=22
1 year patient survival	96%	91%	86%	84%
1 year graft survival	92%	78%	69%	82%

Abstract# 608 Poster Board #-Session: P64-II

RISK FACTORS FOR DEATH ON THE KIDNEY WAITING LIST. Maureen A. McBride,[1] Francis L. Delmonico,[2] H. Myron Kauffman.[1] *[1]Research Department, United Network for Organ Sharing, Richmond, VA; [2]Renal Transplantation, Massachusetts General Hospital, Boston, MA.*

Background. As the kidney waiting list continues to grow, median waiting times (MWT) have become progressively longer and are now greater than four years. Currently, any patient with a creatinine clearance < 80 ml/min and no absolute contraindication (e.g., various active infections or current malignancy) may be placed on the waiting list. Since a longer duration of dialysis adversely affects post-transplant graft and patient survival, we examined the medical characteristics and mortality of candidates waiting on the list to assess the profile of comorbidities of the patient who has died waiting on the list.

Methods. All adult primary kidney alone registrations added to the OPTN/UNOS waiting list (N = 62,470) between 1/1/97 and 12/31/00 were evaluated. Patients were censored at the time of transplant or removal, or on October 31, 2003. Deaths occurring within 180 days of removal from the list for reasons other than transplant or death were considered waiting list deaths. Death rates were calculated and a multivariate Cox regression model was used to estimate relative risks (RR) of death for various combinations of recipient risk factors. Four groups of patients were analyzed: age 18-59 (N = 9,729) with no dialysis, age 18-59 (N= 39,997) on dialysis, age≥60 with no dialysis (N = 2,036), age≥60 on dialysis (N = 10,708).

Results. Actual mortality for patients age 18-59 with no dialysis was 7.9% and with dialysis was 14.3%. The mortalities for patients age≥60 with no dialysis were 16.1% and with dialysis, 24.3%. Additional comorbidities increased mortality as shown in the table below for patients age≥60. Relative risks are shown relative to patients aged 18-59 who were not on dialysis and without any of the listed comorbidities.

Patients Age ≥ 60

Comorbidities	Actual Mortality Rate	RR (p-value)
Dialysis alone	24.3%	3.136 (p<0.0001)
Dialysis & Previous Malignancy	27.1%	3.502 (p<0.0001)
DIalysis & Angina	29.0%	3.752 (p<0.0001)
Dialysis & COPD	29.1%	3.582 (p<0.0001)
Dialysis & Diabetes	29.6%	5.050 (p<0.0001)
Dialysis & Peripheral Vascular Disease	34.3%	4.084 (p<0.0001)

Conclusions. Most wait list deaths occur in a setting of multiple comorbidities. Risk factors such as peripheral vascular disease are associated with significantly increased mortality for candidates on the kidney waiting list. Various comorbidities associated with an increased rate of death on the list may be indicative of a high risk or unsuitable candidate for transplantation.

Abstract# 609 Poster Board #-Session: P65-II

FACTORS ASSOCIATED WITH SURVIVAL OF KIDNEYS FROM NON-HEART-BEATING DONORS. P. Abt,[1] A. Frank,[1] G. R. Stephenson,[1] M. Sellers.[1] *[1]Surgery, University of Pennsylvania, Philadelphia, PA.*

Introduction: Renal allografts from non-heart-beating donors (NHBD) demonstrate similar long term survival to those obtained from brain dead donors; however, variables influencing graft outcome have not been well defined. We examined the relationship of donor-, recipient-, and center-related covariates with graft failure. Methods: The UNOS database was queried to identify NHBD kidney pairs procured between 1993 and 2001. Donors were grouped according to the following: both functioning at last follow-up (Group 1), one kidney of a pair had failed (Group 2), and both kidneys had failed (Group 3). Factors influencing graft survival were identified by chi-square, t-test, and ANOVA as appropriate. A subanalysis in Group 2 was performed to control for donor-related variables. Results: Overall graft survival in 840 recipients at 1 and 5 years was 89.% and 74.5%, respectively. Donors from group 1 (N=294), Group 2 (N=109), and Group 3 (N = 17) were similar in age, gender, and race and had similar warm ischemic times. Cold ischemic time was the only factor different between the groups and increased incrementally from Group 1 (22 hours) to Group 3 (29 hours, P = 0.004). The only recipient factor that predicted graft failure within Goup 2 was pre-transplant PRA (18.5% vs. 9.8%, P=0.007). Conclusions: NHBDs provide a valuable resource with acceptable outcomes. These results can be improved further with efforts to minimize cold ischemia and with increased utilization in less sensitized recipients.

EXPERIMENTAL TOLERANCE INDUCTION I

Abstract# 610 Poster Board #-Session: P66-II

MOBILIZED PERIPHERAL BLOOD STEM CELLS SHOW LOWER TOLEROGENIC POTENTIAL THAN BONE MARROW CELLS IN RECIPIENTS OF COSTIMULATION BLOCKADE. Zvonimir Koporc,[1] Sinda Bigenzahn,[1] Peter Blaha,[1] Friedrich Wrba,[2] Ferdinand Muehlbacher,[1] Megan Sykes,[3] Thomas Wekerle.[1] *[1]Div. of Transplantation, Vienna General Hospital/Univ. of Vienna, Austria; [2]Inst. of Clinical Pathology, Vienna General Hospital/Univ. of Vienna, Austria; [3]Transplantation Biology Research Center, Massachusetts General Hospital/Harvard Medical School.*

Background: Mixed chimerism and tolerance can be induced without cytoreduction through transplantation of 200 million bone marrow cells (BMC) under costimulation blockade. Since such high numbers of BMC are clinically not feasible, we substituted mobilized peripheral blood stem cells (mPBSC) for BMC.

Materials and methods: (1) Groups (n=3-9) of B6 mice received increasing numbers of Balb/c mPBSC (20, 75 or 200x10*6, d0), non-myeloablative total body irradiation (TBI; 0 to 3Gy, d-1) and anti-CD154 mAb (1mg, d0) plus CTLA4Ig (0.5mg, d+2). (2) One group received a mixture of 20x10*6 BMC and 60x10*6 mPBSC (with 3Gy TBI, anti-CD154 and CTLA4Ig). (3) One group received 200x10*6 mPBSC with an intensified conditioning regimen (3Gy TBI, donor-specific transfusion under anti-CD154 [d-6], immunosuppression [d0-d27; rapamycin plus methylprednisolone plus mycophenolate mofetil], increased doses of anti-CD154 [total 4mg] and CTLA4Ig [total 2.5mg]).

Results: Unexpectedly, no mouse in the groups transplanted with up to 200x10*6 mPBSC after non-myeloablative TBI developed long-term chimerism under standard doses of costimulation blockade. After co-injection of mPBSC together with BMC under costimulation blockade, donor cells were not detected by flow-cytometry even as early as 1 week after transplantation, while all mice of a control group transplanted with BMC only developed chimerism. Recipients of mPBSC plus BMC demonstrated preserved donor-reactivity in MLR assays at 7 weeks (mean stimulation index 2.4 vs. 0.7 for control group, p<0.001). The transplantation of 200x10*6 mPBSC under an intensified conditioning regimen led to high levels of chimerism in 7 of 7 mice (85% CD4 and 52% B cell chimerism at week 1) which persisted long-term (23 weeks). 5 of 7 mice showed donor-specific tolerance by accepting donor skin grafts (MST>44d) but promptly rejecting third party grafts.

Conclusion: Doses of costimulation blockers and TBI allowing chimerism with allogeneic BMC are not sufficient to prevent the rejection of mPBSC. mPBSC appear to contain a deleterious APC as they trigger rejection of BMC when transplanted in combination. The lower tolerogenic potential of mPBSC in recipients of costimulation blockade warrants consideration in the development of (pre-)clinical tolerance protocols employing mPBSC.

Abstract# 611 Poster Board #-Session: P67-II

INDIRECT ANTIGEN PRESENTATION BY LIVER SINUSOIDAL ENDOTHELIAL CELLS CONTRIBUTES TO ALLOREACTIVE T CELL TOLERANCE INDUCED BY PORTAL INJECTION OF DONOR SPLENOCYTES. Daisuke Tokita,[1] Hideki Ohdan,[1] Toshimasa Asahara.[1] *[1]Department of Surgery, Division of Frontier Medical Science, Hiroshima University, Hiroshima, Hiroshima, Japan.*

Although it has been well known that portal injection (PI) of donor cells leads to tolerance to allografts, the mechanism has not been fully understood. The difficulty in determining the precise mechanisms may be due to a difference between tolerizing mechanisms in T cells with direct allospecificity and those with indirect allospecificity. In the present study, we have demonstrated that PI of class II-deficient donor splenocytes led to indefinite acceptance of subsequently transplanted donor-type heart allografts. This finding indicates that PI of donor cells efficiently induces tolerance at least among T cells with indirect allospecificity. PKH-26-labeled B6 splenocytes ($30x10^6$ cells/mouse) that were treated with 30-Gy irradiation were injected via the portal vein into Balb/c mice, so that host antigen presenting cells that had taken up the injected splenocytes could be identified as cells that have PKH-26 labeling. At 12 hours after PI, approximately 60% of host liver sinusoidal endothelial cells (LSECs) cells had taken up PKH-26-labeled splenocytes. FCM analyses revealed that naive LSECs were MHC class II^+, $CD40^+$, $CD80^+$ and $CD86^+$ phenotypes that are consistent with those of antigen presenting cells (APCs). In contrast, LSECs capturing allogeneic splenocytes lost CD40 expression and inductively expressed Fas-ligand on their surface. To test tolerizing capacity of host LSECs capturing allogeneic splenocytes toward T cells with indirect allospecificity, we examined the effect of antigen presentation by LSECs to naive T cells on the responsiveness of those T cells to subsequent antigen presentation by professional APCs. Balb/c mouse splenocytes first underwent transmigration across the LSECs from Balb/c mice that had been treated with PI of either naïve or irradiated allogeneic B6 splenocytes, enabling direct interaction between T cells and LSECs. The transmigrated T cells, which were predominantly $CD4^+$ T cells, were subsequently stimulated with splenic APCs from Balb/c mice that had been stimulated with intravenous injection of B6 splenocytes. The proliferative response of $CD4^+$ T cells that had transmigrated across the LSECs from mice that had been treated with PI of irradiated splenocytes was significantly reduced compared with that of LSECs from untreated mice. As far as we know, these are the first demonstration that indirect antigen presentation by LSECs contributes to alloreactive T cell tolerance induced by portal injection of donor splenocytes.

Abstract# 612 Poster Board #-Session: P68-II

VIRAL IL-10 GENE THERAPY VIA AUTOLOGOUS HSC PRIOR TO ORGAN TRANSPLANTATION CAN PREVENT ALLOGRAFT REJECTION. Shashikumar Salgar,[1] Dinghua Yang,[1] Phillip Ruiz,[2] Joshua Miller,[1] Andreas Tzakis.[1] *[1]Surgery; [2]Pathology, University of Miami, Miami, FL.*

Background: Viral interleukin-10 (vIL-10) can impair antigen presenting cell function. In this study, a novel gene therapy approach to induce transplant tolerance was designed. **Materials and Methods:** The vIL-10 (BCRF1) coding sequence from Epstein-Barr virus genome was cloned into pMSCVneo a retroviral vector, and packaged in PT67 cells. Murine hematopoietic stem cells (HSC; Lin^-) isolated from bone marrow were cultured in serum-free medium containing SCF and IL-3. HSC were transduced with *rv*IL-10-retrovirus. Autologous (syngeneic) vIL-10 transduced HSC were injected into lethally (950 cGy) or sub-lethally (400 cGy) irradiated CBA/J mice. After 6 weeks, vascular heterotopic allogeneic heart (C57BL/6) transplantation (Tx) was performed. **Results:** HSC enriched fraction (Lin^-) constituted ~30% $CD34^+$ and ~41% $Sca\text{-}1^+$ cells. *Ex vivo,* vIL-10 production was 2-8 ng/ml of culture supernatant. *In vivo,* serum vIL-10 was 0-750 pg/ml (187±205 pg/ml) during 3-10 weeks after vIL-10-HSC administration. Cardiac allograft survival was prolonged ($P<0.004$) in lethally (71 ±40 days; n=8) and sub-lethally (114 ± 15 days; n=3) irradiated mice that received autologous vIL-10-HSC compared to controls that received unengineered (UE) HSC or vector-DNA-HSC (12-16 days; n=6). However, cardiac allograft survival was 22±13 days (n=5) in sub-lethally irradiated mice (CBA/J) that received donor (C57BL/6) derived vIL-10-HSC compared to controls that received donor derived vector-DNA-HSC (12 ± 2 days; n=3). Further, in lethally irradiated CBA/J mice that received autologous vIL-10-HSC, allogeneic (C57BL/6) skin graft survival were marginally prolonged (13.3 ± 0.6 days; n=3) compared to vector-DNA-HSC administered controls (11.5 ± 0.7 days; n=2). Secondary skin graft survival in primary graft (heart) tolerant animal (>100 days) lasted for only 16 days. In autologous vIL-10-HSC administered group, histopathology demonstrated mild arteritis/venulitis (grade 0.7) and mild acute cellular rejection (grade 1.00). Intragraft expression of co-stimulatory molecules (CD80, CD86), cytokines (IL2, IL4, mIL10, IFNγ), and iNOS molecules was markedly lower in tolerant grafts (vIL-10-HSC treated) compared to rejected grafts (vector-DNA-HSC or UE-HSC treated). Further, T lymphocytes derived from vIL-10-HSC treated graft tolerant recipient demonstrated hyporeactivity to donor and 3rd party antigens in MLR cultures. **Conclusion:** Administration of autologous vIL-10 engineered HSC prior to organ Tx prolonged cardiac allograft survival significantly.

Abstract# 613 Poster Board #-Session: P69-II

DIFFERENTIATION OF $CD25^-$ REGULATORY T CELLS IN LONG TERM SURVIVORS RATS FOLLOWING DONOR-SPECIFIC TRANSFUSION (DST) IN RATS. Nicolas Degauque,[1] David Lair,[1] Cecile Braudeau,[1] Alexandre Dupont,[1] Fabienne Haspot,[1] Fabien Sebille,[1] Sophie Brouard,[1] Jean-Paul Soulillou.[1] *[1]U.437, INSERM, Nantes, France.*

Background. Induction of specific tolerance for donor antigens is one of the most actively explored field in transplantation immunology.In adult rats, long term survival (LTS) of MHC incompatible heart can be obtained by priming the recipients with donor specific transfusion (DST) 14 and 7 days before transplantation.In this study, we analyzed the mechanisms of maintenance of tolerance in LTS rats by focusing on *in vitro* regulatory patterns of splenic T cells harvested from LTS rats.

Materials & Methods. Direct-pathway type Mixed Lymphocyte Reaction (MLR) were performed by culturing irradiated enriched allogeneic LEW.1W ($RT1^u$) dendritic cells with purified T lymphocytes from naive or LTS rats LEW.1A ($RT1^A$).Inhibition assays were performed by adding to this readout system purified CD25- or purified CD4+CD25- or purified CD8+CD25- T cells from LTS rats.Transwell culture were used to investigate the need of cell-cell contact.In addition, the effect of IL2 addition on these MLR as well as anti-IL10, anti-TGFb antibodies and IDO and iNOS inhibitors were tested.Finally, Complementary Determinant Region 3 – length distribution (CDR3-LD) was analyzed using Immunoscope.

Results. CD25- T cells from LTS rats exhibit a reduced proliferation following direct-allostimulation compared to naïve CD25- T cells.This hyporesponsivness could be bypassed by addition of IL2.In MLR, CD25- T cells from LTS rats were able to inhibit proliferative response of naive alloreactive CD25- T cells.Addition of IL2 to this coculture also restored proliferative response.In contrast, when CD4+CD25- or CD8+CD25- T cells from LTS were independantly tested, no inhibition of the MLR was detected.Thus, both subsets are needed for optimal proliferation inhibition.Inhibitory property of CD25- T cells from LTS did not involve soluble factors (IL10, TGFb, IDO and iNOS) but required cell-cell contact as shown by the transwell culture system.Finally, analysis of regulatory CD25- T cells repertoire of LTS rats did not reveal alteration of the CDR3-LD.Thus we could not associate the appearance of clonal selection and regulatory properties in the differentiated CD25- subset in LTS rats.

Conclusion. This study provides the first evidence of existence of dominant regulatory function in the CD25- T cells subset in long term survivors rats following DST.Functionnal *in vitro* regulatory properties may be in part responsible of maintenance of heart graft acceptance in this model.

Abstract# 614 Poster Board #-Session: P70-II

THE ROLE OF B CELLS IN TOLERENCE INDUCED BY ANTI-CD45RB. Muhammad M. Mohiuddin,[1] Daniel J. Moore,[1] Xiaolun Huang,[1] Ergun Velidedeoglu,[1] Moh M. Lian,[1] Major K. Lee,[1] Yong Suk Bae,[1] Adam M. Frank,[1] Meredith Chiaccio,[1] Haiying Chen,[1] James F. Markmann,[1] Shaoping Deng.[1] *[1]Surgery, University of Pennsylvania, Philadelphia, PA.*

Background: We have demonstrated previously that tolerance to cardiac allografts can be induced by recipient treatment with anti-CD45RB antibody and that this tolerance is uniquely dependent on the presence of the host thymus and B-lymphocytes. In this study, we evaluated the role of B-lymphocytes in two separate models of tolerance. **Methods:** In our first model, B cell deficient B6-mMT recipients were reconstituted with purified B cells from normal C57Bl/6 mice. This model was used to track B cells, to monitor their proliferation and to determine their role in graft survival. B6-HEL mice (in which >90% of B cells are HEL specific) were employed as recipients to assess the functional requirement of B cells for tolerance induction. To further understand the role of B cells, we used a B cell deficient TCR transgenic system ($TS1/JH^{-/-}$) in which we can study the response to a specific antigen (HA). Transplanted mice were treated with 5 doses of 100 mg anti-CD45RB. For trafficking studies, injected B cells were labeled with CFSE. **Results:** B6-mMT mice rejected C3H hearts without B cell reconstitution (MST = 9 days; n=6). Anti CD45 treatment alone slightly prolonged the survival but did not induce tolerance (MST = 14 days; n=10). Indefinite survival was obtained when normal B6 B cells were supplied (MST = >100 days; n=10). In tolerant animals, infused B cells were detected at all time points (to >100 days), found in all lymphoid organs including the thymus, and revealed evidence of homeostatic proliferation. That the surviving B cells were of the B cell donor and not derived from graft passenger cells was confirmed by class I labeling. C3H hearts in HEL transgenic mice were promptly rejected (MST = 12 days; n =2). $TS1\ JH^{+/+}$ mice treated with anti CD45RB accepted HA^+ grafts indefinitely (MST= >100 days; n=18). In contrast, $TS1/\ JH^{-/-}$ mice acutely rejected HA^+ grafts (MST = 12 days; n=16) and anti-CD45RB therapy did not prolong survival beyond MST = 30 days (n=6). **Conclusion**: Our results demonstrate conclusively that B cells are necessary for anti CD45RB induced transplantation tolerance. Also, transferred B cells provided stable reconstitution and were functionally active. Tolerance is not induced in B6-HEL transgenic mice indicating a possible role for cognate antigen presentation or of protective donor-specific antibodies in this system. These findings define a unique role for B cells in anti-CD45RB induced tolerance.

Abstract# 615 Poster Board #-Session: P71-II

THE ROLE OF CTLA-4 ENGAGEMENT IN THE INDUCTION OF PROLONGED ALLOGRAFT SURVIVAL BY COSTIMULATION BLOCKADE. Luuk B. Hilbrands,[2] Jeroen J. A. Coenen,[1] Hans J. P. M. Koenen,[1] Esther van Rijssen,[1] Irma Joosten.[1] *[1]Dept. of Bloodtransfusion and Transplantation Immunology, UMC Nijmegen, Netherlands; [2]Dept. of Nephrology, UMC Nijmegen, Netherlands.*

Costimulatory blockade is a promising strategy for the induction of transplantation tolerance. Previously, we observed that costimulation blockade induced functional dominance of regulatory $CD4^+CD25^+$ T cells by reduction of the alloreactive effector pool *in vitro*. It can therefore be hypothesized that regulatory $CD4^+CD25^+$ T cells play an active role in the induction of tolerance by costimulation blockade *in vivo*. Regulatory $CD4^+CD25^+$ T cells (Treg) constitutively express CTLA-4, but considerable controversy still exists about the importance of engagement of CTLA-4 in the activation of $CD4^+CD25^+$ regulatory T cells. *In vivo*, we found that additional anti-CTLA-4 antibody (4F10) abrogated prolonged cardiac allograft survival by anti-CD40L and anti-CD86 over a full MHC-mismatch (MST 36 days vs. MST > 90 days respectively). In *in vitro* MLR we examined the role of CTLA-4 engagement and Treg in this phenomenon. It was observed that anti-CD40L and anti-CD86 resulted in profound inhibition of the alloresponse in MLR (>80% inhibition), whereas additional anti-CTLA-4 partially abrogated this inhibition (<50% inhibition). Subsequently, responder cells were depleted for Treg at the start of MLR. Depletion of Treg impaired the inhibition by anti-CD40L and anti-CD86 to a similar degree as was observed for the addition of anti-CTLA-4. After depletion of Treg, additional treatment with anti-CTLA-4 had no further effect, which suggests an effect of anti-CTLA-4 primarily on Treg. We propose that anti-CD40L and anti-CD86 facilitate immunoregulation by regulatory $CD4^+CD25^+$ T cells *in vivo* and that engagement of CTLA-4 of these cells is pivotal for their activation. Strategies that are aimed to enhance CTLA-4 signalling of Treg may be useful for immunoregulation in transplantation and auto-immune diseases.

Abstract# 616 Poster Board #-Session: P72-II

SENSITIZATION VS. ANGERY/REGULATION: A COMPARISON OF THE IMPACT OF EXPOSURE TO NON-INHERITED MATERNAL ANTIGENS (NIMA) IN FOUR MOUSE MODELS. J. Andrassy,[2] M. L. Molitor,[1] B. R. Marthaler,[1] L. D. Haynes,[1] K. W. Jauch,[2] H. W. Sollinger,[1] W. J. Burlingham.[1] *[1]Surgery, Transplant Division, University of Wisconsin, Madison, WI; [2]Surgery, Ludwig-Maximilians-University, GH, Munich, Germany.*

Background: Recently, we were able to replicate the clinical NIMA-effect in a mouse model (JI 2003; 171:5554). Offspring (*bxb*) from breedings of a B6 (*bxb*) male and a B6D2F1 (*bxd*) female are exposed to mothers $H\text{-}2^d$ antigens. When transplanted with fully allogeneic hearts from DBA/2 (dxd), a > 50% tolerance rate is observed, while control offspring from NIPA-breedings of a B6D2F1 father and a B6 mother uniformly reject (MST = 10d).

Hypothesis: The NIMA-effect is dependent on: 1) the degree of CD4+ and CD8+ T cell anergy induced via the direct pathway, and 2) the development of CD4+ T regulatory cells specific for NIMA induced via the indirect pathway. The strain background is likely to influence these 2 parameters of the allo-immune response of the offspring.

Methods: Four NIMA + NIPA control breedings were initiated (Table). Only homozygous bxb or kxk offspring were transplanted heterotopically with fully allogeneic hearts from DBA/2, C3H or B6 to test the impact of NIMA d, k, or b exposure; GST > 100d was considered tolerant. T-cell function was analyzed by *in vivo* proliferation of CFSE stained cells in semi-allogeneic hosts and by measurement of cytokine producing T cells in MLC responses using ELISpot. ***Results:*** An *in vivo* NIMA effect was seen only in the two NIMAd models (Table). CFSE-analysis of CD4 and CD8 T cells from NIMAd1 (bxb) mice showed a significantly reduced proliferation compared with NIPA. A decrease of IFN-g and IL-2 producing T cells and an increase of IL-10 producing T cells in response to fully- and semi-allogeneic stimulators was observed. In contrast, NIMAk exposed T cell responses were only reduced slightly in bxb offspring, and NIMAb mice were sensitized to NIMA, as indicated by significantly higher pre-transplant IFN-g alloresponses. ***Conclusion:*** Only the exposure to NIMAd was able to induce tolerance. The impact of the NIMA effect seems to be dependent on the balance between sensitization of NIMA-specific effector T cells, on the one hand, and induction of NIMA-specific anergic and regulatory T cells, on the other.

NIMA-models and transplant data

	Male	Female	Transplant	NIMA Tolerance Rate	NIPA Tolerance Rate	P Value
NIMA d1	B6 (bxb)	B6D2F1 (bxd)	DBA/2	57% (11/21)	0% (0/7)	<.004
NIMA d2	C3H (kxk)	C3D2F1 (dxk)	DBA/2	50% (5/10)	16% (1/6)	<.05
NIMA k	B6 (bxb)	B6C3F1 (bxk)	C3H	0% (0/7)	0% (0/5)	NS
NIMA b	C3H (kxk)	B6C3F1 (bxk)	B6	0% (0/8)	0% (0/4)	NS

Abstract# 617 Poster Board #-Session: P73-II

PERIPHERAL CD4 T CELL TOLERANCE INDUCED BY GENE THERAPY. Daron Forman,[1] Eun-Suk Kang,[1] Chaorui Tian,[1] John Iacomini.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

Background:

Reconstitution of lethally irradiated B10.AKM ($H\text{-}2K^k$) mice with syngeneic bone marrow cells infected with retroviruses carrying the allogeneic MHC class I gene $H\text{-}2K^b$ resulted in stable and lifelong expression of K^b on bone marrow-derived cells. More importantly, these mice were specifically tolerant to $H\text{-}2K^b$ skin grafts while still retaining the ability to reject third party skin grafts. Using CD8 transgenic mice, we discovered that alloreactive CD8 T cells underwent negative selection in the thymus, leading to the absence of these potentially alloreactive T cells in the periphery

Methods:

Tg361 CD4 transgenic mice expressing a TCR specific for the MHC class I gene $H\text{-}2K^b$ were utilized to determine the mechanism by which genetic engineering of bone marrow induces donor specific CD4 T cell tolerance after bone marrow transplantation. Lethally irradiated B10.AKM mice were reconstituted with a 6:1 ratio of mock or K^b-transduced B10.AKM bone marrow to Tg361 bone marrow.

Results:

Lethally irradiated recipients that received CD4 transgenic bone marrow in combination with autologous bone marrow transduced with virus encoding $H\text{-}2K^b$ displayed stable and long-term expression of K^b on bone marrow-derived cells. Alloreactive CD4 transgenic T cells were readily detectable in the peripheral blood, spleen and thymus of chimeric recipients; although their levels were 2-fold lower than mock-transduced bone marrow recipients. Despite the presence of potentially alloreactive CD4 T cells in the periphery, chimeric recipients were specifically tolerant to $H\text{-}2K^b$ expressing skin grafts. Interestingly, chimeric mice which received K^b -transduced and CD4 transgenic bone marrow expressed CD25 on 20-30% of Tg361-derived CD4 T cells, compared to only 6% of Tg361-derived CD4 T cells in mock transduced controls.

Conclusions:

We conclude that genetic engineering of bone marrow induces donor specific CD4 T cell tolerance through deletional and non-deletional mechanisms. We further hypothesize that expression of K^b on bone marrow derived cells induces CD4 T cells capable of inhibiting alloreactive T cells.

Abstract# 618 Poster Board #-Session: P74-II

RATIONAL DESIGN OF DONOR DERIVED DENDRITIC CELLS FOR TOLERANCE INDUCTION. Kym R. Garrod,[1] Todd V. Brennan,[1] Sang-Mo Kang.[1] *[1]Surgery, Division of Transplantation, University of California, San Francisco, San Francisco, CA.*

Background: Accumulating evidence that dendritic cells (DC) are important regulators of peripheral immune tolerance has led to the concept that DC may be useful therapeutically. Engineered expression of immunomodulatory genes to create tolerogenic DC has led to promising results *in vitro*; however, *in vivo* results have been disappointing. A major obstacle in previous attempts to induce tolerance using genetically modified DC has been the relative inefficiency of previous gene transfer methods. In addition, the gene transfer methods themselves appear to trigger the maturation of DC, antagonizing the effects of immunomodulatory genes. Another problem in the field of DC immunotherapy is the relative paucity of information on how migration patterns of infused DC affect the subsequent immunologic outcome. We have sought to address these issues by 1) developing a robust method for producing pure populations of genetically modified, immature DC and 2) studying how enhanced lymphoid migration of infused DC affects the subsequent immune response.

Methods/Results: To circumvent DC maturation, cycling stem cells were transduced *prior* to differentiation into DC using an MLV-based retroviral vector, sorted, and placed into DC medium. Remarkably, up to 1×10^9 transduced DC can be generated from a single experiment, with 90-95% purity. Transduced DC were homogeneously immature by both functional and phenotypic analysis. Using this technique, we have expressed immunomodulatory genes such as vIL-10, IDO and PD-1L on DC and confirmed their ability to down-regulate antigen specific immune responses *in vitro* (MLR, cytokine production) and *in vivo* (DTH model). IV infusion of DC is known to result in poor migration to lymphoid compartments. We therefore tested whether engineered expression of the lymphoid homing receptor CCR7 could improve the migration of immature DC to lymph nodes and spleen. Using both histologic and FACS analysis, we have found a >50-fold increase in homing by DC transduced with CCR7, compared to controls. Analysis of CD4 T cell responses using an adoptive transfer model demonstrates enhanced interaction of CCR7 expressing DC with antigen specific T cells. We are currently studying the role of immunomodulatory genes and potential synergy of targeted DC delivery in various transplant models.

Conclusion: We have developed a method for the systematic manipulation of DC gene expression and homing. We hope to elucidate the determinants of immunity and tolerance in response to DC infusion, in order to develop a rational approach to DC based immunotherapy.

Abstract# 619 Poster Board #-Session: P75-II

THE ROLE OF HOST THYMIC FUNCTION IN THE INDUCTION OF TOLERANCE ACROSS FULL MHC BARRIERS BY FK506. Hanzhou Hong,[1] Kazuhiko Yamada,[1] Akira Shimizu,[1] Chisako Kamano,[1] Hitoshi Arakawa,[1] Emma Samelson-Jones,[1] Shannon Moran,[1] David H. Sachs,[1] Martin Hertl.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

Background: Previous studies have demonstrated the importance of an intact thymus in induction of tolerance to class I-mismatched renal allografts by Cyclosporin in miniature swine. A short course of FK506 has subsequently been shown to induce tolerance to fully mismatched renal allografts in this large animal model. In the present study, we assessed whether thymectomy likewise interferes with induction of this tolerance. **Methods:** Recipients of fully MHC disparate renal allografts were treated with a 12-day course of FK506 (0.15 mg/kg/day by continuous i.v. infusion), with doses adjusted to maintain trough levels of 30-50 ng/ml. Four experimental animals were thymectomized 21 days prior to transplantation, while two control animals remained euthymic. Tolerance was tested *in vivo* in animals with surviving grafts at day 100 by re-transplantation using another donor-matched kidney and no immunosuppression. **Results:** Both euthymic controls accepted their renal allografts for >100 days and accepted second grafts long-term with stable renal function. They also demonstrated donor-specific hypo-responsiveness in CML and MLR assays and showed no evidence of IgG or IgM deposition in kidney biopsies, confirming that they were tolerant. In contrast, all four thymectomized recipients developed evidence for acute and chronic rejection. The first animal rejected its graft on day 17, autopsy demonstrating a swollen and necrotic graft with patent vessels. The second animal maintained its kidney >100 days, but rejected a second donor-matched allograft acutely on day 20. The third animal was euthanized on day 99 due to chronic rejection with unstable renal function. The fourth animal accepted both its first and second renal allografts, but remnants of the native thymus were detected on autopsy, indicating incomplete thymectomy. *In vitro* assays of the first three (but not the fourth) animals demonstrated responsiveness to donor MHC by CML and MLR and both IgG and IgM deposition were found in the kidneys histologically. **Conclusion:** The host thymus is required for induction of transplant tolerance across a two-haplotype full MHC barrier by a short course of FK506, although even a small amount of thymic tissue may be sufficient to provide the required thymic function.

Abstract# 620 Poster Board #-Session: P76-II

BLOCKADE OF PD-1 LIGATION REVERSES INHIBITION OF T CELL RESPONSES BY HEPATIC STELLATE CELLS. Cheng-Hsu Chen,[1] Lina Lu,[1] John J. Fung,[1] Shiguang Qian.[1] *[1]Thomas E. Starzl Transplantation Institute, Surgery, University of Pittsburgh, Pittsburgh, PA.*

Liver transplant tolerance in mice is associated with activated T cell apoptosis in liver grafts, suggesting unusual interactions between the hepatic milieu and immune cells. Hepatic stellate cells (HSC) are known to actively participate in the fibrogenesis in liver disease, but little is known of the role in regulating immune responses. In this study, HSC were isolated from B10 ($H2^b$) livers, and cultured in plates for 2d (quiescent) or 7-10d (activated). We have demonstrated that activated HSC fail to stimulate allogeneic T cell (C3H, $H2^k$) proliferation, but the addition of activated HSC to a DC/T allogeneic MLR culture significantly inhibited T cell proliferative responses in a HSC dose dependent manner, which appeared to be related to HSC activation as quiescent HSC had no inhibitory activity. The inhibition was so powerful that 50% of suppression was achieved at T:activated HSC ratio of 1:40, and 90% inhibition at a ratio of 1:20. This inhibition was unlikely MHC restricted because HSC from C3H (syngeneic to T cells) also markedly suppressed T cell proliferative responses. The underlying mechanisms remain unclear. Quiescent HSC expressed very few surface molecules, while activation by culture in uncoated plastics induced strong expression of PDL-1, a ligand for PD-1. Expression of PDL-1 on activated HSC was enhanced by further stimulation, including IFN-γ and activated allogeneic T cells. To determine whether expression of PDL-1 on HSC plays a role in mediating the inhibition of T cells responses, we established a system in which proliferation of C3H T cells was triggered by anti-CD-3ε mAb. T cell proliferative responses were noticeably inhibited by the addition of activated HSC in a dose dependent fashion. Addition of the blocking anti-PDL-1 mAb (BioScience) to the culture reversed the HSC-induced inhibition of T cell proliferation when the Ab concentration was higher than 10 μg/ml. PDL-1 is a ligand of PD-1, a member of the CD28/CTLA4 family expressed on activated lymphoid cells. PD-1 contains an immunoreceptor tyrosine-based inhibitory motif. Mice deficient in PD-1 develop autoimmune disorders suggesting a defect in peripheral tolerance. Our data suggest that that activated HSC are capable to suppress T cell responses, which is, at least partially, mediated by PD-1 ligation between activated HSC and T cells. This may be an important mechanism involved in hepatic tolerance in mice.

Abstract# 621 Poster Board #-Session: P77-II

MECHANISTIC STUDIES ON T CELL APOPTOSIS TRIGGERED BY ALLOGENEIC LIVER $B220^+$ DENDRITIC CELLS. Xiaoyan Liang,[1] Lina Lu,[1] Lianfu Wang,[1] John J. Fung,[1] Shiguang Qian.[1] *[1]Thomas E. Starzl Transplantation Institute, Surgery, University of Pittsburgh, Pittsburgh, PA.*

Mouse liver allografts are spontaneously accepted, which is associated with activated T cell apoptosis. The underlying mechanisms are unclear. We have identified novel $B220^+CD205^+CD11c^-CD11b^-$ dendritic cells (DC) in mouse livers that were phenotypically mature, but stimulated poor [^{3}H]TdR incorporation in allogeneic T cells. However, cell cycle analysis indicated that proliferation of T cells stimulated by liver $B220^+$DC was similar to BM-derived myeloid DC. Low thymidine uptake was a result of extensive activated T cell apoptosis. ~30% of activated T cells were TUNEL positive in liver $B220^+$ DC group (evenly distributed in $CD4^+$ and $CD8^+$ populations), only <10% in myeloid DC group. In contrast to myeloid DC that exacerbated allograft rejection, administration of B10 ($H2^b$) liver $B220^+$ DC (2×10^6) dramatically prolonged survival of B10, but not third party (BALB/c; $H2^d$) heart allografts in C3H ($H2^k$) recipients (MST 37 days vs. 10 days in control and third party groups). This was associated with a higher incidence of apoptotic cells in draining lymph nodes and spleen. T cell apoptosis induced by *gld* (FasL deficient) $B220^+$ DC was reduced about 30%, indicating a partial role of Fas ligation. Liver $B220^+$ DC induced similar apoptosis in TNFR (either p55 or p75) deficient T cells, suggesting that TNF and lymphotoxin (LT)a may not be important ligands. We examined the role of LTβ, encouraged by a RNase protection assay result that expression of LTβ in liver $B220^+$ DC was extraordinary high, and found that liver $B220^+$ DC from $LT\beta^{-/-}$ mice were poor apoptosis inducers, and stimulated profound T cell proliferation, suggesting a critical role of LTβ in mediating the apoptosis. T cell thymidine uptake in a liver $B220^+$DC/T culture was markedly restored by addition of z-VAD-fmk, a common caspase inhibitor peptide, suggesting an involvement of caspase cascades. To determine the involved pathways, proteins were isolated from sorted T cells at different time point of culture, and incubated with substrates (AC-DEVE-AFC for caspase 3, AC-IETD-AFC for caspase 8 and AC-LEHD-AFC for caspase 9). The caspase activity determined by an OD value showed that liver $B220^+$DC, not myeloid DC, activated caspase 3 and caspase 8, but not caspase 9 in T cells. RNase protection assay data delineated that $B220^+$ DC, not myeloid DC, inhibited T cell mRNA expression of Bcl-w and Bfl-1 (anti-apoptosi), but enhanced Bak and Bad (pro-apoptosis) expression. The data suggest an involvement of multiple apoptosis pathways.

Abstract# 622 Poster Board #-Session: P78-II

MECHANISMS OF $CD8^+$ T CELL TOLERANCE AFTER BONE MARROW TRANSPLANTATION WITH NON-MYELOABLATIVE CONDITIONING USING ANTI-CD40 LIGAND AND DAY-1 TBI. Thomas Fehr,[1] Yasuo Takeuchi,[1] Josef Kurtz,[1] Megan Sykes.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

Aim. To investigate mechanisms of $CD8^+$ T cell tolerance in a model for induction of mixed chimerism and tolerance with bone marrow transplantation (BMT) after non-myeloablative conditioning involving anti-CD40 ligand antibody and low dose total body irradiation (TBI).

Methods. Recipient C57BL/6 mice were treated with TBI (3 Gy, d-1), one injection of anti-CD40 ligand antibody (MR1, d0) and BMT from a fully MHC-mismatched donor (B10.A, d0) to induce mixed chimerism, which was followed by FACS analysis of peripheral blood. Immunologic tolerance was assessed by donor and third party skin grafts followed over 100 days. To assess the role of interferon-γ and Fas, mice deficient for these molecules were used as recipients, and in the case of interferon-γ, also as donors. The role of CTLA4 was explored by anti-CTLA4 antibody treatment. To follow the fate of specific alloreactive cells, syngeneic chimeras expressing the transgenic alloreactive 2C T cell receptor on about 10% of peripheral $CD8^+$ T cells were prepared and subsequently transplanted as described above. Deletion of specific alloreactive cells was followed by FACS analysis of peripheral blood using an anti-clonotypic antibody.

Results. Long-lasting mixed chimerism and permanent donor-specific skin graft acceptance was achieved with BMT after conditioning with TBI and anti-CD40 ligand, but no T cell depletion. The same regimen with TBI on d0 instead of d-1 did not reliably induce mixed chimerism, unless $CD8^+$ T cell depletion was added to the regimen. Thus, $CD4^+$ T cells are readily tolerized by the d0 TBI regimen, but $CD8^+$ T cell-mediated alloreactivity is critical to overcome. When alloreactive 2C $CD8^+$ T cells of recipients conditioned with d-1 TBI, anti-CD40 ligand antibody and BMT were followed in peripheral blood, they showed rapid and complete deletion by d7. $CD8^+$ T cell tolerance was dependent on the presence of $CD4^+$ T cells, since $CD4^+$ T cell depletion abolished the achievement of chimerism and tolerance. In contrast, neither interferon-γ nor Fas/Fas ligand interactions were necessary. $CD8^+$ T cell tolerance could be blocked by one injection of anti-CTLA4 antibody or by cyclosporin A treatment for the first 14 days.

Conclusion. $CD8^+$ T cell tolerance was achieved by BMT after conditioning with anti-CD40 ligand and low dose TBI on day-1. It involved rapid peripheral deletion and was dependent upon $CD4^+$ T cells, the calcineurin pathway and CTLA4, but not upon Fas/Fas ligand interactions or interferon-γ.

Abstract# 623 Poster Board #-Session: P79-II

DETERMINING THE DEVELOPMENTAL FATE OF B CELLS PRODUCING ANTI-αGAL ANTIBODIES USING IMMUNOGLOBULIN KNOCK-IN MICE. Joel Kaye, Nathalie Cretin, Denise Malkowski, John Iacomini. *Transplantation Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA.*

Background: To further our understanding of how B cells producing αGal-specific antibodies (Ab) are developmentally regulated , we generated immunoglobulin (Ig) knock-in (KI) mice in which heavy and light chain variable regions encoding an αGal specific Ab were inserted into the endogenous Ig heavy and light chain loci of mice on either an αGal deficient or sufficient background.

Materials and Methods: The rearranged V_H and V_L chain genes were cloned from M86, an αGal specific IgM hybridoma derived from αGal knockout mice. Heavy and light chain KI mice were generated separately by gene targeting in embryonic stem cells. Resulting KI mice were then intercrossed to generate M86Ig mice and then bred with αGal knock-out mice to generate mice on either a αGal^+ (KI-Gal$^+$) or αGal^- (KI-Gal$^-$) background. Production of αGal specific Ab was then analyzed, as was development of B cells producing αGal specific antibodies.

Results: KI-Gal$^-$ mice contain in their serum high titers of αGal specific IgM, which is capable of mediating hyperacute rejection of *H-2* matched αGal^+ heart transplants. Essentially all B cells which spontaneously secrete αGal specific Ab express the KI heavy and light chain genes, reside in the spleen, and are IgM^{hi},IgD^{lo},$CD21^{hi}$ marginal zone B cells. αGal specific Ab were undetectable in the serum of KI-Gal$^+$ mice. Analysis of KI-Gal$^-$ mice revealed that in the bone marrow, B lineage cells capable of binding αGal were pre-B or newly formed B cells. We were unable to detect αGal binding B lineage cells in the bone marrow of KI-Gal$^+$ mice, suggesting that B cells expressing the knocked-in transgene are tolerized during their development. The frequencies of B cell lineage cells expressing the knocked-in heavy chain alleles in the bone marrow of both KI-Gal$^+$ and KI-Gal$^-$ mice were similar. Thus, B cells which express the $M86V_H$ region were not deleted in KI-Gal$^+$ mice. Furthermore, culturing Il-7 expanded pre-B cells from KI-Gal$^-$ BM on αGal^+ BM-derived stromal cells resulted in up-regulation of Rag-2 transcripts. Together, these data suggest that the main mechanism of B cell tolerance in this system is receptor editing rather than deletion.

Conclusion: Our data suggest that αGal specific B cells are programmed to undergo MZ development. In the presence of αGal, B cells undergo receptor editing rather than deletion to become tolerant to self. The novel mouse strains we have developed will allow us to examine development of MZ B cells, and the role of αGal specific MZ B cells in host immunity.

Abstract# 624 Poster Board #-Session: P80-II

INTERLEUKIN-10 BUT NOT TRANSFORMING GROWTH FACTOR-β ESSENTIAL FOR BOTH INDUCTION AND MAINTENANCE OF REGULATORY CELLS BY INTRATRACHEAL DELIVERY OF ALLOANTIGEN. Osamu Aramaki,[1] Nozomu Shirasugi,[2] Tadatoshi Takayama,[1] Ko Okumura,[3] Hideo Yagita,[3] Masanori Niimi.[2] *[1]Third Department of Surgery, Nihon University, Tokyo, Japan; [2]Department of Surgery, Teikyo University, Tokyo, Japan; [3]Department of Immunology, Juntendo University, Tokyo, Japan.*

Background. We previously reported that intratracheal delivery of alloantigen induced regulatory cells in mouse heart grafting model. Immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor (TGF)-β were down-regulator of immune responses. In several studies focused on mechanisms of induction or function of regulatory cells, IL-10 and/or TGF-β are thought to play critical roles. Here, we investigated the roles of IL-10 and TGF-b in the induction and effector phase of the regulatory cells.

Methods. CBA (H-2^k) mice were pretreated with intratracheal delivery of C57BL/10 (H-2^b) splenocytes and administration of neutralizing anti-IL-10 or anti-TGF-β monoclonal antibody (mAb). Seven days after the pretreatment, naive CBA mice were given adoptive transfer of splenocytes from the pretreated mice and underwent heart grafting from C57BL/10 mice the same day as the adoptive transfer. To determine the role of these cytokines in the effector phase of regulatory cells, anti-IL-10 or anti-TGF-β mAb was administered weekly into the second recipients with the adoptive transfer.

Results. Untreated CBA mice rejected C57BL/10 cardiac grafts acutely (median survival time [MST]: 7 days). Pretreatment with intratracheal delivery of C57BL/10 splenocytes prolonged graft survival significantly (MST: 65 days).

In induction phase, administration of anti-IL-10 mAb abrogated prolonged survival induced by the adoptive transfer from mice pretreated with intratracheal delivery of alloantigen (MST: 20 days), whereas concurrent administration of anti-TGF-β mAb could not abrogate this effect (MST: 88 days).

In effector phase, secondary recipients which received adoptive transfer plus anti-IL-10 mAb did not prolonged survival of C57BL/10 cardiac grafts (MST, 27 days), whereas anti-TGF-β mAb could not abrogate the function of regulatory cells (MST, 53 days).

Conclusion. IL-10 but not TGF-β was required in the induction and effector phase of the regulatory cells by intratracheal delivery of alloantigen.

Abstract# 625 Poster Board #-Session: P81-II

COMBINING LFA-1 BLOCKADE WITH EVEROLIMUS OR CD40L BLOCKADE FOR ALLOGENEIC BONE MARROW TRANSPLANTATION INDUCES HEMATOPOIETIC CHIMERISM AND CENTRAL TOLERANCE TO SOLID TISSUES IN MICE. Barbara Metzler, Patrick Gfeller, Marc Bigaud, Jianping Li, Grazyna Wieczorek, Christoph Heusser, Philip Lake, Andreas Katopodis. *Transplantation & Immunology Research, Novartis Institute for Biomedical Research, Basel, Switzerland.*

Introduction. Central transplantation (Tx) tolerance through hematopoietic chimerism initially requires peripheral immunmodulation to prevent rejection of allogeneic stem cells or bone marrow (Bm). We investigated the role of the T cell adhesion and costimulatory molecule LFA-1 as a target for immunmodulation at the time of fully MHC-mismatched Bm transfer.

Methods. After mild cytoreduction with busulfan, C57BL/6 (B6) recipient mice received 2 x10^7 BALB/C Bm cells, and a one week treatment with single agents or combinations of antibodies against LFA-1, CD40L, and everolimus [RAD, 40-O-(2-hydroxyethyl)-rapamycin]. The kinetics of hematopoietic chimerism formation was monitored by flow cytometric analysis, as was the allo-Bm dependent depletion of host allo-specific Vβ11$^+$T cells. Central transplantation tolerance was tested with both full-thickness skin grafts and heterotopic heart grafts 3 and 5 months after Bm transfer, respectively.

Results. Under these conditions no chimerism was observed with any of the reagents anti CD40L, anti LFA-1 or everolimus when given alone. In marked contrast, combinations of anti LFA-1 + anti CD40L or everolimus + anti CD40L consistently induced high levels of stable multi-lineage chimerism. The combination of anti LFA-1 +RAD resulted in declining chimerism in about 50% of Bm recipients, with either complete loss or stabilization of chimerism at a lower level. Furthermore, these outcomes appeared predictable by an earlier significant loss of Vβ11$^+$ T cells in those Bm recipients that would stabilize but not in those that would eventually loose their chimerism. Skin and heart Tx 3 and 5 months after BmTx, in the absence of any further treatment, revealed effective central transplantation tolerance. Strikingly, even very low levels (<1%, if without busulfan conditioning) of stable T cell chimerism were sufficient to prevent skin graft and chronic heart graft rejection.

Conclusions. Combinations of any 2 out of the 3 reagents anti-LFA-1, anti CD40L, and everolimus induced hematopoietic chimerism in fully allo-MHC mismatched Bm recipients. Stable chimerism alone, even at very low levels, prevented skin and heart graft rejection, including protection from vascular intimal thickening ('chronic rejection').

Abstract# 626 Poster Board #-Session: P82-II

LIVER SINUSOIDAL ENDOTHELIAL CELLS CONSTITUTIVELY EXPRESSING FAS LIGANDS IN LIVER ALLOGRAFTS TOLERIZE HOST-REACTIVE T CELLS BY DIRECT RECOGNITION. Takashi Onoe,[1] Hideki Ohdan,[1] Daisuke Tokita,[1] Hidetaka Hara,[1] Yuka Tanaka,[1] Wendy Zhou,[1] Kohei Ishiyama,[1] Hiroshi Mitsuta,[1] Kentaro Ide,[1] Toshimasa Asahara.[1] *[1]Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.*

Although livers transplanted across MHC barriers in mice are normally accepted without recipient immune suppression, underlying mechanisms remain to be clarified in detail. To identify the cell type and mechanism that contributes to induction of such a tolerance state, we established an allogeneic mixed hepatic constituent cell-lymphocyte reaction (MHLR) assay. Hepatic constituent cells (HCs) were isolated from B6 and Balb/c mice as stimulators, and splenocytes were isolated from B6 mice as responders. Irradiated HCs were co-cultured with fluorescent dye (CFSE)-labeled B6 splenocytes. In the allogeneic MHLR, whole HCs did not promote proliferation of allo-reactive T cells. The MHLR resulted in marked proliferation of both allo-reactive CD4$^+$ and CD8$^+$ T cells only when CD105$^+$ cells, which are exclusively liver sinusoidal endothelial cells (LSECs), were depleted from whole HCs by magnetic cell sorting. Such proliferation of allo-reactive T cells was inhibited by returning LSECs to the MHLR. Physical separation of LSECs from the responder-stimulator cells by using a dual chamber transwell culture system in the MHLR eliminated LSEC-induced inhibitory effects on allo-reactive T cell proliferation. Administration of a third party of LSECs did not affect allospecific T cell proliferation. To test the tolerizing capacity of LSECs toward allo-reactive T cells, B6 splenocytes that had transmigrated through the monolayer of either B6 or Balb/c LSECs were restimulated with irradiated Balb/c splenocytes. Non-responsiveness of T cells that had transmigrated through allogeneic Balb/c LSECs and marked proliferation of T cells that transmigrated through syngeneic B6 LSECs were observed after the restimulation. These findings indicate that allogeneic LSECs have a capacity to induce allo-reactive T cell tolerance through sufficient cell contact. To address how LSECs tolerize allo-reactive T cells, we analyzed the phenotype of the LSEC. Naïve LSECs constitutively expressed FasL. FasL blocking by mAbs eliminated the tolerizing capacity of the LSECs. Consistently, in allogeneic MHLR using whole HCs (including the LSECs) as stimulators, T cells at the early period of cell division expressed phosphatidylserine, which is expressed on the surface of apoptotic cells. Thus, FasL-induced apoptosis participate in tolerization of allo-reactive T cells by LSECs of liver allografts.

Abstract# 627 Poster Board #-Session: P83-II

T CELL DEPLETION BUT NOT T CELL REGULATION PLAYS A ROLE IN TRANSPLANTATION TOLERANCE IN MICE WITH REDUCED T CELL-INTRINSIC NF-kB ACTIVATION. Ping Zhou,[1] Mona Mashayekhi,[1] Samuel J. Balin,[1] David A. Palucki,[1] Maria-Luisa Alegre.[1] [1]*Medicine, University of Chicago, Chicago, IL.*

NF-kB is a key regulator of transcription following TCR and co-stimulatory receptor ligation. To determine the role of T cell-intrinsic NF-kB activation in acute allograft rejection, we have used IkBaDN-Tg mice (H-2b) that express an inhibitor of NF-kB restricted to the T cell compartment. We have previously shown that these mice permanently accept fully allogeneic cardiac grafts and secondary donor skin grafts. Our current study investigates the mechanisms of tolerance in this setting. Mixed lymphocyte reactions and ELISpot assays performed using splenocytes from tolerant animals showed reduced T cell responses, suggesting that alloreactive T cells are either hyporesponsive or deleted. We hypothesized that reduced NF-kB activation in T cells during development may result in increased generation or function of CD4+/CD25+ regulatory T cells (Treg). However, IkBaDN-Tg mice did not have increased number or function of Treg either before or after cardiac transplantation. Similarly, transfer of wildtype splenocytes into tolerant IkBaDN-Tg mice freshly transplanted with a second heart of donor origin led to cardiac allograft rejection. Taken together, these experiments suggest that regulation is not a major mechanism by which IkBaDN-Tg mice achieve tolerance.

NF-kB activation has been linked to survival of many cell types in vitro. Therefore, to determine if deletion of alloreactive T cells was one of the mechanisms of tolerance operating in transplanted IkBaDN-Tg mice, these animals were crossed with mice (H-2b) expressing the antiapoptotic Bcl-xL protein as a transgene in T cells. To determine the impact of Bcl-xL transgenic expression in vivo, allogenic hearts were transplanted into wildtype, IkBaDN-Tg, Bcl-xL-Tg and IkBaDN/Bcl-xL-Tg littermates. In contrast to IkBaDN -Tg mice that accepted allogeneic cardiac grafts indefinitely, IkBaDN/Bcl-xL-Tg mice effectively rejected their allografts. Thus, overexpression of Bcl-xL in IkBaDN-Tg T cells was sufficient to promote acute allograft rejection. Together, our results suggest that apoptosis of alloreactive T cells plays an important role and is necessary for the transplantation tolerance observed in mice with defective T cell-intrinsic NF-kB activation. Therefore, reduced NF-kB activation in T cells favors transplantation tolerance at least in part by limiting T cell survival rather than by inducing T cell regulation.

Abstract# 628 Poster Board #-Session: P84-II

CD25$^+$ REGULATORY T CELLS ARE INVOLVED IN LIVER TRANSPLANT TOLERANCE INDUCTION IN MICE. Wei Li,[1] Xin Xiao Zheng,[2] James D. Perkins.[1] [1]*Department of Surgery, Division of Transplantation, University of Washington, Seattle, WA;* [2]*Department of Medicine, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA.*

Liver allografts in mice are accepted spontaneously in all MHC strain combinations without the requirement for immunosuppression. The mechanisms underlying this phenomenon remain largely undefined. Recently, CD4$^+$CD25$^+$ cells have been shown to represent a unique population of immunoregulatory cells and play an important role in the downregulation of T cell activation and maintenance of transplant tolerance. In this study, we examined the role of CD25$^+$ regulatory T cells in liver transplant tolerance induction by in vivo administration of anti-CD25 monoclonal antibody. **Methods:** Mouse MHC mismatched orthotopic liver transplantation was performed from B10 ($H2^b$) donors to C3H ($H2^k$) recipients. The rat anti-mouse CD25 mAb (PC61) was given to the donors or recipients at 250 µg/d, pre-transplant day -6, -4, -2 or to the recipient post-transplant day 0, 2, 4, by intraperitoneal injection. Liver graft rejection was determined by recipient survival. The apoptotic activities of liver graft infiltrating cells (GIC) and recipient spleen cells (SC) were examined by in situ TUNEL staining. **Results:** Anti-CD25 mAb pre-treatment to the donor did not significantly affect liver allografts survival, whereas the liver grafts from anti-CD25 mAb either pre- or post-transplant-treated recipients were rejected acutely in comparison to the indefinite graft survival (>100 days) of the controls (Table). Histological evaluation of liver grafts from anti-CD25 mAb treated recipients showed markedly increased graft infiltrating cells in both portal triad and parenchymal areas. The frequency of apoptotic cells was less in treated mice compared with that of control mice by TUNEL staining. **Conclusions:** The recipients' CD4$^+$CD25$^+$ regulatory cells play a very important role in spontaneous liver allograft acceptance. Depletion of recipient, but not donor CD4$^+$CD25$^+$ regulatory cells results in liver allograft acute rejection and associated with reduced apoptosis of liver GIC and SC.

Groups	Treatment		Graft survival days	Mean	SD
	Donor	Recipient			
1			> 100, >100, >100, >100	>100	
2	d -6, -4, -2		29, 30, >100, >100, >100	71.8	38.6
3		d -6, -4, -2	5, 7, 7, 15, 15, 17, 17, 36	14.9	9.8
4		d 0, 1, 2, 3	7, 10, 14, 30, 35	19.2	12.5

Abstract# 629 Poster Board #-Session: P85-II

ANTI-γC AND ANTI-IL-2Rβ MONOCLONAL ANTIBODIES INHIBIT T-CELL PROLIFERATION AND INDUCE APOPTOSIS AND H-Y SKIN GRAFT ACCEPTANCE IN MURINE MODELS. Sheng Chang,[1] Bicheng Chen,[1] Dunfeng Du,[1] Hongmin Zhou,[1] Jie Zhou,[1] Zhonghua Klaus Chen.[1] [1]*Institute of Organ Transplantation, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.*

Background: The common γ chain (γc) is an essential signaling component shared by T-cell growth factor (TCGF) receptors (i.e., IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, IL-21R). The γc signals play a critical role in regulating proliferation, differentiation, and apoptosis of peripheral T-cells. Here we intended to investigate whether blocking γc signals can induce transplant tolerance and its likely mechanism. **Methods**: **I.** Splenocytes (5×10^6 cells) from C57BL/6 male mice were transfused into syngeneic female mice. On day 2 and 4, recipients received i.p. injection of mixture of anti-IL-2Rβ mAb (TMβ-1,0.5mg) and anti-γc mAbs (i.e., 4G3, 3E12 and TUGm2, 0.5mg, respectively), or control rat IgG2a (isotype control group, 2.0mg). On day 7, HY-mismatched skin grafts were performed. **II.** Splenocytes (5×10^6 cells) from C57BL/6 ($H\text{-}2^b$) mice were harvested and transfused into T-cell deficient Balb/c ($H\text{-}2^d$) nude mice that were reconstituted with syngeneic T-cells (3×10^7 cells from wild-type Balb/c mice) labeled with CFSE. On day 2, recipients received the mixture of mAbs or control rat IgG2a (isotype control group) as described in Part I. The labeled T-cells were isolated and harvested from recipient spleen after 12 hrs or 48 hrs. T-cell proliferation was examined with fluorescent dye labeling technique and T-cell apoptosis was detected with Annexin V staining. FACS was used for analyzing the results. **Results**: **I.** All recipients treated with mixture of mAbs in combination with donor-splenocytes transfusion accepted HY skin grafts over 60 days without evidence of rejection (*still under investigation*, n=7). Animals received control rat IgG2a rejected grafts within 17 days after grafting (MST=14.7 days, n=7). The prolongation of graft survival was significant difference ($P<0.001$). **II.** T-cell proliferation was markedly inhibited and apoptotic T cells could be detected 12 hrs after the mAbs injection. The proliferation was constantly inhibited, but no more apoptotic T-cells were found in 48 hrs. In isotype control group, however, T-cells actively proliferated and no apoptosis was detected at both time points. **Conclusions**: Our study shows that blockade of γc signaling combined with donor-splenocytes pretreatment can significantly prolong HY skin graft survival. This result may be associated with inhibition of antigen-specific T-cell proliferation and induction of apoptosis. We anticipate that this protocol may develop a novel approach to induce donor-specific tolerance.

Abstract# 630 Poster Board #-Session: P86-II

ESTABLISHMENT OF A BONE MARROW (BM) EXPANSION SYSTEM FOR EX VIVO GENERATION OF CELLS WITH POTENT CAPACITY TO PROLONG SKIN GRAFT SURVIVAL. Edip Akpinar,[1] Jenny Park,[1] Douglas A. Hale.[1] [1]*Transplantation Branch, NIDDK-National Institutes of Health, Bethesda, MD.*

Background: The eventual application of tolerance induction regimens employing cadaveric donor BM is limited since insufficient BM can be procured for use in all 7 potential recipients. In protocols in which the administration of donor BM is delayed, strategies for expanding BM can overcome this limitation and provide an opportunity to alter the BM inoculum to increase efficacy and decrease associated morbidity. **Methods:** BM, ficoll processed BM (FBM), immunomagnetically isolated c-kit+lin+ or lineage negative cells (lin-), obtained from BALB.C mice were placed in culture for 7 days respectively. Iscove's Modified Dulbeccos Medium (IMDM) with 10% Fetal Calf Serum (FCS) and IL3, IL6, SCF, FLT3 and M-CSF was used as Full Media (FM). The effect of feeder cells (STO and HUBEC) was tested using transwell plates. Cell yield and phenotype were determined after 7 days of culture by flow cytometry. Expanded cells were infused 7 days following skin grafting in C57Bl/6 recipients of BALB.C skin conditioned with antilymphocyte serum (ALS) and sirolimus. Colony Forming Unit assays (CFU) were performed in semisolid methylcellulose medium including IL3, IL6, SCF and EPO. **Results**: BM, FBM, lin-, and ckit+lin+ cells yielded 8, 12, 100 and 250-fold expansion respectively when placed in FM. Removal of FCS completely abolished the proliferative effects of the FM. Addition of IL3 alone increased cell proliferation by 3 fold, other cytokines did not change the cell number when added individually. Culture in transwell plates did not alter the proliferation rate in FM indicating that feeder layers are unnecessary. Phenotyping revealed that cultured cells differentiate predominantly into myeloid series with 90% expressing CD11b. No difference in adhesion receptor expression was detected between fresh and cultured cells. Fresh BM, FBM, lin –, ckit+lin+, expanded BM and expanded ckit+lin+ cells produced 76, 120, 360, 895, 190, 305 colonies with >50 cells respectively in semisolid media. Median skin graft survival in mice receiving 25, 100 or 150 million expanded c-kit+lin+ cells was 145, 185 and 245 days respectively. **Conclusion:** Culture of early committed progenitor (c-kit+lin+) cells yields a 250-fold expansion of cell number over 7 days. The expanded cells proliferate rapidly and are capable of producing long-term skin graft survival in mice conditioned with ALS and sirolimus and may be useful adjuncts in the development of a tolerance induction regimen.

Abstract# 631 Poster Board #-Session: P87-II

PROMOTION OF ORGAN TRANSPLANT TOLERANCE BY A SINGLE PREOPERATIVE INFUSION OF IN VIVO-MOBILIZED PRE-PLASMACYTOID DENDRITIC CELLS, IN COMBINATION WITH ANTI-CD154 mAb. Angus W. Thomson,[1,3] P. Toby H. Coates,[1,3] Zhiliang Wang,[1,3] F. Jason Duncan,[1,3] Pia Bjorck.[2] *[1]Surgery and Dermatology, University of Pittsburgh, Pittsburgh, PA.*

Background: There is as yet little information regarding the influence of plasmacytoid dendritic cells (pDC) on alloimmunity. Recent in vitro data suggest that human and murine pDC can regulate alloreactive T cell responses. We have examined the influence of freshly-isolated in vivo-mobilized donor pDC, administered alone or with CD40-CD154 blockade, on the outcome of vascularized heart transplantation.

Methods: pDC and classic myeloid (m) DC were mobilized in C57 BL/10 (B10; $H2^b$) mouse donors by administration of the hemopoietin fms-like tyrosine kinase 3 ligand (Flt3L; Amgen; 10 µg/day for 10 days). pDC ($CD11c^+ B220^+ CD11b^-$) and mDC ($CD11c^+ B220^- CD11b^+$) were sorted from spleens (>97% purity) using a high speed flow sorter. The cells were administered (2.10^6 i.v.) to C3H He/J (C3H; $H2^k$) hosts, 7 days before vascularized intra-abdominal heart transplantation. Anti-CD154 mAb (MR1) or control rat IgG was administered (250 µg i.p.) together with the pDC and on the day of transplantation. To test for the induction of T regulatory cells, host splenic bulk $CD4^+$ (or $CD4^+ CD25^+ CD45RB^{lo}$) cells were flow-sorted from DC-treated recipients 25 days post transplant, and transferred ($8x10^6$ or $5x10^5$, respectively) to naïve C3H recipients that received B10 heart grafts one day later.

Results: Freshly-isolated donor mDC prolonged graft survival significantly from 10.3 ± 1.0 to 19.7 ± 11.0 days ($p< 0.01$). Under the same conditions, a single infusion of donor pDC prolonged graft survival to 36.4 ± 29.5 days ($p< 0.01$). The superior therapeutic effect of pDC was significantly enhanced by anti-CD154 mAb administration (68.3 ± 42.4 days, $p< 0.0001$) that alone prolonged survival to 26.0 ± 6.6 days ($p<0.01$). 50% of pDC + anti-CD154 mAb-treated mice achieved graft survival >100 days. Third party (BALB/c) challenge skin grafts were rejected in these animals. Transfer of splenic $CD4^+$ T cells from pDC-treated mice, 25 days post transplant, failed to prolong heart graft survival in naïve C3H recipients.

Conclusion: A single infusion of growth factor-mobilized donor pDC exhibits superior efficacy to immature mDC in prolonging fully MHC-mismatched organ allograft survival in the absence of immunosuppressive therapy. Anti-CD154 mAb administration enhances the therapeutic effect of pDC, and the combined treatment promotes indefinite (>100 day) donor-specific graft survival. The mechanism(s) underlying the tolerogenic property of pDC in this model remain to be fully elucidated.

Abstract# 632 Poster Board #-Session: P88-II

LONGTERM REGULATION OF $CD8^+$ T CELLS BY SHORT-TERM IMMUNOTHERAPY TARGETING LFA-1 AND CD40/CD40L COSTIMULATION. Keri E. Lunsford,[1] Anna M. Eiring,[1] Mitchel A. Koester,[1] Donghong Gao,[1] Ginny L. Bumgardner.[1] *[1]Department of Surgery, Division of Transplantation, The Ohio State University Medical Center, Columbus, OH.*

We and others have demonstrated that immune damage by alloreactive (CD4-independent) $CD8^+$ T cells is difficult to suppress by strategies which readily regulate alloreactive $CD4^+$ T cells. Recently, we have discovered that targeting LFA-1 alone preferentially suppresses CD8-dependent versus CD4-dependent rejection of allogeneic hepatocytes. In addition, short-term immunotherapy targeting both LFA-1 and CD40/CD40L costimulation produced synergistic effects and resulted in suppression of CD8-dependent rejection such that longterm survival (LTS) of hepatocytes up to 90 days was achieved in the majority of recipient mice. The purpose of this study was to determine whether recipient mice with LTS induced by short-term immunotherapy targeting LFA-1 and CD40/CD40L costimulation were resistant to immune damage when challenged with naïve $CD8^+$ T cells.

Methods: Two million FVB/N (hA1AT transgenic, $H\text{-}2^q$) hepatocytes, were transplanted into CD4 KO ($H\text{-}2^b$) mice and treated with anti-LFA-1 mAb (0.3mg, d0-6) and anti-CD40L mAb (1mg, d0, 2, 4, 7) by ip injection. Hepatocyte survival was monitored by detection of serum reporter product, hA1AT, by ELISA. Hepatocyte recipients with longterm survival (>60 days) were challenged by adoptive transfer of $2x10^6$ naive $CD8^+$ T cells.

Results: Combined treatment with anti-LFA-1 and anti-CD40L mAbs significantly prolongs hepatocyte allograft survival in CD4 KO mice (N=19) such that 95% of recipients achieved hepatocyte survival >60 days. A subgroup of recipient mice induced to achieve hepatocyte survival >60 days by this combined treatment strategy were subsequently challenged with $2x10^6$ naïve $CD8^+$ T cells. Continued survival of longterm hepatocellular allografts (>30 days) despite adoptive transfer of $CD8^+$ T cell was observed in 4 of 5 CD4 KO recipients. Control SCID mice with functioning hepatocellular allografts which were adoptively transferred with $2x10^6$ naive $CD8^+$ T cells rapidly rejected hepatocytes with MST of 17 days (N=5).

Conclusion: Targeting of both CD40/CD40L and LFA-1 not only suppresses (CD4-independent) CD8-dependent hepatocyte rejection, but also appears to induce immunoregulation resistant to challenge with naïve alloreactive $CD8^+$ T cells.

Abstract# 633 Poster Board #-Session: P89-II

DEVELOPMENT OF A MIXED CHIMERISM-TOLERANCE INDUCING NONMYELOABLATIVE REGIMEN RELEVANT TO CADAVER DONOR TRANSPLANTATION. Ichiro Koyama,[1] Tatsuo Kawai,[1] Siew-Lin Wee,[1] Svetlan Boskovic,[1] Ognjenka Nadazdin,[1] Rex-Neal Smith,[2] Megan Sykes,[3] Robert B. Colvin,[2] David H. Sachs,[3] A. Benedict Cosimi.[1] *[1]Transplant Unit, Massachusetts General Hospital, Boston, MA; [2]Pathology, Massachusetts General Hospital, Boston, MA; [3]Transplant Biology Research Center, Massachusetts General Hospital, Charlestown, MA.*

Purpose: A previously reported nonmyeloablative conditioning protocol (Standard Regimen) that can induce mixed chimerism and renal allograft tolerance in primates, requires a 6-day preparative period before transplant, and is consequently limited to recipients of living donor allografts. In this study, we aimed to modify the regimen for cadaveric donor application. **Method:** The standard regimen consists of total body irradiation (TBI), thymic irradiation (TI), antibody treatments and donor bone marrow (DBM), followed by a one-month course of cyclosporine. Various timimg or dosages of TBI, TI and antibodies were studied in six groups. **Results:** Compression of 3 Gy of TBI and 7 Gy of TI into a 24-hour period led to unacceptable toxicity (Regimens A and C). Delayed irradiation and DBM failed to induce chimerism even with additional aCD154 (Regimen B). A single 3 Gy of TBI induced chimerism in 2/3 recipients without TI when combined with aCD154 and ATG (Regimen D). Nevertheless, all recipients treated with Regimen D eventually rejected their allografts. Addition of TI (4Gy) to Regimen D again resulted in toxicity (Regimen E). An antibody combination of ATG, aCD154 and aCD8 in conjunction with reduced dose TBI consistently induced mixed chimerism, less infectious complications, and prolonged graft survival (Regimen F). **Conclusions:** A single dose of TBI appears to be more effective for inducing chimerism than fractionated dosages. An antibody combination of ATG, aCD154 and aCD8 combined with TBI 2.5 Gy improved consistency of chimerism induction with less risk for infectious complications and appears promissing for induction of tolerance following a less than 24 hours pre-transplant conditioning protocol.

The conditioning regimens and mixed chimerism

Regimen	TBI (Gy)	TI (Gy)	DBM	antibody	chimerism	graft survival (days)
standard regimen	D-6,-5 (1.5x2)	D-1 (7)	D0	ATG	8/13	>3478, >2569, 834[a],771, 405[a],260[d],198[a],196[a],137[a],72[d], 44[d],40[d],37[d]
A	D-1 (1.5X2)	D-1 (7)	D0	ATG	0/6	23[a],34[b],46[c],49[b],69[a],120[c]
B	D0, +1 (1.5x2)	D+5 (7)	D+6	ATG, aCD154	0/4	13[d],14[d],16[d],78[d]
C	D-1 (1.5x2)	D-1 (7)	D0	aCD154, aCD8	0/2	17[b],22[b]
D	D-1 (3x1)	none	D0	aCD154,aCD8	2/3	53[d],90[d],110[d]
E	D-1 (3x1)	D-1 (4)	D0	aCD154,aCD8	1/3	24[b],29[b],34[d]
F	D-1 (2.5x1)	D-1 (4)	D0	ATG, aCD154, aCD8	5/5	26[b],60[d],117[a],280[c],>376

[a]ureteral complication,[b]infection,[c]lymphoma,[d]acute rejection, [c]chronic rejection

Abstract# 634 Poster Board #-Session: P90-II

BLOOD T CELL POPULATIONS PHENOTYPES IN DRUG-FREE "OPERATIONALLY TOLERANT" HUMAN KIDNEY RECIPIENTS. Stephanie Louis,[1] Magali Giral,[1] Alexandre Dupont,[1] Jean-Paul Soulillou,[1] Sophie Brouard.[1] *[1]INSERM U437, Institut de Transplantation et de Recherche en Transplantation, Nantes, France.*

Interruption (PTLD or uncompliance) of immunosuppression (IS) in long-term graft recipients usually results in a rejection. However, some recipients who stopped IS keep a good graft function ("operationally tolerant"). We previously showed that kidney recipients without IS and minimally immunosuppressed displayed more $CD25^+CD4^+$T cells than patients with chronic rejection. In this study, we performed a more exhaustive analysis of potentially relevant T cell phenotypes in these patients versus recipients with chronic rejection. **Methods**: Four groups were studied: 1) "operationally tolerant" recipients with a functional graft, drug-free (DF) for more than 3 years (n=4), 2) patients under low doses of steroid monotherapy (<10mg)(Ster,n=7), 3) patients with chronic rejection (CR,n=6) and 4) normal individuals (NL,n=5). Using four-color flow cytometry, we analyzed $CD25^+CD4^+$T cells for major regulatory-associated molecules and some chemokines receptors in $CD4^+$ and $CD4^-$ T cells. **Results**: GITR, TLR4 and CD103 are not overexpressed in $CD25^+CD4^+$T cells, whatever the groups. Same conclusion was obtained when $CD25^{hi}CD4^+$T cells were analyzed. In half of DF patients, 30% of $CD25^+CD4^+$T cells stained positive for intracellular CTLA4 compared to 8.1 and 11% for CR and Ster recipients. In 3 out of 4 DF patients, $CCR7^+CD25^+CD4^+$T cells were low (12% +/-2 versus 57% +/-19 for CR group, $p<0.05$). In one DF patient (#60), 70% of $CD25^+CD4^+$T cells were $CCR7^+$, mimicking the profile observed in CR patients. Ster patients split in two groups displaying 50% and 18% of $CCR7^+$. Other Th1/Th2 chemokine receptors (CCR9,CCR5,CXCR3,CCR4) did not exhibit difference of expression. Finally, when $CD4^+$ and $CD4^-$T cells were examined, no significant difference was observed. However, again, patient #60 had a distinct profile with increased $CCR9^+CD4^+$T (17%) and $CD40L^+CD4^+$T cells (36.8%) versus all other patients (within 4-5% and 5-6% respectively), a profile which has been associated

with Tr1 cells. **Conclusions**: Blood of "operationally tolerant" patients were characterized by an increase in $CD25^+CD4^+$T cells. $CCR7^+CD25^+CD4^+$T cells were usually low. Some patients exhibited an increase in intracellular CTLA4 in $CD25^+CD4^+$T cells. In addition, one DF recipient had a unique blood phenotype with increased $CCR7^+CD25^+CD4^+$, $CCR9^+CD4^+$ and $CD40L^+CD4^+$T cells. Thus, operationally "tolerant" patients may develop distinct patterns possibly related to mechanisms of unresponsiveness.

Abstract# 635 Poster Board #-Session: P91-II

METASTABLE TOLERANCE IN THE LIVER TRANSPLANT — TGFB LATENT+ CD4 T CELLS INFILTRATING THE HEPATIC PARENCHYMA CORRESPOND TO ALLOPEPTIDE (DONOR HLA-B)- SPECIFIC T-REG CELLS IN PERIPHERAL BLOOD. William J. Burlingham,[1] Ewa Jankowska-Gan,[1] Junglim Lee,[1] Hans W. Sollinger,[1] Munci Kalayoglu,[1] Stuart J. Knechtle,[1] Jose Torrealba.[1] *[1]Surgery and Pathology, University of Wisconsin, Madison, WI.*

Peripheral tolerance to a liver allograft is **metastable**. For ex.,patients weaned off IS may retain their graft initially, but only 20% will be able to retain graft function without rejecting. We have previously shown that anti-donor unresponsiveness in trans-vivo DTH assays of PBMC from liver transplant recipients can be broken by donor soluble HLA class I antigen (dAg) challenge, along with cytokine (TGFβ or IL-10) neutralization. **Hypothesis**: CD4+ TGFβ(latent)+ T regulatory cells specific for donor soluble HLA- class I allopeptide, promote liver transplant survival by taking up residence within the graft parenchyma, where they inhibit local DTH reactions. **Methods**: Liver transplant (LTx) recipient LVR1, a 6 HLA mismatch with her donor, was transplanted under OKT3 induction, and biopsied at 2 yrs post-Tx. PBMC were obtained 1-6 yrs post-transplant. Soluble donor-type HLA-B62 antigen , and B62-derived 18mer allopeptides p37,p106, and p149 were used as dAgs. Control PBMC were obtained from:a) healthy age-matched normals, b) 2 LTx recipients **not** mismatched for B62, and c) a tolerant kidney transplant recipient , KDY#1, mismatched with his donor for the B62 antigen, but having different HLA class II alleles(DR4,6 vs. DR2,- for LVR#1). Liver biopsy sections were immunostained for TGFβ(latent)+, CD4 +, CD8+ and double+ T cells by immunofluorescence(IF). **Results**: We found that soluble B62 and allopeptide p149, but not p37 or p106, triggered suppression of recall DTH responses by patient LVR#1 PBMC. In contrast , DTH regulation to donor sHLA-B62 was also seen in renal Tx patient KDY#1, but the dominant epitope was p37. No regulation to sB62 or its allopeptides was found in healthy controls or in non-B62-mismatched LTx pts. All the 'indirect pathway' regulatory activity in LVR#1 and KDY#1was found to be localized to the CD4+ T cells; neutralization of TGFβ in the DTH assay revealed a strong anti-donor effector T cell population, also CD4+ ,with similar epitope specificity. Dual IF staining of the biopsy revealed CD4+TGFβ+ T cells a) adhering to the intima of blood vessels and b) closely adjacent to the hepatocytes. **Conclusion:** CD4+ TGFβ(latent)+ T regulatory cells are specific for donor soluble HLA-class I/ allopeptide; similar cells traffic to the liver transplant itself. This is consistent with the idea that promote survival by inhibiting local DTH response.

Abstract# 636 Poster Board #-Session: P92-II

B CELLS ARE ASSOCIATED WITH INCREASED SERUM IL-10 IN THE EARLY STAGES OF PRIMATE TOLERANCE. Anne Hutchings,[1] Jianguo Wu,[1] Clement Asiedu,[1] Stacie Jenkins,[1] Jin He,[1] Karen J. Goodwin,[1] Stephanie Le Bas-Bernardet,[1] Francis T. Thomas,[1] Richard L. George,[1] Judith M. Thomas.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL.*

We have previously reported that sustained elevated levels of systemic IL-10 are associated with the generation and maintenance of specific allograft tolerance in non-human primates (NHP) induced by a combination of anti-CD3 immunotoxin (IT) with Deoxyspergualin (DSG). This study was performed to determine the phenotype of the cells responsible for the early IL-10 dominated cytokine milleu. Peripheral blood samples were collected from NHP within 3 months of tolerance induction with IT and DSG, and compared to samples from normal control animals. Serum cytokines were quantified with multiplex kits on a Luminex® and cells were stained with rhesus-reactive fluorescent antibodies for flow analysis. RT-PCR was used to examine mRNA expression in peripheral lymphocytes. Compared to normal animals, the IT and DSG treated allograft recipients had significantly elevated levels of serum IL-10 (72.6 pg/ml ± 8 vs. 26.4 ± 7, $p<0.03$). In addition, significantly more B cells from NHP treated with IT and DSG expressed intracellular IL-10 (15.7% ± 4 vs. 7.8% ± 1, $p<0.004$). There were similar increases in IL-10 expressing monocytes (72.6% ± 8 vs. 26.4% ± 7, $p<0.004$) and NK cells (43.9% ± 12 vs. 19.6% ± 6, $p<0.008$). Of note, only the percentage of individual recipients' IL-10 positive B cells correlated with their serum IL-10 levels ($r=0.90$, $p<0.04$). TRAF3 expression was decreased in the mononuclear cells of treated NHP recipients compared to normal controls. Since TRAF3 inhibits the IL-10 promoter, we postulate that downregulation of TRAF3 by DSG unleashes early IL-10 expression to foster a milieu favorable for tolerance development. Moreover, the prominence of IL-10 producing non-T cells suggests that preservation of B cells, monocytes and NK cells is crucial for the synergy between DSG and T cell depletion therapy in promoting tolerance.

Abstract# 637 Poster Board #-Session: P93-II

EXTRACORPOREAL PHOTOPHORESIS ALLOWS RECIPIENT SPECIFIC TRANSFUSION (RST) TO PROLONG CARDIAC ALLOGRAFT SURVIVAL. T. Gonzalez,[1,3,4] S. Prange,[3] D. Zhou,[3,4] D. Lian,[3,4] C. Du,[1,2,4] Z. Yin,[1,4] Q. Guan,[1,4] P. J. O'Connell,[1,3] R. Zhong,[1,2,3,4] A. House,[3,4] A. M. Jevnikar.[1,2,3,4] *[1]RRI; [2]LHRI; [3]Med, Surg, Micro&Imm, Univ. West. Ont; [4]MOTS, LHSC, London, ON, Canada.*

Extracorporeal photophoresis (ECP) involves the ex vivo treatment of peripheral blood leukocytes with a photosensitizing agent (8-methoxysporalen, MOP) with UVA before re-infusion into patients. ECP has been used to treat refractory acute rejection in cardiac transplants, suggesting a profound effect on circulating immune cells. Although ECP induces apoptosis of lymphocytes and apoptotic bodies can exert immunosuppressive effects, the immunomodulatory mechanism(s) of ECP in solid organ transplantion remains unknown. We therefore tested the ability of ECP-treated splenocytes undergoing apoptosis to inhibit allogeneic responses. We first confirmed that ECP induced apoptosis in 70% of CD3+ T-cells in spleen cell cultures by 24 hours, using FACS-Annexin V labelling. Although 30% of T-cells remained viable, they were unresponsive to both ConA and alloantigen stimulated proliferation. In contrast, bone-marrow derived dendritic cells (DC) were resistant to apoptosis. We then tested the ability of ECP-treated splenocytes (ECP-S) to inhibit allogeneic responses in MLC. Addition of ECP-S from B6 mice (B6-ECP-S) suppressed allo-specific responses of non-treated B6 responders to BALB/c stimulators (SI=0.8) as compared to control MLC (SI-22, $p<0.05$). Similar treatment using ECP-S from BALB/c stimulators had no effect. Pre-incubation of ECP-S with non-treated syngeneic responders for 24 hours maximally suppressed allo-responses, suggesting the primary effect is on responder cells. To test effects in vivo, we performed murine heterotopic cardiac transplants using B6 recipients and BALB/c donors. Infusion of B6- ECP-S to B6 recipients at at transplant did not prolong survival compared to controls (mean=d 8). However, infusion of ECP-S 24 h before transplant prolonged survival (mean=d 20, $p<0.0002$)). Increase in graft survival was more modest using BALB/c recipients and B6 donors (d10,11,11). Infusion of BALB - ECP-S to BALB/c recipients 24 h before transplant along with cyclosporine (15 mg/kg/day) increased allograft survival to beyond d19 compared to mice given cyclosporine alone (d 16.5 ±0.8). These data demonstrate that ECP can be used to inhibit allogeneic responses in vitro and in vivo. In contrast to donor specific transfusion (DST) strategies, ECP of recipient or recipient specific transfusion (RST) prior to transplantation may be useful in preventing allograft rejection.

Abstract# 638 Poster Board #-Session: P94-II

RENAL TRANSPLANTATION IN SWINE: TOLERANCE AND RENAL FUNCTION UNDER SHORT TERM CYA TREATMENT. SYNERGISTIC EFFECT OF DONOR SPLEEN TX. Marcello O. Maestri,[1] Johannes Rademacher,[2] Annalisa Gaspari,[2] Stefania Crespi,[2] Luca M. Lenti,[2] Laura Cansolino,[2] Giuseppe Novelli,[2] Domenico Agoglitta,[2] Sara Segreti,[2] Paolo Dionigi.[2] *[1]Surgery, IRCCS San Matteo Hospital, Pavia, PV, Italy; [2]Surgery, University of Pavia, Pavia, PV, Italy.*

Recently several Authors have shown that CyA in "*in vivo*" models can induce a state of tolerance, even in large mammalians. A few studies demonstrated a possible tolerogenic role for spleen as a whole graft. Aim of this study is to demonstrate that a short term, high dose treatment based on CyA can effectively induce tolerance after kidney Tx in swine when combined with spleen Tx. 49 outbred swine (all females, weight range Kg 27-30) had a renal graft from unrelated donors (all males, weight range Kg 25-28). Each couple got a MLR test a few days before surgery to assess the expected alloreactivity. Surgery was performed under general anesthesia. The recipient's native kidneys were removed and the donor graft implanted intraperitoneally (Renal vein to inferior v. cava. Renal artery to aorta. Ureter to bladder extramucosal anastomosis). The animals were stratified in groups, according to table 1, which details CyA administration and spleen implant.The renal grafts were hystologically examined at sacrifice. Table 2 details the results of this study. Data were evaluated by non parametric tests ($p<0.05$ was considered significant). Spleen Tx has a synergistic effect with immunosuppressive treatment. In this design, the spleen cells were administered through the portal vein (isolated cells) or a donor spleen graft was anastomosed to the recipient portal system. Both techniques show a prolonged graft survival when compared to immunosuppression alone ($p<0.05$) and to controls ($p<0.05$). Group 4 and 6 have a significantly better hystology compared to controls. Further studies will help to elucidate a possible role of spleen cells in induction and stability of tolerance to unrelated renal grafts.

Table 1

Group	N	Surgery	CyA
1	6	KTx	-
2	7	KTx and DST	-
3	13	KTx	9 mg/Kg/day (12 POD)
4	10	KTx and DST	9 mg/Kg/day (12 POD)
5	8	KTx and STx	-
6	5	KTx and STx	9 mg/Kg/day (12 POD)

DST: Donor Splenocytes Transfusion via portal vein. KTx: kidey Tx. STx: Donor spleen Tx as whole graft, anastomosed to recipient's portal system. CyA was given i.v. and stopped after twelve postoperative days

Table 2

Group	Median Survival (days)	Acute Rejection	Chronic Rejection	No Rejection
1	8	6/6	0/6	0/6
2	60	3/7	4/7	0/7
3	42	5/13	8/13	0/13
4	60	0/10	0/10	10/10
5	7.5	6/8	2/8	0/8
6	50	0/5	0/5	5/5

median survival and onset of rejection

Abstract# 639 Poster Board #-Session: P95-II

THE NEW STANDARD OF CARE: ALEMTUZUMAB INDUCTION WITH TACROLIMUS AND SIROLIMUS MAINTENANCE IMMUNOSUPPRESSION TO LIMIT ACUTE AND CHRONIC ALLOGRAFT REJECTION. Steven C. Hoffmann,[1] Robert L. Kampen,[1] Jonathan P. Pearl,[1] Douglas A. Hale,[1] Lynn M. Jacobson,[2] David E. Kleiner,[3] Roslyn B. Mannon,[1] Rececca J. Muehrer,[1] S. J. Swanson,[4] Bryan N. Becker,[2] Allan D. Kirk.[1] *[1]Transplantation Branch, NIDDK, Bethesda, MD; [2]Medicine, University of Wisconsin Medical Center, Madison, WI; [3]Laboratory of Pathology, NCI, Bethesda, MD; [4]Organ Transplant Service, Walter Reed Army Medical Center, Washington, DC.*

The choice of optimal immunosuppressive drug combinations following human renal allotransplantation remains challenging. Regimens incorporating Alemtuzumab induction with Tacrolimus (TAC) or Sirolimus (SLR) maintenance monotherapy have recently gained prominence, significantly increasing patient and allograft survival. Therefore, we examined functional and transcriptional differences in protocol biopsies and PBMC from renal transplant patients given Alemtuzumab induction with either SLR or TAC monotherapy. Biopsies were processed for cell phenotyping and RNA was extracted from biopsies and PBMC for real-time PCR. Patients treated with Alemtuzumab alone displayed a single depletion-resistant effector memory phenotype (CD3+CD4+CD45RA-CD62L-) that were uniquely prevalent within the first month post-transplant and during rejection. These cells were resistant to steroids, Deoxyspergualin and SLR, in vitro, but were TAC responsive. Transcripts for inflammatory cytokines (IL-2, TNFa) and T_H1, cytotoxic T-cells (T-bet, FasL, RANTES) were significantly upregulated in SLR biopsies despite lymphopenic conditions. However, patients placed on TAC monotherapy had significantly decreased transcripts for costimulatory markers (CD80, CD86, CD154, ICOS), cytotoxic T-cell transcripts (IFNg, FasL, Gr B) and also chemokines (RANTES, MIP1a, MIG, IP-10); without evidence of acute rejection thus far (>6 months). In contrast, biopsies from Alemtuzumab depleted patients on SLR displayed significantly decreased transcripts for Smad-3, TGFb, VEGF and type I collagen at 6 and 12 months post-transplant compared to biopsies from TAC patients. Transcriptional profiling of patient biopsies has shown that TAC decreases leukocyte chemotaxis and activation early post-transplant while SLR has beneficial effects on long-term outcomes by reducing early expression of fibrotic mediators of chronic rejection. Indeed, such molecular analyses and data now advocate for a combined approach to maintenance immunotherapy following pronounced depletion with Alemtuzumab and warrant significant study as the potential new standard of care.

Abstract# 640 Poster Board #-Session: P96-II

ALLOANTIBODY DEVELOPS COINCIDENT WITH REJECTION OF SKIN AND RENAL ALLOGRAFTS IN NON-HUMAN PRIMATES TREATED WITH THE ANTI CD-154 MONOCLONAL ANTIBODY IDEC-131 COMBINED WITH DONOR SPECIFIC TRANSFUSION (DST) AND SIROLIMUS. Kiran K. Dhanireddy,[1,2] He Xu,[1] Edwin H. Preston,[1,2] John P. Pearl,[1] Frank V. Leopardi,[1] Lynt B. Johnson,[2] Allan D. Kirk.[1] *[1]Transplantation Branch, NIDDK/NIH, Bethesda, MD; [2]Department of Surgery, Georgetown University Hospital, Washington, DC.*

Background. We have demonstrated that IDEC-131 in combination with DST and sirolimus prolongs both renal and skin allograft survival. Alloantibody development preceded skin allograft rejection in some monkeys treated with the anti-CD154 monoclonal antibody hu5c8. The objective of this study was to determine the timing of alloantibody development in relation to graft rejection using IDEC-131, DST and sirolimus.

Methods. Outbred rhesus monkeys were used as donor-recipient pairs based on genetic non-identity at MHC and pretransplant MLR responsiveness. Renal and skin allografts were performed as previously described. IDEC-131 (20 mg/kg/dose) was given IV on day –1, 0, 3, and 7 then weekly for 8 weeks. Sirolimus was given orally (1mg/kg/day) for 90 days. Donor specific whole blood (7 cc/kg) was given IV on day –1. Alloantibody determination was performed on donor lymphocytes by flow cytometric cross match.

Results. Alloantibody development was coincident with rejection in monkeys treated with IDEC-131, DST and sirolimus (n=7). Three monkeys receiving triple therapy have not rejected renal allografts and have not developed alloantibody. Monkeys receiving IDEC-131 and sirolimus (n=4) did not develop alloantibody while on therapy even if the allograft was rejected. Two of 4 monkeys receiving only IDEC-131 developed alloantibody, one of which was still on therapy. Monkeys receiving sirolimus alone developed alloantibody at the time of rejection, while still on therapy (n=2). Two triple therapy renal transplant monkeys were challenged with donor skin grafts after >1 year. These monkeys did not develop alloantibody 10 months after skin grafting.

Therapy	Rejection-free Survival	Timing of Alloantibody Development
IDEC-131, DST, sirolimus	168, 185, 233, 274, >290, >724, >743	168, 185, 210, 231, —, —, —
IDEC-131, sirolimus	20, 29, 86, >519	90, 90, —, —
IDEC-131 alone	9, 14, 44, 455	—, 14, —, 469
sirolimus alone	9, 11	7, 7

Conclusions. IDEC-131 in combination with DST and sirolimus prolongs renal and skin allograft survival. In addition, alloantibody formation is delayed until the time of allograft rejection. In monkeys that have not rejected their grafts, alloantibody has not developed even in response to a secondary immunologic challenge. There is no evidence of durable tolerance, humoral tolerance, or split tolerance.

Abstract# 641 Poster Board #-Session: P97-II

ASSESSMENT OF IMMUNOLOGIC RISK BY ACCURATE, SENSITIVE AND QUANTITATIVE MEASUREMENT OF ANTI-HLA ANTIBODY IN A SINGLE TUBE ASSAY. Brian Susskind,[1] Paul Brailey,[1] Prabir Roy-Chaudhury,[2] Michael Cardi,[3] Joseph Buell,[4] Rino Munda,[4] Sharad Goel,[3] Joseph Austin,[3] Michael Hanaway,[4] E. Steve Woodle.[4] *[1]Transplantation Immunology Division, Hoxworth Blood Center, Cincinnati, OH; [2]Department of Nephrology, University of Cincinnati College of Medicine, Cincinnati, OH; [3]Kidney and Hypertension Center, The Christ Hospital, Cincinnati, OH; [4]Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, OH.*

Flow cytometry single HLA antigen panel beads (Labscreen™) provide an accurate and sensitive HLA antibody detection method (Transplantation 2003, 75:43). We combined this technology with the the Luminex™ multi-analyte microfluidics platform and conversion of Fluorescence Intensity (FI) to Molecules of Equivalent Soluble Fluorescence (MESF) using Quantum™ 27 microbeads (Bangs Laboratories) as a means to "titer" the individual antibodies in a single tube assay. Initial experiments compared results with a flow cytometry single antigen panel on 8 sera from highly allosensitized patients, and 13 serologically well-defined sera. Results comparing flow and Luminex™ versions of the Labscreen™ beads were highly concordant. Compared to cytotoxic PRA, the Labscreen single antigen panel for Luminex identified the same specificities, as well as reactive and nonreactive low frequency antigens within the broader CREG groups.

Labscreen results in combination with MESF quantitation were useful in explaining unexpected crossmatch findings, e.g., negative in highly sensitized patients, or positive results by uncovering veiled specificities.

In serum from six patients undergoing late humoral rejection (1 – 8 years post-transplant) of their kidney transplants, Labscreen PRA beads consistently identified antibodies to mismatched, donor-specific HLA antigens, whereas the recipients' HLA were nonreactive. Moreover, differential levels of donor-specific anti-HLA antibodies (DSA) and their responses to anti-rejection therapy were observed, and no DSA was detected in six other cases where the biopsies were either negative (N=4) or rejection was read as strictly cellular (N=2).

Four patients with low levels of DSA (<40,000 MESF) and a positive FCXM were deemed suitable to transplant in a "high risk" immunosuppression protocol of pre-transplant plasmapheresis plus FK506, MMF and Thymoglobulin. Post transplant monitoring showed a post-transplant increase in DSA of 3- to 7- fold above pre-transplant levels within two weeks, but then a rapid decline and disappearance of DSA.

In conclusion, combination with single HLA antigen beads provides a useful method for assessment of immunologic risk due to anti-HLA antibodies in allosensitized patients and in post-transplantation monitoring.

Abstract# 642 Poster Board #-Session: P98-II

DONOR SPECIFIC ANTI-HLA CLASS I ANTIBODY DETECTION BY AN ELISA-BASED TRANSPLANT MONITORING SYSTEM (TMS®). Brian Susskind,[1] Rubina Karim,[1] Paul Brailey,[1] Prabir Roy-Chaudhury,[2] Michael Hanaway,[3] Michael Cardi,[4] E. Steve Woodle.[3] *[1]Transplantation Immunology, Hoxworth Blood Center, Cincinnati, OH; [2]Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, OH; [3]Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, OH; [4]The Kidney and Hypertension Center, The Christ Hospital, Cincinnati, OH.*

Disadvantages to most HLA crossmatch (XM) methods are requirements for viable target cells and detection of antibodies (Abs) against non-HLA antigens. Recently alternative approaches based on ELISA have been described in which donor HLA antigens solublized from leukocytes are adsorbed onto microtiter plates (Transplant Monitoring System®, GTI Corporation, Brookfield WI). We compared results of TMS with our standard T-cell flow cytometry XM (T-FCXM) and C'-dependent cytotoxic (CDC) XM on 66 sera and 20 donors. Correlation between TMS and T-FCXM (Figure 1) was 76% ($p<0.05$). Relative to FCXM, Sensitivity of TMS (likelihood of TP vs. FN) = 67%, Specificity = 82% (TN vs. FP); Pos Predictive Value = 72% (TP vs. FP); Neg Predictive Value = 78% (TN vs. FN). Because FN may be due to antibodies toward non-HLA antigens (TMS undetectable), "FN" samples devoid of anti-HLA class I antibodies by FC PRA were excluded. The high level of FN results shown suggests that T-FCXM is the more sensitive assay. Comparing TMS with CDC XM in terms of ultimately prediction of a positive T-FCXM demonstrated that PPV of TMS=72% vs. 30% for CDC. Thus, the use of TMS in the preliminary screening XM for cadaveric donors could reduce the number of final, T-FCXM positive cases, saving time, labor, and resources. In one case of a patient known to have anomalous non-HLA Abs which reacted in FCXM and on flow PRA beads, TMS was negative XM on a donor with which the patient was successfully transplanted. Thus, TMS appears potentially beneficial for use with patients having auto- or non-HLA antibodies, and because it does not require viable target cells, for monitoring post-transplantation donor-specific antibodies using frozen lysates of donor antigens. Given these important advantages over standard methods, further testing of the clinical relevance of TMS XM is warranted.

		TMS Results	
		Pos	Neg
FCXM	Pos	18 True Pos (TP)	9 False Neg (FN)
	Neg	7 False Pos (FP)	32 True Neg (TN)

Abstract# 643 Poster Board #-Session: P99-II

DRB GENOTYPING OF CYNOMOLGUS AS A WAY TO SELECT HISTOINCOMPATIBLE RECIPIENT-DONOR PAIRS INVOLVED IN THERAPEUTIC ASSAYS FOR PREVENTION, OR TREATMENT, OF ALLOGRAFT REJECTION. Antoine Blancher,[1] Pierre Tisseyre,[1] Marianne Dutaur,[1] Pol-André Apoil,[1] Claudine Maurer,[2] Valérie Quesniaux,[2] Marc Bigaud,[2] Michel Abbal.[1] *[1]Laboratoire d'Immunogenetique Moleculaire, Universite Paul Sabatier, Toulouse, France; [2]Novartis Institute for Biomedical Research, Novartis PharmaAG, Bale, Switzerland.*

Introduction : *Macaca fascicularis* (cynomolgus) is used to test new drugs or therapeutic protocols to prevent allograft rejection. Knowledge of histoincompatibility of cynomolgus donor-recipient graft pairs is crucial to assess efficiency of new therapies. Histoincompatibility can be checked by performing *in vitro* mixed lymphocyte reaction (MLR) or by MHC genotyping. In Man, DRB is responsible for positive *in vitro* MLR responses, and is sufficient to induce *in vivo* alloimmunization and allograft rejection. We have focused our work on cynomolgus DRB genotyping which is based on the extensive allelic polymorphism of DRB exon 2 sequences. **Methods :** Allelic DRB exon 2 amplicons were separated by denaturating gradient gel electrophoresis (DGGE) and sequenced. Simple or double way MLR were carried out classically with peripheral blood mononuclear cells (PBMC) enriched by Ficoll flotation. **Results :** By studying 504 unrelated animals, we have characterized 67 different DRB exon 2 sequences. Most of them were either identical with DRB exon 2 from macaque species other than cynomolgus (*M. mulatta, M. nemestina, M. arctoides, M. silenus*), or differed from the latter by less than four nucleotide positions. Three of our exon 2 sequences were either identical (2 sequences), or differed by only one nucleotide (1 sequence), from prosimian DRB (*Galago seleganensis*). Finally, two sequences differed significantly from all other human or primate DRB exon 2. One-way MLR carried out with lymphocytes from animal pairs of identical DRB were always negative (stimulatory index mean (SIm)=0.82 N=8). One-way MLR with animal pairs having different but compatible DRB genotypes were also negative (SIm=1.3 N=21). By contrast, 239 out of 355 (67 %) MLR performed with incompatible DRB animal pairs were strongly positive (SIm=38.7). MLR performed with haplo-identical pairs of animals resulted in SI (SIm=53.9 N=24) higher than those obtained with full DRB mismatched pairs (SIm=36.3 N=215). The remaining 116 out of 355 incompatible MLR were negative (SI<10), most probably because of inhibition of MLR due to erythrocyte contamination of PBMC. **Conclusion :** Cynomolgus DRB genotyping by DGGE-sequencing of DRB exon 2 allows to assess histoincompatibility and to select histoincompatible pairs of animals required to experiment new treatments of allograft rejection.

Abstract# 644 Poster Board #-Session: P100-II

MEASUREMENT OF CHIMERISM IN CYNOMOLGUS MONKEYS USING HUMAN SPECIFIC SHORT TANDEM REPEAT (STR) BASED ASSAY. Edip Akpinar,[1] Jodie M. Keary,[2] Roger Kurlander,[2] Douglas A. Hale.[1] *[1]Transplantation Branch, NIDDK-NIH, Bethesda, MD; [2]Hematology DLM, CC-NIH, Bethesda, MD.*

Background: Prior to their application in humans, tolerance induction protocols have to be successfully vetted in nonhuman primate (NHP) models. One particularly promising approach for tolerance induction involves the generation of a state of mixed donor/recipient chimerism. Application of this approach in a cynomolgus macaque model would be facilitated by the establishment of a reliable technique for the quantitative assessment of peripheral blood mononuclear cell chimerism. **Methods:** A short tandem repeat (STR) kit (GenePrint® Fluorescent Monoplex STR Systems, Promega Corp, Madison, WI) developed for use in humans was tested to determine if it could be used to detect chimerism in NHPs. Peripheral blood samples were obtained from cynomolgus macaques prior to their use in experimental tolerance induction protocols. Genomic DNA was isolated from the samples and its concentration was determined. A total of 1 nanogram of genomic DNA was amplified with respective primers. The end product was mixed with allelic ladders and the results were read in an ABI PRISM® 3100 Avant Genetic Analyzer. **Results:** Since it is based on detection of non-expressed genomic sequences, the technique was found to be simple and rapid. Preliminary assays were first performed to define the presence of working discriminating alleles in macaque genome. These pilot assays revealed that the TPOX and CFS1PO alleles exist in both rhesus and cynomolgus species. After definition of the working alleles, we sought to determine whether these STR loci were sufficiently informative to permit discrimination between potential donor/recipient pairs. Out of 40 cynomolgus macaques investigated thus far, 2 pairs demonstrated close identity at both of these alleles and could not be discriminated by the technique. This rate implies that this technique should interfere minimally with the assignment procedure of donor-recipient pairs based upon molecular based tissue typing or highly reactive mixed lymphocyte cultures. In primates demonstrating evidence of chimerism following treatment, a quantitative assessment of the level of chimerism present can be determined by comparison of peaks obtained prior to and following treatment. **Conclusion:** Assessment of chimerism is feasible in cynomolgus monkeys, using human specific STR kits specific for TPOX and CFS1PO alleles.

Abstract# 645 Poster Board #-Session: P101-II

A NOVEL AND RAPID PHARMACODYNAMIC (PD) ASSAY MEASURES COMPLEX IMMUNOSUPPRESSIVE DRUG EFFECTS IN RATS, MONKEYS AND HUMANS. Christoph Burkhart,[1] Marc Bigaud,[1] Christoph Heusser,[1] Randall E. Morris,[1] Friedrich Raulf,[1] Gisbert Weckbecker,[1] Gabriele Weitz-Schmidt,[1] Welzenbach Karl.[1] *[1]Novartis Institutes of Biomedical Research, Dept. Transplantation & Immunology, Novartis Pharma AG, Basel, Switzerland.*

Introduction: Since T cells are central to the immune response that leads to the rejection of transplanted organs, we have established a novel sensitive pharmacodynamics (PD) assay. This assay monitors the effect of drugs applied both in vitro and as in vivo on T-cell function in whole blood. Thus, measuring the direct effect of a new potential immunosuppressant drug on T-cell function, this novel PD assays may offer a first line approach to correlate drug effects and observations in experimental animal models with clinical outcome. The goal of this study is to test the PD assay to monitor the immunosuppressive effects of NIBR-0071 a novel kinase inhibitor in rat, monkey and human.

Methods: Whole blood was stimulated with polyclonal stimuli and then assessed for T-cell activation markers and intracellular effector cytokines by 4-colour flow cytometry. Systematic exploration of stimulation protocols enabled the definition of an optimized PD assay that was then applied in rats and monkeys to analyze the effects of NIBR-0071 on T-cells in vivo. As an alternative readout to flow cytometry, quantitative PCR after in vitro stimulation was employed.

Results: In vitro stimulation with PMA/anti-CD28 mAb resulted in fast (<24hrs) and reliable up-regulation of CD25 and CD69 and increased the expression of intracellular IL-2 and TNFa in T-cells. This activation was sensitive to inhibition by NIBR-0071 with IC_{50} values in the range of 0.1 mM for rat and 1 mM for monkey and human blood. In rats, the effective exposure level of NIBR-0071 at a dose inducing prolongation of graft survival could be correlated with the inhibition of T-cell activity markers TNFa and CD25. In cynomolgus monkeys inhibitory effects of at therapeutic drug dose of NVP-2 were observed in 3 out of 4 monkeys for CD25, TNFa and IL-2 and in 2 out of 4 monkeys for CD69.

Using RT-PCR as a readout, the human T-cell activation markers IL-2, TNFa, CD154, OX40, and ICOS showed suitable stimulation index and sensitivity to standard immunosuppressants.

Conclusions: Our studies demonstrate that PD assays can predict drug efficacy after administration in vivo in rats and monkeys. Thus, PD studies using whole blood assays may be useful to select dose and type of immunosuppressants in animals and patients. In addition, this novel PD assay may accelerate the selection of lead compounds as well as foster the discovery of new mechanisms of action for compounds.

Abstract# 646 Poster Board #-Session: P102-II

IN VITRO EVALUATION OF THE EFFECTS OF CANDIDATE IMMUNOSUPPRESSIVE DRUGS: FLOW CYTOMETRY AND QUANTITATIVE REAL-TIME PCR AS TWO INDEPENDENT AND CORRELATED READ-OUTS. Mona Flores,[1] Sally Zhang,[1] Ann Ha,[1] Bari Holm,[1] Randall Morris,[1] Bruce Reitz,[1] Dominic Borie.[1] *[1]Transplantation Immunology, Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA.*

Background: Immune monitoring performed on peripheral blood from transplant recipients may use flow cytometry or molecular biology techniques. Flow cytometry assays cells that are phenotypically characterized, whereas gene monitoring techniques predominantly start with RNA extraction from unfractionated cell populations. We therefore investigated how the effects of immunosuppressive drugs on cytokine production in stimulated whole blood, as determined by flow cytometry, would correlate with those measured with quantitative real-time PCR (TaqMan® RT-PCR).

Methods: Blood drawn from cynomolgus monkeys was exposed to incremental amounts of cyclosporine (CsA; 300, 600, 900 and 1200 ng/ml) or tacrolimus (TRL; 8, 20, 40 and 80 ng/ml) before lectin stimulation *in vitro*. Blood was in parallel either stained for CD3, IFN-γ, IL-2, IL-4, and TNF-α and analyzed on a flow cytometer with various gating strategies (lymphocyte gate, $CD3^+$ gate, or an extended gate including all distinct populations) or submitted to RNA extraction for quantitation of the above mentioned cytokines mRNA transcripts using TaqMan® RT-PCR.

Results: Both methods revealed a parallel dose-dependent inhibition of cytokine production in stimulated and treated blood. The 50% inhibitory concentrations (IC_{50}'s) for T-helper 1 cytokines ranged from 511-771 ng/ml (CsA) and 15-29 ng/ml (TRL) with flow cytometry, and from 275-529 ng/ml (CsA) and 11-48 ng/ml (TRL) with TaqMan® RT-PCR. Both assays correlated well (r=0.76, Table).

Drug	Cytokine	Pearson Product Moment Correlation		
		$CD3^+$ Gate	Lymphocyte Gate	Extended Gate
Drugs (combined analysis)	IL-2	0.91	0.93	0.68
	IFN-γ	0.86	0.97	0.32
	TNF-α	0.71	0.82	0.77
	IL-4	0.72	0.84	0.71
	All Cytokines	0.76	0.85	0.54

Extending gating from the $CD3^+$ to the lymphocyte gate improved correlation (r=0.85) for all cytokines investigated. Further extending gating resulted in lower correlations. Independent of gating strategy, a high correlation (r=0.97) was observed between both assays when drug IC_{50}'s were considered.

Conclusion: Flow cytometry and TaqMan® RT-PCR may be used interchangeably to monitor the effects of candidate immunosuppressive drugs on cytokine mRNA production in lectin-stimulated whole blood. Although yet to be verified, the current data suggests that pharmacodynamic assessment of peripheral cytokine gene expression in immunosuppressed transplant recipients might use either method.

Abstract# 647 Poster Board #-Session: P103-II

PHARMACODYNAMIC MONITORING OF I.V. AND ORAL CYCLOSPORINE THERAPY IN RENAL TRANSPLANTATION. Paul A. Keown,[1] Vivien Wu,[1] Olaf Heisel,[1] Jean Shapiro, John Gill. *[1]Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada.*

Background: Pharmacokinetic (PK) monitoring and targeted exposure of CsA substantially reduces the risk of acute rejection and minimizes the risk of drug toxicity and renal injury. We postulate that pharmacodynamic (PD) monitoring will add critical information of biological effect that may facilitate the individualization of immunosuppression during the process of host-graft accommodation.

Methods: A total of 15 renal transplant patients were studied with combined PK and PD profiling. Those receiving PK-modeled iv. CsA (1.5 mg/kg bid. over 2 hours, days 1-5) were studied on days 3, 7, 14 and 28, and stable patients receiving oral CsA (mean 2 mg/Kg bid) were studied at months 3-12 and > 12 post-transplant. PD analysis was performed by measurement of intracellular IL-2 production in a single-step FACS assay, while CsA concentrations were measured using a specific Mab assay.

Results: In patients receiving iv CsA, intracellular IL-2 expression declined rapidly during the first 2 hr of the dosing interval reaching a nadir at the time of maximum CsA concentration (2 hr; 1193± 377 µg/L). On day 3, IL-2^+ $CD3^+$ cells fell from 19± 8% pre-dose to 1.1± 0.2% by 2 hr and 0.9± 0.4% by 3 hr before returning towards baseline. Pre-dose IL-2 expression declined slowly throughout the first month (day 28: 12± 10% IL-2^+ $CD3^+$ cells), and was uniformly highly suppressed at 2 hr (0.8± 0.6% IL-2 $CD3^+$ cells). There was a marked reduction in pre-dose IL-2 expression in stable patients, resulting in an almost complete disappearance of IL-2^+ $CD3^+$ cells from the peripheral circulation throughout the dosing interval. Values at 3-12 months (pre-dose: 4± 3%, and 2hr 3± 3%) and > 12 months (pre-dose: 4± 3% and 2hr 0.8± 0.1%) were similar, suggesting that immunological accommodation occurs by 3 months in quiescent patients and is reflected by sustained inhibition of IL-2 production. Molecular expression of IL-2/cell also declined markedly throughout the dosing interval although was not extinguished, (day 3: 3906± 1538 pre; 1462± 115 2 hr: day 28: 4144± 1216 pre; 1581± 763 2hr; > 12 months: 2633± 1299 pre; 2037± 859 2h).

Conclusion: PD measurement of IL-2 production (a) offers a simple, rapid and reproducible measure of biological effect, (b) corresponds closely to the PK concentration throughout the dosing interval in early phase treatment and (c) remains markedly and continuously suppressed in stable patients possibly reflecting immune accommodation; (4) a sub-set of $CD3^+$ cells remain refractory to inhibition by CsA. Their function remains uncertain.

Abstract# 648 Poster Board #-Session: P104-II

INHIBITORY ROLE OF CYCLOSPORIN A AND ITS DERIVATIVES ON REPLICATION OF HEPATITIS C VIRUS. Kunitada Shimotohno, Koichi Watashi. *Department of Viral Oncology, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Kyoto, Japan.*

HCV-associated liver failure is common indication for liver transplantation, and infection often recurs after transplantation. Histologic evidence of recurrence is apparent in approximately 50% of HCV-infected recipients in the first postoperative year. Exposure to corticosteroids is associated with higher mortality, increased HCV viremia. By contrast, it is not clear whether calcineurin inhibitors such as cyclosporin A (CsA), FK506 or azathioprine affect the histologic recurrence of HCV.

The development of anti-HCV agents has been accelerated by the establishment of cell lines in which HCV genome RNA self-replicates efficiently (referred to as HCV replicon cells). We examined the effects of various compounds on the replication of the HCV genome using HCV replicon cell lines, and observed a suppressive effect of CsA on HCV genome replication. Treatment with 1 micro gram /ml CsA for 7 days decreased the amount of HCV NS5A and NS5B proteins to undetectable levels. A similar reduction of HCV protein and RNA synthesis was not observed after treatment with FK506 (1 micro gram /ml).

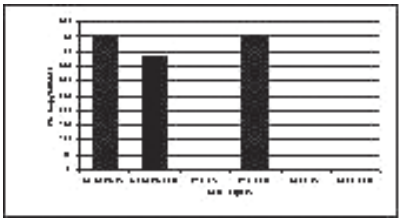
CsA seemed to exert the anti-HCV activity by the pathway independent from calcineurin-inducible signaling. This was supported by an analysis of HCV replication using the CsA derivatives, NIM811 and PSC833. NIM811 binds cyclophylins but does not inhibit calcineurin or the downstream NF-AT pathway, whereas PSC833 associates with neither cyclophilins nor the CN/NF-AT pathway. HCV replicon cells treated with NIM811 inhibited replication of HCV genome, whereas PSC833 did not affect the efficiency of HCV replication. These data indicate that the inhibitory function of CsA is mediated by a mechanism independent of its immunosuppressive function, but rather by impairment of cyclophilin function.

Cyclophilins are evolutionally conserved proteins with peptidyl-prolyl isomerase (PPI-ase) activity, which is essential for protein folding and which recently has been suggested to have a role in the regulation of transcription and differentiation. Mammalian cells contain more than 10 species of cyclophilins, and not all cyclophilin functions are explained by PPI-ase activity. Thus, identification of a cyclophilin that is essential for replication of the HCV genome sheds further light on the mechanism of HCV replication. Furthermore, our data showing an inhibitory effect of CsA on HCV genome replication highlight the importance of selecting an appropriate immunosuppressive agent in liver transplant recipients at increased risk of recurrent HCV infection.

Abstract# 649 Poster Board #-Session: P105-II

TROPISM OF BK VIRUS TO RENAL CELLS AND THE EFFECT OF IMMUNOSUPPRESSANTS ON VIRAL CYTOPATHIC EFFECT IN A TISSUE CULTURE MODEL. Brahm Vasudev,[1] Rimas Orentas,[2] Joanna Walczak,[2] Sundaram Hariharan.[1] *[1]Department of Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]Department of Pediatric Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI.*

Background: BK Virus (BKV) infection in Renal Transplant recipients manifests as BK Virus Nephropathy (BKVN). We hypothesized that BKVN occurs due to a combination of viral tropism and pharmacological effects of immunosuppressive agents.
Methods: Human Embryonic Kidney Cells (HEK293), Proximal Tubular Epithelial (PTE) and HeLa cells (Non-renal) were analyzed for susceptibility to BKV. These cells were exposed to BKV stocks propagated in HEK293 cells. Eleven days following infection, culture supernatant and cells were collected and DNA was isolated for the detection of BKV by real time quantitative PCR. Pharmacological studies were carried out with HEK cells in tissue culture. At 60% confluency, cell monolayers were infected with BKV in the presence of 1000 ng/ml CsA (Well A), 125 ng/ml Tac (Well B), 25 micro mol/ml MMF (Well C) and BKV alone (Well D/Control). On day 6 cells were fixed and stained with methylene blue and cytopathic effects were examined.
Results: TROPISM: Both supernatant and cell-derived DNA from HEK293 contained BKV. In the PTE cells, only cell-derived DNA demonstrated BKV DNA. No viral copies were detected in HeLa cell cultures (Figure 1). CYTOPATHIC EFFECT: Differential cytopathic effects were seen in that maximum cytopathic effects was seen in well C (BKV+MMF) with almost complete cytolysis and clearing of cells and little or no cytopathic effect was seen in wells A, B and D.
Conclusions: These experiments demonstrate that 1) BKV has tropism to renal cells as the infection occurred in HEK293 and PTE cells and not in HeLa cells. 2) Viral cytopathic effects were more pronounced in tissue cultures with BKV and MMF and less pronounced with BKV and CsA/Tac. These experiments show that susceptibility to BKV cytopathic effects is likely a combination of viral tropism to renal cells and potentially the pharmacological effect of immunosuppressive agents.



Abstract# 650 Poster Board #-Session: P106-II

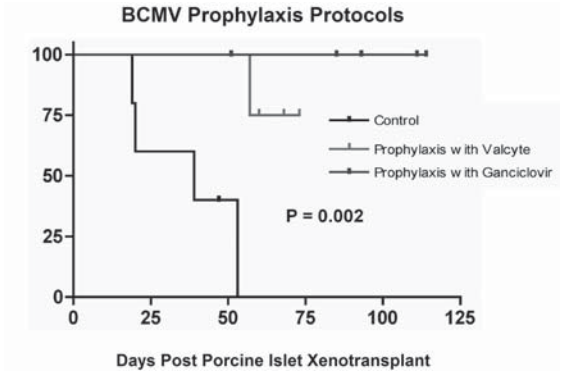
REGULATION OF ANTI-EBV AND ANTI-CMV CYTOTOXIC T-CELL RESPONSES BY IMMUNOSUPPRESSIVE MEDICATIONS. Raju K. Radha,[1] Tetsu Sado,[1] Andy Pao,[1] Stanley C. Jordan,[1] Mieko Toyoda.[1] *[1]Pediatric Nephrology, Cedars-Sinai Medical Center, Los Angeles, CA.*

Introduction: Immunosuppressive drugs are associated with increased risk for viral infections. Immunosuppression inhibits viral-specific immunity, but this is difficult to quantify. Calcineurin inhibitors such as CsA primarily suppress T cells but have also been shown to inhibit B cells, macrophages and dendritic cells which can affect anti-viral immunity. In this study, we examined the effects of CsA and FK506 on anti-CMV and anti-EBV T cell responses using Cytokine Flowcytometry (CFC).
Methods: Blood samples from 3 normal healthy subjects who had demonstrable Anti-viral T cell responses were used for the study. Inhibition of anti-viral activity by CsA and FK506 was examined at trough and C2 concentrations (CsA 250, 1000 ng/ml; FK 10, 40 ng/ml respectively). Mycophenolic Acid (MPA) and Methyl Prednisolone were used at 20 mcg/ml and 100 mcg/ml respectively. For CFC assays, whole blood was incubated with CMV or EBV lysates and Brefeldin A for 6 hours, which inhibits Golgi secretion of cytokines and allows intracellular staining with Anti-IFNg antibody. All measurements are described as Mean ± SEM values. Percentage inhibition was calculated as the decrease in IFN-g positive population.
Results: At trough levels, CsA decreased cytokine positive cells by 2.3 ± 2.3 % in the CD8 T cell population and 7 ± 4.3% in the CD4 T cell population. Similar levels of inhibition was seen with FK (8.3 ± 4.6% of CD8 T cells and 10 ± 5.29% of CD4 T cells). At C2 levels, CsA caused severe inhibition of cytokine responses in CD8 and CD4 T cells (63.9 ± 15.1% of CD8 and 63.3 ± 12.8% of CD4 respectively). However, inhibition was lower with FK (20.3 ± 7.4% of CD8 and 10 ± 8.08% of CD4 respectively). Addition of MPA and methyl prednisolone to trough levels of CsA or FK completely inhibited virus-specific T cell cytokine production.
Conclusions: 1. Cytokine Flowcytometry can be used to measure the effects of CsA and FK on anti-viral T cell responses. 2. Inhibition of CD8 and CD4 anti-viral activity was minimal at acceptable trough levels. At C2 levels, there was profound inhibition with CsA and significantly lower inhibition with FK. 3. Addition of MPA and steroids causes further reduction in T cell activity. Taken together, these results suggest a cyclical pattern of anti-viral activity based on pharmacokinetics in transplant recipients. 4. CFC may provide an objectiveand quantifiable immunologically relevant parameter to monitor and possibly reduce immunosuppression in transplant recipients with viral infections.

Abstract# 651 Poster Board #-Session: P107-II

GANCICLOVIR (GCV) PROPHYLAXIS AGAINST BABOON CYTOMEGALOVIRUS (BCMV) INFECTION IN PIG-TO-NON HUMAN PRIMATE (NPH) ISLET XENOTRANSPLATION. Tun Jie,[1] Melanie G. Thompson,[1] Tor C. Aasheim,[1] Martin Wijkstrom,[1] Sue Clemmings,[1] Henk Schuurman,[2] Bernhard J. Hering.[1] *[1]Department of Surgery, University of Minnesota, Minneapolis, MN; [2]Immerge BioTherapeutics, Boston, MA.*

BCMV infection has significant negative impact on graft function and recipient survival in pig-to-NHP islet xenotransplantation. This study sought to establish a safe and effective prophylaxis protocol against BCMV in NPH porcine islet xenograft recipient. Previously, 5 cynomolgus monkeys received porcine islet transplant after diabetes induction with streptozotocin. 3 of 5 animals developed clinical signs of CMV pneumonia and died with functional islet grafts (post transplant day [PTD]19, 20, 39). The two remaining animals were sacrificed because of graft rejection (PTD 47, 53), and one was found to have severe systemic CMV disease postmortemly. CMV infections were confirmed by PCR analysis for BCMV DNA on tissues from lung, liver and kidney. Since then, we instituted a BCMV prophylaxis protocol to cover the duration while animals received immunosuppressive mediations. 4 NHPs received either oral valganciclovir (15 – 20 mg/kg per day) and occasion intravenous GCV (5 – 7.5 mg/kg per day). Only one animal developed CMV disease and was sacrificed because of xenograft rejection (PTD 57). Since the bioavailability of oral valganciclovir was uncertain in NHP, we modified the protocol (as suggested by M. Jonker) to intramuscular injection of GCV (2.5 mg/kg per day), covering only the peri-induction period (PTD -7 to +21). Six NHPs that received this prophylaxis protocol remained free of BCMV infection (no sign of CMV disease and negative PCR analysis for BCMV on these animals' PBMC). Functional porcine islet xenografts were documented beyond 51 days. In addition, no side effects were seen in animals received valganciclovir or GCV.
GCV is a safe and effective prophylactic agent against BCMV infection in NHP xenograft recipients. PCR analysis for BCMV is a valuable diagnostic test for BCMV disease in NHPs.



Abstract# 652 Poster Board #-Session: P108-II

RAPAMYCIN-INDUCES ENDOTHELIAL CELL DEATH, LEADING TO TUMOR-VESSEL THROMBOSIS AND ANTITUMOR ACTIVITY. Edward K. Geissler,[1] Gudrun E. Koehl,[1] Markus Guba,[2] Markus Steinbauer,[1] Karl-Walter Jauch,[2] Christiane J. Bruns.[2] [1]*Surgery, University of Regensburg, Regensburg, Germany;* [2]*Surgery, Ludwig-Maximilians-University, Munich, Germany.*

Background: Recently we have shown rapamycin (RAPA) inhibits tumors by an antiangiogenic effect. Here we examined the effect of RAPA on human pancreatic cancer in immunodeficient mice, and took a closer look at the vascular development and dynamics within the tumor. **Methods**: L3.6pl human pancreatic cancer cells were orthotopically implanted into Balb/c nu/nu (nude) mice. Tumors were established for 7d before beginning treatment with 1.5 mg/kg/d (i.p.) RAPA. Tumor size and histology (H/E) were evaluated on d28. Tumors were also double-stained with anti-CD31 antibody (endothelial cell) and TUNEL to examine endothelial cell apoptosis. To study tumor angiogenesis, L3.6pl cells were implanted into dorsal skin-fold chambers (DSFC) of nude mice. Tumor vascularization, under daily RAPA treatment, was evaluated by intravital microscopy. In addition, thrombosis was induced in DSFC tumors by a method whereby i.v. FITC-dextran and UV-light (1010 mW/cm²) promote vessel clotting in a chosen microscopic field. Thrombosis was visualized by observing the flow of fluorescently-labeled RBCs (i.v.-injected). **Results**: RAPA treatment of established tumors markedly decreased their volume (388±5 mm³, n=10), vs. saline-treated controls (1,672±144 mm³, n=8, p<0.0001). H/E staining revealed a striking feature that RAPA-treated tumors develop extensive vascular thrombosis. Immunohistochemically, endothelial cells ($CD31^+$) of tumor vessels with thrombosis were TUNEL positive, suggesting apoptosis. Adjacent normal pancreatic tissue showed no evidence of either thrombosis or endothelial-cell death. DSFC analysis of L3.6pl tumor vessel growth under RAPA treatment showed abnormally dilated, irregular, vascular structures. Interestingly, following thrombosis induction with FITC-dextran and UV-light, saline-injected controls showed complete blood-flow blockage at 18.3±2.1 min (n=3), but RAPA-treatment caused thrombosis by 6.7±1.1 min (n=3). When thrombosis was induced in normal vessels outside the tumor area, no acceleration of thrombosis occurred with RAPA use. **Conclusion**: We conclude that: (1) RAPA effectively inhibits the growth of a human pancreatic tumor in nude mice, (2) blood vessels within pancreatic tumors are susceptible to thrombosis with RAPA treatment, suggesting a novel antitumor mechanism, (3) thrombosis with RAPA correlates with endothelial cell apoptosis, and (4) the thrombogenic effect of RAPA appears to affect developing, but not established, blood vessels.

Abstract# 653 Poster Board #-Session: P109-II

RAPAMYCIN AMELIORATES THE EFFECT OF COLD ISCHEMIA IN EXPERIMENTAL TRANSPLANT ARTERIOSCLEROSIS. Felipe G. Balbontin,[2] Jim R. Write,[1] Weimin You,[1] Bao You Xu,[1] Ping Wu,[1] Rasha Salih,[1] Albert Fraser,[1] Joseph G. Lawen.[1] [1]*Kidney Transplant Program, Queen Elizabeth II Health Sciences Centre, Halifax, NS, Canada;* [2]*Urology, Hospital del Salvador, Santiago, Chile.*

Cold ischemia time (CIT) and its main consequence, delayed graft function, have been recognised as significant contributing factors for chronic rejection and its hallmark feature, transplant arteriosclerosis (TA). Rapamycin (RPM) has been shown to inhibit growth factor action on immune and non-immune cells. The aim of this study is to evaluate the impact of CIT and RPM on graft aortic arteriosclerosis in a syngenic rat model devoid of immunologic effects.

Male Lewis rats (weight 250-300grs) served as donors and recipients of syngenic aortic interposition grafts. Segments of thoracic aorta, 1-1.5cms were transplanted end to end to infrarenal recipient aortas. Grafts were preserved in cold (4°C) Eurocollins solution for 0 or 24 hrs. RPM (2mg/kg) in the form of Rapamune was administrated daily by gavage. Controls received only Rapamune base (Phosal). After 8 weeks animals were sacrificed and computer assisted morphometric studies were performed. Area of intimal thickness (IT), media and its relation to total vessel were calculated. Results are expressed as percent intima or media area / media + intima area (mean ± SD). At least 3 different segments of aorta were assessed in each animal. RPM levels were measured at 12 weeks. Four experimental groups were formed: Grp A: CIT 0 hr (n=4), Grp B: CIT24 hr (n=8), Grp C: CIT 0hr + RPM (n=3), Grp D: CIT 24 hr + RPM (n=5).

RPM level at 2 months was 4.02 ± 2.84ng/L. Addition of RPM significantly decreased IT and medial atrophy and was significantly different from controls.

Percent of Intimal Thickness (IT) and Media Area in Four Experimental Groups

Experimental Groups	IT(%)*	Media (%)*
A. CI 0 hr. + Phosal	0.22± 0.08	0.77± 0.081
B. CIT 24 hr + Phosal	0.52± 0.064	0.48± 0.06
C. CIT 0 hr + RPM	0.18± 0.034	0.82± 0.034
D. CIT 24 hr + RPM	0.37± 0.071	0.63± 0.07

*p<0.01 A vs. B and B vs. C. p<0.05 B vs. D, A vs. D and C vs. D. p=N.S A vs. C

RPM decreased cold ischemia induced TA in this syngenic rat aortic transplant model.

Abstract# 654 Poster Board #-Session: P110-II

DIRECT PORTACAVAL SHUNTING AT THE TIME OF 95% HEPATECTOMY PREVENTS HYPERPERFUSION INJURY IN THE RAT MODEL. Tao Liu,[1] Ryan A. McTaggart,[1] John P. Roberts,[1] Sang-Mo Kang.[1] [1]*Division of Transplantation, University of California at San Francisco, San Francisco, CA.*

Introduction

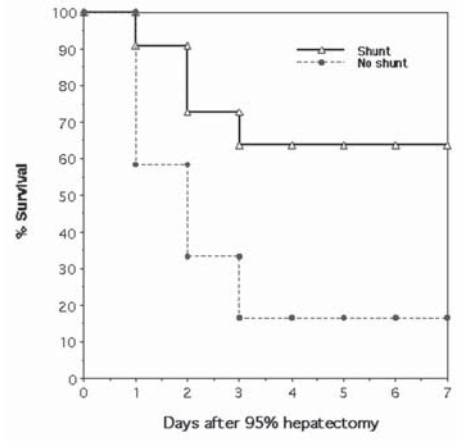
Massive hepatectomy and small-for-size liver transplantation carry a high risk of liver failure and mortality. The clinical picture is that of worsening liver function, indicating a progressive injury to the liver. Histologically, this injury is characterized by sinusoidal congestion, terminal portal venous dilatation, and fatty degeneration of hepatocytes. It has been hypothesized that the increased portal venous flow per unit liver mass leads to a "hyperperfusion injury"(HI). Prevention of HI may have great clinical impact by increasing the potential living donor pool, allowing for use of smaller liver segments, and improving the feasibility of split liver transplantation. Previous reports have suggested that creation of a portasystemic shunt can prevent HI. However, these studies were limited by the need to use large animals or, in the rat, the use of subcutaneous splenic transposition to model a portasystemic shunt. To develop a useful pre-clinical animal model, we tested whether a direct surgical portacaval shunt (PCS) placed at the time of massive (95%) hepatectomy would prevent HI in rats.

Methods and Results

Male Wistar rats underwent either hepatectomy alone (n=12) or side-to-side PCS immediately prior to hepatectomy (n=11). All animals were sacrificed at 7 days. Only 17% of control rats survived 7 days, compared to 63% in the PCS group (Figure). All livers were examined at death or sacrifice. Remarkably, no evidence of portal venule and sinusoid expansion was found in the PCS group, with nearly normal liver lobules. Regeneration was maintained in the PCS group, with an approximate 12 to 16 fold increase in liver weight by day 7 in the survivors.

Conclusion

We have developed a rat model of HI as well as a pre-clinical model of HI prevention. Hopefully, this model will allow a detailed analysis of the physiological and molecular mechanisms underlying HI, and begin to reveal potential methods for its prevention.



Abstract# 655 Poster Board #-Session: P111-II

EX VIVO PROTEIN TRANSDUCTION: SUCCESSFUL DELIVERY OF A BIOLOGICALLY ACTIVE PROTEIN INTO DONOR LIVER. Takashi Kaizu,[1] Khaja K. Rehman,[2] Paul D. Robbins,[2] David A. Geller,[1] Noriko Murase.[1] [1]*Surgery, University of Pittsburgh, Pittsburgh, PA;* [2]*Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA.*

Background: TAT protein transduction domain (PTD) has been shown to efficiently deliver fused proteins in both *in vitro* and *in vivo* models. We have previously shown excellent adenoviral gene transfection to donor liver grafts. Although viral vectors offer effective gene transduction, complications associated with viral vectors remain as the major concern. To further expand the transduction method, this study investigated TAT fusion protein delivery method to liver grafts. **Methods**: Orthotopic syngeneic LEW rat liver transplant (OLT) was performed with 18 hrs cold preservation in UW. TAT-β-gal marker protein (120 kDa, 73 μM/liver) or anti-apoptotic TAT-Bcl-XL protein (32 kDa, 0.08 - 8.0 μM/liver), was injected into liver grafts with clamp technique (CT) where fusion proteins were *ex vivo* infused into the harvested liver via portal vein and hepatic artery. By clamping outflow, infused TAT fusion proteins were trapped in the liver during the cold preservation. A non-fused TAT or control β-gal (without TAT) was used as controls. Efficacy of transduction was assessed by western blot and immunohistochemistry during preservation period and after OLT. Serum AST and

histopathology were assessed to confirm non-toxic nature of fusion protein transduction method. **Results**: Injection of TAT-β-gal to the excised liver induced rapid β-gal protein transduction at 1 hr after injection. X-gal stain was maintained for 18 hrs of preservation period (18±2.5 % at 18 hrs), however, positive staining was decreased to 6.8±1.5 % at 6 hrs after OLT, and no X-gal stain was observed at 48 hrs. Administration of TAT-Bcl-XL also induced marked transduction of Bcl-XL protein in donor liver, which was retained during whole preservation period. Quantity of protein transduction in the liver appeared to be dose-dependent. Immunohistochemistry of X-gal and Bcl-XL revealed that proteins were transduced into both sinusoidal endotherial cells and hepatocytes. There was no positive stain in control TAT- or β-gal-injected liver grafts. Fusion protein injections did not exacerbate cold hepatic I/R injury assessed by serum AST levels and histopathology. **Conclusion**: These results demonstrate that *ex vivo* TAT fusion protein delivery to the donor liver with CT leads to successful transduction of not only β-gal control peptide but also of functional anti-apoptotic Bcl-XL protein without hepatic toxicity. Although the transduction period is brief, *ex vivo* fusion protein delivery system could be a safety therapeutic method for transplantation.

Infection and Non-Organ Specific Immunosuppression

Abstract# 656 Poster Board #-Session: P112-II

SEROLOGIC IMMUNITY TO DIPHTHERIA, TETANUS, AND MEASLES AFTER PEDIATRIC SOLID ORGAN TRANSPLANTATION. Camille Sabella,[1] Lara A. Danziger-Isakov,[1] Robert J. Cunningham III,[1] Richard Sterba,[1] Deepa Chand,[1] Carla Saracusa,[1] Julie H. Corder,[1] Johanna Goldfarb.[1] *[1]Pediatrics, The Children's Hospital, The Cleveland Clinic, Cleveland, OH.*

Background: It is unclear whether pediatric solid organ transplant recipients maintain serologic immunity to pre-transplant immunizations. **Methods**: Children undergoing orthotopic heart transplantation (OHT) or renal transplantation (RT) at our institution between 11/97 and 6/02 underwent serologic testing for diphtheria, tetanus, and measles. Pre-transplant, 1 y and 2 y post-transplant titers were measured. **Results**: Thirty-seven children underwent OHT or RT during this time period (27 OHT and 10 RT). The mean age was 8.9 y (range 1m-20y). 34/37 patients had completed their primary immunization series for diphtheria and tetanus and 32/37 for measles prior to transplantation. Pre-transplant titers to diphtheria and tetanus were available for 29 patients; 28 demonstrated immunity pre-transplant and all 28 maintained serologic evidence of immunity at 1 year post-transplant. At two years, 20/21 and 18/19 maintained immunity to diphtheria and tetanus, respectively. Pre-transplant titers to diphtheria and tetanus were not available for 8 patients, but all had post-transplant serologic evidence of immunity at one or two years post-transplant. Pre-transplant titers to measles were available for 23 patients; 18 demonstrated immunity pre-transplant. Immunity persisted in 17 of 18 and 11 of 11 available at one and two years, respectively. Pre-transplant titers to measles were not available for 13 patients. Eight of these were subsequently shown to have immunity at one or two years post-transplant. The 5 patients who did not have post-transplant immunity to measles had never been vaccinated with measles vaccine. Pre- and post-transplant geometric mean titers (IU/ml) were similar for diphtheria (0.78 vs 0.41), tetanus (2.47 vs 3.7), and measles (2.6 vs 2.3). These titers were not significantly different for the OHT vs RT group. **Conclusions:** Pediatric patients undergoing solid organ transplantation maintain serologic evidence of immunity post-transplantation. This underscores the importance of pre-transplant vaccination in patients being evaluated for cardiac and renal transplantation.

Abstract# 657 Poster Board #-Session: P113-II

EFFECTIVENESS OF LOW DOSE VALGANCICLOVIR IN KIDNEY AND LIVER TRANSPLANT RECIPIENTS. Robert Dupuis,[1] Derrick Van Beuge,[1] Ruthann Conoley,[1] Kenneth Andreoni,[1] David Gerber,[1] Jeffrey Fair,[1] Randy Detwiler,[2] Roshan Shrestha,[2] Robert Watson,[1] Mark Johnson.[1] *[1]Transplant Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Introduction: Valganciclovir has recently been approved for prevention of CMV in transplant recipients at a starting regimen of 900mg per day. Limited data suggested a lower dose of 450mg per day may be effective.

Purpose: To evaluate our experience with a low dose regimen of Valganciclovir in kidney and liver transplant recipients for CMV prevention.

Methods: All kidney or liver transplant recipients who received Valganciclovir between 7/02 and 8/03 were included in this analysis. All patients were started on a regimen of 450mg once a day, adjusted for renal function, for three months after transplant. All patients were monitored via CMV pp65 antigen levels every 2 weeks.

Kidney transplants received tacrolimus, mycophenolate, prednisone +/- thymoglobulin or basiliximab. Liver transplants received tacrolimus, prednisone +/- thymoglobulin.

Results: There were 91 patients; 60 kidney and 31 liver transplant recipients who received Valganciclovir prophylaxis. The length of followup was on average 8 months (range 4-18 months) after transplant. Further results are presented in table below.

	Kidney	Liver
CMV status (%)		
D+/R-	33	13
D+/R+	37	55
D-/R+	22	22
D-/R-	8	10
CMV antigenemia &/or disease (%)	12/60 (20%)	4/31(13%)
	6/12 D+/R-	2/4 D+/R-
	3/12 D+/R-	2/4 D+/R+
	3/12 D+/R+	
Number of days to CMV (mean)	100	67
range	42-154	14-150

All patients who developed antigenemia &/or sypmtoms did so within 6 months of transplant. However 3 kidney transplants and 2 liver transplants had evidence of CMV while on low dose valganciclovir. All 4 liver transplants had only positive antigenemia., whereas 7/12 kidney transplants had both positive antigenemia and symptoms. All 4 liver transplants with CMV had received thymoglobulin whereas 10/12 kidney transplants received thymoglobulin. Overall adverse effects included leukopenia (4 patients), pancytopenia (1 patient) and drug fever (1 patient).

Conclusion: Low dose valganciclovir was effective in the majority of patients. However patients who were D+/R- and who received thymoglobulin were more likely to develop CMV and in some cases while on therapy. In these patients a higher dose regimen may be more appropriate.

Abstract# 658 Poster Board #-Session: P114-II

ORAL GANCICLOVIR AND VALGANCICLOVIR PROPHYLAXIS MAY PREVENT REACTIVATION OF NON-β HERPESVIRUSES IN CYTOMEGALOVIRUS (CMV) D+/R- SOLID ORGAN TRANSPLANT (SOT) PATIENTS. Raymund R. Razonable,[1] Robert A. Brown,[1] Atul Humar,[2] Emma Covington,[3] Emma Alecock,[3] Carlos V. Paya,[1] the PV16000 Study Group. *[1]Infectious Diseases, Mayo Clinic, Rochester, MN; [2]Infectious Diseases, University of Toronto, Toronto, ON, Canada; [3]Medical Science, Roche Products Limited, Welwyn Garden City, United Kingdom.*

BACKGROUND: CMV D+/R- SOT patients are at high risk of CMV, EBV, and other viral infections. The resulting CMV and EBV interaction increases the risk of EBV-PTLD. Oral ganciclovir (OGCV) and valganciclovir (VGCV) prophylaxis were shown in a randomized trial to be effective in reducing CMV replication and disease in CMV D+/R- SOT patients. We performed this follow up study to evaluate the impact of anti-CMV prophylaxis on EBV replication. We also assessed whether OGCV and VGCV prophylaxis modified the natural history of clinical and subclinical human herpesvirus (HHV)-8 and varicella zoster virus (VZV) reactivation, both of which occur in up to 20% of predisposed SOT patients.

METHODS: 263 liver, heart, kidney, and kidney-pancreas SOT patients who received OGCV (n=95) or VGCV (n=168) for 100 days were enrolled. Peripheral blood samples were collected from all patients prior to (baseline) and during (days 14, 42, 70, and 100) antiviral prophylaxis, and at months 4, 4.5, 6, 8, and 12 post-SOT and were quantified for EBV, HHV-8, and VZV DNA using a LightCycler-based PCR assay (lowest limit of detection, 1 copy).

RESULTS: HHV-8 and VZV DNAemia were not detected in any of the 2,232 blood samples tested. In contrast, EBV DNAemia was common; it was detected in 56% of patients. The overall prevalence of EBV DNAemia was comparable between patients who received OGCV and VGCV and it was highest (23.6%) at day 14 and lowest (9.5%) at the end of prophylaxis. The degree of EBV DNAemia was low-level in the majority of patients; EBV DNA ≥10^3/ml was detected in only 7.6%. Patients who received OGCV were more likely than those who received VGCV to have higher levels of EBV DNAemia (EBV DNA ≥2x10^3/ml: 6.3% OGCV vs. 2.4% VGCV; EBV DNA ≥5x10^3/ml: 5.3% OGCV vs. 0.6% VGCV).

CONCLUSIONS: This study highlights the absence of HHV-8 and VZV viremia during the first year post-SOT in a large international cohort of patients who received OGCV or VGCV prophylaxis. It is possible that the anti-CMV prophylaxis have also prevented the reactivation of HHV-8 and VZV. In contrast, EBV DNAemia was common, although higher levels of EBV replication were observed only in a minority of CMV D+/R- SOT patients. Indeed, VGCV may have prevented higher levels of EBV replication. The correlation between EBV replication and other clinical outcomes is being investigated.

Abstract# 659 Poster Board #-Session: P115-II

USE OF VALGANCYCLOVIR IN THE PROPHYLAXIS; PREEMPTIVE AND DIRECTED THERAPY FOLLOWING SOLID ORGAN TRANSPLANTATION. Hugo Bonatti,[1] Ferguel Cakar,[1] Daniel Hoefer,[2] Claudia Boesmueller,[1] Elfriede Ruttmann,[2] Herwig Antretter,[2] Ludwig Mueller,[2] Wolfgang Steurer,[1] Paul Hengster,[1] Christian Geltner,[2] Clara Larcher,[3] Guenther Laufer,[2] Raimund Margreiter.[1] *[1]Department of General and Transplant Surgery, University Hospital, Innsbruck, Austria; [2]Cardiac Surgery, University Hospital, Innsbruck, Austria; [3]Institute for Hygiene, University Hospital, Innsbruck, Austria.*

BACKGROUND: CMV is the most common virus complicating solid organ transplantation. Oral Gancyclovir is poorly absorbed, however recently the Valin Ester of the drug with improved oral availability was introduced into clinical practice.

AIM: To retrospectively review our experience with Valgancyclovir (VGCV) in the prophylaxis and treatment of CMV infection and disease on a multiorgan transplant unit.

PATIENTS AND METHODS: Between 1.6.2002 and 31.6.2003 a total of 288 solid organ transplants were performed at the Innsbruck University hospital. Standard immunosuppression consisted of Calcineurin inhibitor based triple drug therapy with/ without ATG or IL2 receptor antagonist induction. A total of 98 patients (34% of patients transplanted during the study period) received VGCV including 20 kidney, 15 pancreas, 14 liver, 21 heart, 24 lung, 2 bowel, 1 islet and one limb recipient. CMV mismatched lung transplants also received anti CMV hyperimmunoglobulin.

RESULTS: Valgancyclovir was given for prophylaxis for 3 months in the case of CMV mismatched transplantation (n=40), retransplants (n=8), and high level of immunosuppression (n=28). The latter included all heart and all lung transplants except for CMV negative/negative match and the limb recipient. The remaining patients received ValGCV pre-emptively due to a positive CMV assay and in one case for CMV disease. All cases of CMV infection were successfully treated and none of the patients who received prophylaxis developed CMV infection while on VGCV. Six cases of neutropenia were observed. Thirteen patients developed CMV infection/disease after withdrawal of VGC prophylaxis. Three patients developed breakthrough CMV disease, only in one case a UL97 mutant (GCV resistant strain) was isolated and this patient was successfully treated with Cidofovir. The other two patients responded to i.v GCV followed by a second course of VGCV. All ten cases of CMV infection responded to a second course of i.v. GCV or VGCV.

CONCLUSIONS: VGCV was found highly active in prevention and treatment of CMV infection and disease in SO recipients. Nevertheless, in the CMV mismatched population CMV replication after withdrawal of VGCV is common. Due to the favourable pharmacokinetics the drug should replace conventional poorly orally absorbed GCV.

Abstract# 660 Poster Board #-Session: P116-II

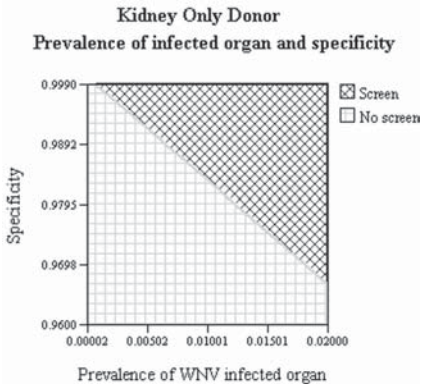
SCREENING FOR WEST NILE VIRUS IN DECEASED ORGAN DONORS. Bryce Kiberd,[1] Kevin Forward.[1] *[1]Medicine, Dalhousie University, Halifax, NS, Canada.*

Given the recent reports of WNV transmission by an organ donor, screening may become mandatory. This study examined the societal impact of screening deceased organ donors for WNV by medical decision analysis.

In the no screen arm we assumed that the infection rate was similar to that in the (1/4160 range 1/10,000-1/50) blood donor population. If transplanted, disease transmission was 100% with a 50% (25-75%) case fatality rate. In the screen arm, donor organs screening positive were not used for transplant. We projected years of life lost (gained) by the use of such a screening strategy. A range in infected donor prevalence, case fatality rate, and test sensitivities/specificities were explored. Patient survival probabilities for heart, liver and kidney transplant were taken from the literature. Analysis was performed in Data 4.0.

In the base case analysis (RT-PCR test 99.0% specific and 95% sensitive) screening will cause a net loss of life from discarded organs that are false positive for infection. Using 2002 transplant donor rates, up to 694 (Heart 141; Liver 314; Kidney 239) potential life years could be lost. The prevalence of infective donors, test specificity, mortality on wait list and case fatal rate are important input variables. With current prevalence rates the test specificity should exceed 99.9% even in seasonal high risk areas for heart and liver donors. However the test could be less specific for screening **kidney only** deceased donors (see figure).

Better understanding of the test performance and turn around time are required. Strategies might include selective testing in seasonal high-risk areas (kidney donor only) or donor recipient matching (D+/R+). It must be emphasized that this is the societal perspective. From an individual perspective an allocation scheme that is wait time dominant, waiting for the next kidney is not a substantial penalty and screening will be preferred. The issues of screening scarce organs are complex, different for different organs and different than screening blood/tissues.



Abstract# 661 Poster Board #-Session: P117-II

REACTIVITY OF PLATELIA™ *ASPERGILLUS* GALACTOMANNAN (GM) WITH PIPERACILLIN-TAZOBACTAM: CLINICAL IMPLICATIONS BASED ON ACHIEVABLE SERUM CONCENTRATIONS. N. Singh,[1] A. Obman,[1] S. Husain,[1] S. Mietzner,[1] J. E. Stout.[1] *[1]VAMC and Univ.of Pitt, Pittsburgh, PA.*

Background: The detection of GM by Platelia *Aspergillus* EIA has proven useful for the diagnosis of invasive aspergillosis and has recently received FDA clearance. Cross-reactivity with GM of *Penicillium* spp. has been noted and EIA reactivity with drugs of fungal origin is potentially possible (Mycoses 97). We assessed if commonly used antibiotics (of fungal, nonfungal or synthetic origin) tested positive for GM (Index >0.50) and determined if achievable serum concentrations of these antibiotics based on a normal dosing regimen could potentially result in positive tests.

Methods: Antibiotics tested included amoxicillin, ampicillin-sulbactam, nafcillin, piperacillin, piperacillin-tazobactam, cefazolin, ceftazidime, gentamicin, erythromycin and levofloxacin.Drugs that tested positive for GM as undiluted samples were further tested at achievable serum concentrations and minimal serum concentrations. For the latter experiment, the drug was diluted in serum pre-tested and shown to be negative for GM (Index < 0.20). Antibiotic dilutions tested were based on the peak achievable serum level for each drug as the target concentration, with one 2-fold dilution above and 2 serial dilutions below the target level.

Results: Undiluted samples of piperacillin-tazobactam and piperacillin tested positive, whereas, those of amoxicillin, ampicillin-sulbactam, nafcillin, cefazolin, ceftazidime, erythromycin, gentamicin, levofloxacin tested GM negative.However,all 3 lots of piperacillin-tazobactam and all bags within each lot tested GM positive with Index value of >5.168(corresponding to an optical density out of the maximal range of absorbance of the plate reader). At achievable serum concentrations (levels up to 300µg/ml) however, only 1 of 3 lots of piperacillin-tazobactam yielded a positive GM test; concentrations of 75 µg/ml, 150 µg/ml, and 300 µg/ml tested positive, whereas lower concentrations, mimicking the trough levels(10 and 5 µg/ml) tested GM negative. Piperacillin at achievable serum concentrations tested GM negative.

Conclusions:. Clinicians evaluating the results of the GM test should be aware that achievable serum concentrations of piperacillin-tazobactam could potentially result in a positive GM test in patients receiving this antibiotic.The timing of collection of serum samples may influence the test results with reactivity being less likely in samples collected at trough levels or prior to the administration of the dose.

Abstract# 662 Poster Board #-Session: P118-II

HUMAN POLYOMAVIRUS INFECTION IS COMMON IN CYTOMEGALOVIRUS (CMV) D+/R- SOLID ORGAN TRANSPLANT (SOT) PATIENTS. Raymund R. Razonable,[1] Robert A. Brown,[1] Atul Humar,[2] Emma Covington,[3] Emma Alecock,[3] Carlos V. Paya,[1] the PV16000 Study Group. *[1]Infectious Diseases, Mayo Clinic, Rochester, MN; [2]Infectious Diseases, University of Toronto, Toronto, ON, Canada; [3]Medical Science, Roche Products Limited, Welwyn Garden City, United Kingdom.*

BACKGROUND: The natural history and spectrum of clinical manifestations of the polyomaviruses BK (BKV) and JC (JCV) in SOT patients are not fully defined. While BKV is a cause of allograft dysfunction in renal SOT patients, its impact on the outcome of non-renal SOT is not known. Likewise, while JCV causes the rare progressive multifocal leukoencephalopathy, the incidence and impact of subclinical JCV reactivation have not been investigated. In this study, we assessed the incidence and relevance of clinical and subclinical BKV and JCV reactivation in renal and non-renal SOT recipients.

METHODS: 263 CMV D+/R- kidney, liver, heart, and kidney-pancreas SOT patients who received oral ganciclovir (OGCV; n=95) or valganciclovir (VGCV; n=168)

prophylaxis consented to participate in this longitudinal study, which was designed to quantify BKV and JCV DNA in blood samples that were collected prior to (baseline) and during (day 14, 42, 70 and 100) anti-CMV prophylaxis, and at months 4, 4.5, 6, 8 and 12 post-SOT. BKV and JCV DNA were quantified in 2,232 blood samples using a LightCycler-based PCR assay (lowest limit of detection, 1 copy).

RESULTS: BKV or JCV DNAemia occurred in 41 of 263 patients (15.6%) at a median of 100 days post-SOT; four patients had concomitant or sequential BKV and JCV DNAemia. BKV DNAemia (range: 2-7174 copies/ml) was observed in 32 patients (12.2%); five were treated for biopsy-proven acute renal allograft rejection. BKV DNAemia was more common in patients who received OGCV (17.9% vs. 8.9% of patients who received VGCV). Interestingly, BKV DNAemia was observed in renal [24/92 (26.1%)] and non-renal [heart, 3/45 (6.7%); liver, 5/121 (4.1%)] SOT patients. JCV DNAemia (6-220 copies/ml) was also observed in both renal [7/92 (7.6%); kidney-pancreas, 1/5 (20%)] and non-renal [heart, 3/45 (6.7%); liver, 2/121 (1.7%)] SOT patients. JCV DNAemia occurred in 3 patients (3.2%) who received OGCV and 10 patients (5.9%) who received VGCV.

CONCLUSION: This study demonstrates that (1) polyomavirus DNAemia is common during the first year post-SOT; (2) JCV reactivation is more common than what historical data suggests; and (3) BKV DNAemia occurs in both renal and non-renal SOT patients. We are investigating the clinical significance of BKV DNAemia in non-renal SOT patients, and correlating the degree of BKV and JCV reactivation with graft dysfunction, and other clinical manifestations.

Abstract# 663 Poster Board #-Session: P119-II

THE CLINICAL VALUE OF QUANTITATIVE POLYMERASE CHAIN REACTION IN CYTOMEGALOVIRUS INFECTION AFTER SOLID ORGAN TRANSPLANTATION. Josh Levitsky, Alison G. Freifeld, Kim Bargenquast, R. Brian Stevens, Andre C. Kalil. *Hepatology and Infectious Disease, The University of Nebraska Medical Center, Omaha, NE.*

BACKGROUND: Consistent data for using quantitative polymerase chain reaction (QnPCR) to predict diagnosis, disease severity, and treatment response of cytomegalovirus (CMV) infection after solid organ transplantation (SOT) is lacking.

METHODS: Twelve-month retrospective data of SOT recipients who had CMV infection with measurable QnPCR (copies/ml) were analyzed. CMV infection was divided into the following: **CMV viremia (CMV-V):** detectable QnPCR with no signs or symptoms; **CMV syndrome (CMV-S):** detectable QnPCR + ≥1 of the following: fever, malaise, leukopenia, thrombocytopenia; **CMV disease (CMV-D):** detectable QnPCR + evidence of end-organ disease.

RESULTS: 21 recipients (13 renal, 6 liver, 1 lung, and 1 cardiac) were identified: 12 males, 9 females; median age 36 (2-61); Use of FK506 (76%) or Cyclosporine (24%); History of Rejection (32%) or Other Infections (29%). CMV status: D+/R- (63%), Non D+/- (37%). Valganciclovir (59%) or Other prophylaxis (41%) was given for a median length of 90 days (21-610) after SOT. Three patients had CMV-V, two had CMV-S, and 16 had CMV-D. The median number of days from SOT to CMV infection was **2,072** (529-2,544) for CMV-V, **542** (35-1,050) for CMV-S, and **167** (39-3,568) for CMV-D. Patients with CMV-D had a higher median initial QnPCR (**175,000**; 1,670-1.7 million) than patients with CMV-S and CMV-V (**5,100**; 760-36,000) (p=0.04). There were non-statistically significant trends in differences in the median initial QnPCR among the following: Liver SOT (**108,000**; 3,400-1.6 million) vs. Other SOT (**14,000**; 760-1.7 million); Valganciclovir (**19,000**; 1,670-1.7 million) vs. Other Prophylaxis (**180,000**; 3,400-1.6 million); No Prior Rejection (**11,000**; 760-1.7 million) vs. Prior Rejection (**103,000**; 3,400-250,000). Patients with initial QnPCR ≥10,000 had a longer 'diagnosis to no viremia' interval (median **23.5 days**;10-165) than patients with initial QnPCR ≤10,000 (**13.5 days**; 7-25) (p=0.08). All patients have been followed for a median of 165.5 days (45-452) after an undetectable QnPCR.

CONCLUSION: A high initial CMV QnPCR level was associated with a statistically significant higher rate of end-organ disease. In addition, there were potential associations between high initial QnPCR and prolonged clearance of viremia, liver transplantation, prophylaxis other than valganciclovir, and a prior history of rejection. CMV QnPCR may provide useful prognostic information for patients with CMV infection after SOT.

Abstract# 664 Poster Board #-Session: P120-II

SEVEN-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN PEDIATRIC SOLID ORGAN TRANSPLANT RECIPIENTS: INTERIM RESULTS. Upton Allen,[1,2] Anne Dipchand,[2] Diane Hébert,[2] Annie Fecteau,[2] Vicky Ng,[2] David Grant,[2] Lori West,[2] Samia Wasfy.[1] *[1]Division of Infectious Diseases, Hospital for Sick Children, Toronto, ON, Canada; [2]Pediatric Academic Multiorgan Transplant Programme, Hospital for Sick Children, Toronto, ON, Canada.*

Introduction: Transplant recipients are known to be at increased risk of invasive pneumococcal disease. The 7-valent pneumococcal conjugate vaccine is recommended for use in this patient population. However data on safety and immunogenicity are lacking. This study examined the safety and immunogencity of the 7-valent pneumococcal conjugate vaccine (prevnar™) in pediatric solid organ transplant recipients.

Methods: Pediatric SOT recipients were enrolled in this study if they were < 18 years of age and were ≥ 4 months post-transplantation. The vaccination schedule involved 3 doses of prevnar™, followed by the 23-valent polysaccharide vaccine. Safety data for the first week after vaccination were categorized as 1) local reactions, 2) systemic reactions, 3) effects on allograft function. Data were summarized using descriptive statistics and addressed outcomes related to the 7-valent vaccine.

Results: Fifty-six transplant recipients received 116 doses of the 7-valent vaccine (median 3, range 1-3 doses); 35 males and 21 females. The median age of subjects was 5.1 years (range 0.63-17.9 years). There were 25 hearts (45%), 16 liver (29%), 14 renal (25%) and 1 lung (2%) transplant recipients. Vaccination was initiated at a median of 19.6 months post-transplantation (range 6-51.6). Follow-up of 116 doses that were administered have revealed 1 episode of rejection that was temporally associated with vaccination, but which was felt not to be attributable to vaccination. There were no side-effects with 70.7 % of doses of these 116 doses. The most common adverse events were mild local reactions in 12.9%, fever in 7.8% and irritability and crying in 1.7%.

Summary: Data from this pilot study indicated that the vaccine was well tolerated by pediatric transplant recipients. The observed adverse events were generally mild and self-limited. These data along with emerging immunogencity profiles will inform decision-making regarding the optimal use of this vaccine in pediatric transplant recipients.

Abstract# 665 Poster Board #-Session: P121-II

A SURVEY OF DENTAL CARE PROTOCOLS AMONG U.S. ORGAN TRANSPLANT CENTERS. Bijan Eghtesad,[1] Debra Mayher,[1] James Guggenheimer.[2] *[1]Syrgery, Transplantation, University of Pittsburgh, Pittsburgh, PA; [2]School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA.*

Untreated dental disease represents a potential risk for infection in transplant patients, but the vast transplantation literature contains few references of this complication. There is also little information with regard to dental care protocols for patients before and after organ transplantation. To obtain more definitive documentation about the policies that deal with dental care and experience with dental infections, we conducted a survey of US transplant centers. The instrument consisted of eight questions that addressed pre-transplant dental evaluation procedures, incidence of pre-and post-transplant dental infections, and recommendations for antibiotic prophylaxis with dental treatment after transplantation. Questionnaires were sent to 768 medical and/or surgical directors at all U.S. transplant centers.

Responses were received from 294 recipients (38%). Among the respondents, 80% routinely requested a pre-transplant dental evaluation, but 49% of these were only for specific organs. The occurrence of a dental infection prior to transplantation that resulted in a postponement or cancellation was reported by 38% of the respondents. Post-transplantation sepsis from a suspected dental source was acknowledged in 27% of the surveys. Prophylaxis with antibiotics prior to dental care was recommended by 83%; 77% indicated that it be used for all dental procedures, whether invasive or not. Most respondents (96%) recommended the 1997 American Heart Association endocarditis prevention regimen.

A survey of organ transplant centers has provided some information with regard to pre-transplantation dental screening, dental infections and the use of prophylactic antibiotics. Additional studies are needed in order to accrue more definitive data that will assist with the development of standardized and appropriate pre- and post-transplant dental care protocols.

Abstract# 666 Poster Board #-Session: P122-II

PREVALENCE OF BK VIRUS INFECTION IN RECIPIENTS OF NON-RENAL SOLID ORGAN TRANSPLANTS WITH CHRONIC RENAL DYSFUNCTION. Todd D. Barton,[1] Ajit P. Limaye,[2] Alden Doyle,[1] Vivek N. Ahya,[1] James N. Ferrenberg,[3] Emily A. Blumberg.[1] *[1]Internal Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA; [2]Laboratory Medicine, University of Washington, Seattle, WA; [3]Fred Hutchinson Cancer Center, University of Washington, Seattle, WA.*

Purpose: BK virus (BKV) infection is an important cause of chronic renal dysfunction (CRD) in renal transplant recipients, but the possible contribution of BKV infection to CRD in non-renal solid organ transplant (NRSOT) recipients has not been explored. We sought to characterize the prevalence of BKV infection in NRSOT patients with CRD and to describe factors associated with BKV infection in this setting.

Methods: This study was a cross-sectional study at a single U.S. university-based transplant center. Consecutive NRSOT patients with unexplained CRD >3 months post-transplant were identified through database review and provider referral. Medical records were reviewed for details of transplant-related medical history, including rejection, immunosuppression, opportunistic infections, and renal function. PCR was used to amplify BKV-specific sequences from serum and urine samples using previously described methods.

Results: Thirty-four consecutive NRSOT recipients (23 lung, 8 liver, 2 heart, 1 heart-lung) with CRD (median estimated GFR 47 mL/min, range 22-100) were enrolled at a median of 3.5 years (range 0.3-12.5 years) post-transplantation. Twenty-six (76%) patients were male, and the median age was 58 years (range 30-70). Five of 34 (15%) patients had BKV viruria (range 1040-1.8x10^6 copies/mL), but none had BKV viremia. All 5 patients with BKV viruria were recipients of heart or lung transplants. Five of 19 (26%) patients who were receiving mycophenolate mofetil (MMF) had BKV viruria, as compared to none of 15 (0%) patients not taking MMF (p=0.03). Three of 4 (75%) patients with a history of CMV disease post-transplant had BKV viruria in comparison to 2 of 30 (7%) patients with no history of CMV disease (p<0.01). Patients with BKV viruria were more likely to be over age 55 (100% vs. 69%), have a history of rejection (60% vs. 40%), be more than 5 years post-transplant (60% vs. 28%), and have received an intra-thoracic organ (100% vs. 72%), though these differences did not achieve statistical significance in this small cohort. Mean estimated GFR was similar in patients with or without BKV viruria (49 vs. 47 mL/min).

Conclusions: BKV viruria was present in 15% of NRSOT patients with unexplained CRD, all of whom were recipients of intra-thoracic organs and were receiving MMF as part of their immunosuppressive regimen. The possibility that BKV infection might contribute to CRD in this setting warrants further investigation.

Abstract# 667 Poster Board #-Session: P123-II

IMMUNOPHENOTYPIC CORRELATES OF GRAFT ADAPTATION IN STEROID-FREE PEDIATRIC LIVER-INTESTINAL TRANSPLANTATION WITH THYMOGLOBULIN (rATG) PRECONDITIONING. Rakesh Sindhi,[1] Adrianna Zeevi.[1]

Background/Purpose: Pretransplant lymphocyte depletion decreases need for maintenance immunosuppression. However, the timing of drug minimization cannot be determined by current tools for immune monitoring. *Methods*. Mechanistic monitoring was initiated prospectively in 77 consecutive pediatric recipients of Liver (LTx-41) and intestine (SBTx-36), median age 4 years (0.45-19), follow-up10 months (1-28), transplanted with steroid-free rATG preconditioning and Tacrolimus/Sirolimus (TAC/SRL) monotherapy. Patient subsets within this population, in whom longitudinal data could be analyzed were grouped as rejectors (REJ), and those with reduced immunosuppression , i.e. once daily/alternate day TAC/SRL (IMred). Between group comparisons were made with t-tests, Hill equations were used for effect: concentration relationships between drug levels and immunophenotypic parameters, and Support Vector Machine Analysis (SVM) was used to classify Imred and REJ. Lymphocyte subset distribution and function constituted immunophenotypic endpoints. Results: Compared with REJ, IMred was associated with 1. Significant decreases in the frequencies of TH1-priming-Type 1 progenitor dendritic cell (0.32±0.1 vs 0.17±0.17, p=0.05) at 34±22 days after transplantation, 2. Significantly decreased mixed lymphocyte response to donor antigen (stimulation index 16±11 vs 59±42, p=0.05), but not to third party, HLA-mismatched donors in the first month after transplantation, and 3. the ability of B-cell expression of CD54, CD95, CD86, CD25, CD69, and CD71, when used collectively, to yield a computational algorithm from a test dataset (n=9) that correctly distinguished IMred from REJ in a validation dataset of 6 subjects by SVM analysis. 4. Further more, magnetic bead-sorted, pure recipient CD8+28- subpopulations (T-suppressor) induced downregulation of CD86 on donor antigen presenting cell, stimulated with a T-helper cell line transfected to overexpress CD40 ligand in a reference non-rATG subpopulation of 4 subjects who were tolerant (off IM for >1 year). In rATG-treated subjects, T-sup were absent from 3 of 3 subjects with recurrent REJ, and were present in 4 of 8 subjects with IMred. Conclusions: Reduced IM requirement is associated with significantly decreased donor-specific alloreactivity, and TH1-priming pDC1 frequency. Donor-specific T-suppressor cells may identify the timing for safe weaning of immunosuppression in children receiving rATG pretreatment.

Abstract# 668 Poster Board #-Session: P124-II

INTESTINAL TRANSPLANTATION FOR ADULT PATIENTS WITH CAMPATH 1-H INDUCTION. Seigo Nishida,[1] Juan I. Madariaga,[1] David M. Levi,[1] Jang Moon,[1] Tomoaki Kato,[1] Gennaro Selvaggi,[1] Werviston DeFaria,[1] Sergio Santiago,[1] Anthony Gyamfi,[2] Phillip Ruiz,[3] Debbie Weppler,[1] Andreas G. Tzakis.[1] *[1]Surgery, University of Miami, Miami, FL; [2]Anesthesiology, University of Miami, Miami, FL; [3]Pathology, University of Miami, Miami, FL.*

Background: Campath-1H (C1H) is a humanized monoclonal antibody directed against the CD 52 antigen that is present on the surface of T cells, B cells, NK cells and monocyte. It depletes the peripheral blood lymphocytes. The aim of this research was to study the result of intestinal transplantation for adult patients with C1H.

Methods: This is a retrospective review of the 32 intestinal transplants with C1H in 29 adult patients between January 2001 and November 2003 at the University of Miami. C1H (0.3 mg/kg) was administered in four doses: Preoperatively, at the completion of the transplant, and on postoperative day 3 and 7. Tacrolimus levels were maintained at low level (around 5-10 ng/dl). We analyzed the incidence of rejection, use of immunosuppression, complication, and mortality

Results: Thirty-two transplants in 29 patients (17M, 12 F; mean age 34.1 yr) were performed. Eight patients(27%) did not have any rejection episode. Out of 21 patients, 27 rejection episode were treated during the first post operative year. Twenty four rejection were treated with steroid, 3 rejection were treated with OKT3. Three patient developed hemolytic uremic syndrome. One patient developed CMV infection. One patient developed PCP. There were neither GVHD nor PTLD. Eighteen patients are alive and 11 patients are dead. Of the 18 patients who are alive (POD23 to POD940), Ten patients are free of steroid. Current immunosuppression are tacrolimus (n=9), tacrolimus + sirolimus (n=1), tacrolimus + steroid (n=6), and tacrolimus+ sirolimus + steroid (n=2). Six patients died within 3 months. Two were bound to ICU before the transplant. Cause of death included rejection and subsequent sepsis (n=2, POD 21 and 89), pulmonary embolism (n=1, POD 9), necrotizing graft pancreatitis (n=1, POD7), pulmonary edema and sepsis (n=1, POD 1), intra-abdominal bleeding and multiple organ failure (n=1, POD 22). Five patients died at late period. The cause of death included endocarditis and sepsis (n=1, POD 175), opiate overdose (n=1, POD 321), tumor recurrence (n=2, POD 157 and 726), and complication of stoma closure (n=1, POD 477).

Conclusions: Our preliminary data showed the intestinal transplantation with C1H were acceptable result. This regimen allowed the immunosuppression low and could minimize the use of steroid. Incidence and severity of rejection are lower. Incidence of infection was not affected by this regimen.

Abstract# 669 Poster Board #-Session: P125-II

A NEW CROSSMATCH TECHNIQUE ELIMINATES INTERFERENCE BY HUMANIZED AND CHIMERIC DRUGS. Benita K. Book,[1] Christopher H. Bearden,[1] Avinash Agarwal,[1] Richard A. Sidner,[1] Nancy Higgins,[2] Mark D. Pescovitz.[1] *[1]Dept of Surgery, Indiana University, Indianapolis, IN; [2]Clarian Transplant Center, Indianapolis, IN.*

Humanized and chimeric anti-lymphocyte antibodies (Ab) are being used as immunosuppressive agents to prevent or treat rejections. The presence of drugs such as rituximab (RIT) (anti-CD20), daclizumab (DAC) (anti-CD25), and alemtuzumab (ALE) (anti-CD52) in serum interfere with standard antibody detection methods such as complement dependent cytoxicity (CDC) and flow cytometric crossmatch (FCXM). These agents are recognized as anti-human antibody and/or fix complement and cannot be differentiated from alloantibodies. Removal of humanized (Ab) without removing patient Ig would require beads bound to anti-idiotype antibody. A new ELISA crossmatch technique utilizing class I and class II HLA antigens from lysed donor cells, Transplant Monitoring System (TMS) (GTI,Inc.,Waukesha, WI), potentially precludes interference by eliminating the interfering antigens. To test this hypothesis, sera from nonsensitized volunteers alone or sera supplemented with 0.1 µg/mL or 10 µg/ml of RIT, DAC, or ALE (to mimic trough and peak clinical levels) were tested using three crossmatch techniques. T-cell CDC crossmatches used 2 washes with anti-human globulin (AHG); B-cell CDC crossmatches used 1 wash and no AHG; FCXM used a standard 3-color protocol with a mean channel shift of 45 indicating a positive T-cell result and mean channel shift of 150 indicating a positive B-cell result. TMS was performed as per manufacturer's instruction. Equivalent donor cells were used as targets. No reactivity was seen when testing sera with no drug added with any of the 3 crossmatch techniques. At both 0.1 µg/mL and 10 µg/mL RIT interfered with CDC B-cell, but not T-cell crossmatches. RIT at 10 µg/mL, but not 0.1 µg/mL interfered with the B-cell FCXM. No interference was seen with RIT in T-cell FCXM or TMS. ALE interfered with B-cell and T-cell CDC and FCXM, but neither class I nor class II TMS. DAC did not interfere with CDC or FCXM at 0.1 µg/mL, but caused a false positive B-cell result in both FCXM and CDC with some, but not all samples. No interference by was seen DAC with the TMS. While further testing is needed, TMS may be a useful alternative method to differentiate de novo donor specific antibodies after treatment with humanized or chimeric immunosuppressive agents.

Abstract# 670 Poster Board #-Session: P126-II

COMPLICATIONS OF RABBIT ANTITHYMOCYTE GLOBULIN (RATG) IN PEDIATRIC CARDIOTHORACIC TRANSPLANT PATIENTS. Deborah A. Kahler,[1] Brandon K. Knott,[1] K. O. Schowengerdt,[2] F. Jay Fricker,[2] Gary A. Visner,[2] Albert Faro.[2] *[1]Pharmacy, Shands at the University of Florida, Gainesville, FL; [2]Pediatrics, University of Florida, Gainesville, Fl.*

Background: RATG is a polyclonal antibody preparation that decreases the number of circulating lymphocytes and modulates T-cell function. Complications can include infusion related reactions (IRR), bone marrow suppression, and infections. We report our experience using RATG as induction immune suppression, treatment of steroid resistant rejection (SRR) and as addition to corticosteroid treatment of hemodynamic significant heart allograft rejection in 38 pediatric cardiothoracic transplant recipients (age range 12days-23yrs, median 17yrs). **Methods:** Between March 1999 and August 2002 we treated 19 heart (H) transplant, 18 lung (L) transplant and 1 H/L transplant recipient with a total of 54 courses of RATG for induction (8 L, 6 H), SRR (15 L, 3 H, 1 H/L), severe rejection (1 L), OB (2 L) or rejection with hemodynamic compromise (18 H). The number of doses per treatment course ranged from 4 to 15 (median 14) and the dose was 1.5-2 mg/kg per day. Patients were pre-medicated with acetaminophen, diphenhydramine and IV methylprednisolone. **Results:** We observed a total of 35 infectious complications in 16 patients, 6 IRR, and 18 instances of dose reduction because of leukopenia or thrombocytopenia. VIRAL INFECTIONS: 5patients with CMV, 2 invasive (colitis, retinitis); 1 case of genital HSV, 1 adenovirus. EBV seroconversion(1 patient) without complications, and 1 case of PTLD resulting in death were also observed. BACTERIAL:Eight patients had sepsis, bacteremia, clostridium difficile colitis, pneumonia and wound infections. FUNGAL: Infections occurred in 9 patients, and included thrush (1), candida line sepsis (3), fungal eye infection (1), pneumonia (4), UTI (2), trichosporon sepsis (2), trichosporon wound infection (1), candida wound infections (2). Three patients' deaths were directly attributable to bacterial or fungal infection which occurred in proximity to the administration of RATG. IRR included rash/hypersensitivity reaction (3), mild dyspnea with hypotension (1), serum sickness (1), rigors/hypotension/headache (1). Only 1 necessitated discontinuation of the drug after 13 of 14 doses had been administered. Dose reduction occurred for ↓WBC and platelets (2), ↓ platelets (3), ↓ WBC (10), concern for infection (3).

Conclusion: RATG has been an effective agent for INDUCTION and RESCUE immune suppression with an acceptable administration risk profile. In this series its use was associated with fungal and viral infections that caused significant morbidity and mortality.

Abstract# 671 Poster Board #-Session: P127-II

A STUDY OF THE CHANGES IN ABSORPTIVE SURFACE AREA OF TRANSPLANTED SMALL BOWEL. Suman Setty,[1] Gregorio Chejfec,[1] Eunice John,[1] Guiliano Testa,[1] Enrico Benedetti.[1] *[1]Pathology, University of Illinois, Chicago, IL; [2]Pathology, University of Illinois, Chicago, IL; [3]Pediatrics, University of Illinois, Chicago, IL; [4]Transplantation Surgery, University of Illinois, Chicago, IL; [5]Transplantation Surgery, University of Illinois, Chicago, IL.*

INTRODUCTION: Small bowel transplant is a modality used to improve the quality of life of subjects with short gut syndromes of various causes. We have demonstrated that transplanted mucosa shows statistically significant morphologic changes compared with native bowel. These include edema, increased apoptosis, increased goblet cells and villus blunting. Additionally an earlier study of transplanted bowel has demonstrated that there is a gradual increase in villus height over time after transplant which plateaus after several months. Calculations at the light microscopic level have demonstrated that these changes significantly impact the absorptive surface area. In this study, we performed a morphometric analysis of sequential graft biopsies on a patient with a living related small bowel transplant.

METHODS: Serial mucosal biopsies of the intestinal graft were taken weekly for the first post operative month and subsequently based on clinical indication (such as, to rule out rejection or infectious etiologies). We studied the serial small bowel biopsies performed at 1, 2, 5 and 10 week intervals after transplant which showed no evidence of rejection or infection. Relatively preserved villus areas were photographed at a fixed magnification (10x). Measurements of villus height and width were calculated at five points along the x and y axis. Gaussian-based fitting and height/width based calculations of villus surface area were performed.

CONCLUSIONS: The villus architecture was observed to change significantly with time progression. Early changes consisted of shorter broader villi (height/width ratio), with greater variation in height to width ratios. The mucosa was flat with wide spread villus blunting in the earlier biopsies. The villus height increased over time, achieving heights comparable to age-matched controls. The absorptive surface area was calculated using two different techniques; a). the villus as a cylinder b). Gaussian distribution. Our findings showed a significant difference in surface area calculations with the two techniques, up to 20%.We conclude that the significant alteration of villus architecture reaches a plateau and eventually the bowel reacquires its pre-transplant architecture.

Abstract# 672 Poster Board #-Session: P128-II

CHARACTERIZATION OF HUMAN AND NON-HUMAN PRIMATE COMPOSITE TISSUE ALLOGRAFT REJECTION. Linda C. Cendales,[1] David Kleiner,[1] Michael A. Eckhaus,[1] Robert Kampen,[1] Allan D. Kirk.[1] *[1]NIH, Bethesda, MD.*

Composite tissue allotransplants (CTAs) differ from other allografts in their embryologically diverse tissues. Given the infrequent application of CTA, it has not been established whether rejecting tissues have an immunological hierarchy. We have examined specimens from transplanted human limbs in various stages of rejection and compared them with a controlled evaluation of rejecting non-human primate limb CTAs to determine the temporal progression of CTA rejection and evaluate the relative degree to which CTA elements are targeted for immune destruction. Human tissues were received from international transplant centers for histological evaluation. A primate radial forearm flap model was developed for serial histological and transcript RT-PCR analysis. Primate autografts (n=5) and allografts with (n=4) and without (n=4)subtherapeutic immunosuppression have been studied with protocol incisional and excisional biopsies. Primate CTA components studied included native and donor skin, skeletal muscle, artery, vein, nerve and tendon. Human CTA rejection under immunosuppression presented as a rash localized to the allograft, and was characterized by a prominent dermal infiltrate. Given that biopsies were prompted by skin signs, this could indicate that rejection begins in the dermis, or that rejection becomes clinically evident after the infiltrate reaches the dermis. Primates evaluated without immunosuppression had an infiltrate arising within 3 days in the perivenular tissue leading to graft congestion and failure without a prominent dermal infiltrate. Dermal findings were consistent with ischemic injury referable to the early vascular injury. Subtherapeutically immunosuppressed animals rejected in 2 weeks with a marked dermal lymphocytic infiltrate similar ito human cases. The inflammatory lesions in the engrafted tissues were primarily perivascular and CD3+. Transcriptionally most composite tissues showed constituative expression of the regulatory cytokines IL-10 and TGF-B. These increased in native tissues exposed to trauma and in rejecting tissues suggestive of local regulation in response to tissue injury. Allografts were unique in their expression of CD80, CD25, and T-bet consistent with a cytotoxic T-cell mediated event. These was most evident in transplanted artery and skin. Thus, the clinical appearance of a rash occurs relatively late as an extension of vascular alloimmune egress. These data suggest that protocol surveillance of CTAs may be of some benefit in detecting occult alloimmune activity.

Abstract# 673 Poster Board #-Session: P129-II

OUTCOMES AND SYSTEM ERRORS OF PATIENTS EXPERIENCING AN IN-HOSPITAL CRISIS AFTER ORGAN TRANSPLANTATION. Eric L. Marderstein,[1] Michael Devita,[2] R. Scott Braithwaite,[3] Richard L. Simmons,[1] David A. Geller.[1] *[1]Department of Surgery, Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Department of Critical Care Medicine; [3]Department of Medicine.*

BACKGROUND: An important patient safety measure at our institution are the liberal criteria for initiation of a "condition", or crisis, which is designed to enact a rapid response to patients in a pre-arrest crisis state to avoid progression to a full arrest. These "condition" criteria encompass alterations in vital signs and mental status. These "conditions" also act as an important reporting system to identify potentially elusive system errors that result in adverse events. The purpose of this project was to evaluate the outcomes and system errors of in-hospital crisis in transplant patients.

METHODS: Patients having a "condition" at our institution from July 1, 2001 until June 30, 2003 were stored in a database. The electronic charts of these patients were reviewed by one of a team of physicians, and the cases were then presented to the full Condition Review Committee where contributory factors and errors were identified. The Patient Safety Committee provided institutional approval.

RESULTS: 117 patients over the two-year period were admitted to the transplant services and experienced a "condition" or crisis. The hospital mortality of these patients was high (28.2%). 69 major errors were identified in these patients. Medication errors were common (17 patients, 14.5%) and resulted from the use of narcotics (6), immunosuppressives (6), insulin (4) and benzodiazepines (2). Physician knowledge, judgment or delay in treatment contributed to the events in 19 patients (16.2%). Problems with equipment (13 patients, 11.1%) and problems related to patient transport off of the ward (13, 11.1%) occurred with high frequency.

CONCLUSIONS: Despite criteria for the initiation of a rapid response, the hospital mortality of an in-hospital crisis in transplant patients remains high. Our condition database is an effective reporting system for the identification of medical error. Reviewing the course of these patients was an efficient way to identify opportunities for system change to reduce error.

Abstract# 674 Poster Board #-Session: P130-II

PHARMACODYNAMIC MONITORING OF MONOCLONAL ANTIBODY THERAPY IN INTESTINAL TRANSPLANTATION.

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Background: Monoclonal antibodies play an important role in immunosuppressive therapy after intestinal transplantation (ITx). The experience with pharmacodynamic monitoring of daclizumab and infliximab application after ITx is presented.

Material and Methods: Intestinal transplantation was performed in 12 adult patients for irreversible short bowel syndrome. Initial immunosuppression consisted of tacrolimus, rapamycin, prednisolone and daclizumab (n=10) or tacrolimus, alemtuzumab, and steroids (n=2). Daclizumab (1mg/kg body weight) was administered in an individualized fashion according to pharmacodynamic monitoring using serum sIL-2R levels and CD4+CD25+-T-cells. Infliximab was used in two patients as rescue therapy for OKT3-resistant rejection according to serum TNF alpha and serum- LPS binding protein (LBP). Immunological monitoring included sIL-2R, TNF-alpha, LBP, IL-6, IL8, CRP , and CD4+CD25+ - T-cells.

Results: 6- and 12-mths-patient and graft survival were 83% (10/12) and 75% (9/12). Acute rejection (AR) rates were 8% (1/12) within the first 6 mths and 17% (2/12) within on year. One late AR occured after 2 years. All ARs were steroid- and OKT3-resistant. Steroid-resistent ARs were accompanied by a significant increase of serum-TNF alpha and LBP (3/3). In two patients presenting with AR 9 mths and 2 yrs after intestinal transplantation, complete resolution was not achieved despite 5 and 10 days of OKT3 treatment, respectively. They were treated with four, individually timed infusions of 3mg/kg body weight infliximab (chimeric anti-TNF alpha moAb) until complete recovery. Onset of therapy, number of infusions and intervalls between infusions (patient1: 4 wks interval; patient 2: 2 wks interval) were determined according to the course of serum TNF alpha and LBP levels.

The results of daclizumab application according to pharmacodynamic monitoring were as follows: Only one patient recieved the recommended number of five daclizumab applications. The other patients recieved four (n=1), three (n=3), two (n=3) or a single daclizumab infusion (n=2) according to sIL2R- and CD4+-CD25+-T-cell monitoring Conclusion: Therapy with IL2R-antagonists can be individualized by pharmacodynamic sIL2R and CD4+CD25+ T-cell monitoring. Infliximab is a valuable therapeutic option in in a selected group of patients after intestinal transplantation experiencing steroid and OKT3 refractory severe rejection. Its administration can be guided by pharmacodynamic monitoring of serum TNF alpha and LBP.

Abstract# 675 Poster Board #-Session: P131-II

THROMBOTIC MICROANGIOPATHY (TMA) IN THE SIROLIMUS ERA.

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Transplant-associated TMA associated with calcineurin inhibitors is a serious complication in solid organ (SOT) and allogeneic bone marrow (allo BMT) recipients. Recently, TMA in pts on sirolimus has been described. Little is known about the relative risks and clinical differences in TMA associated with these agents. Methods: Records of the Apheresis Unit at a single center were reviewed to identify transplant recipients who had severe TMA requiring apheresis. A cohort from1995-2001 was compared with one from 2002-3 (after introduction of sirolimus). Results: In the 1995-2001 group, 6 SOT (2 lung, 2 kidney, 1 heart, and 1 liver) and 14 allo BMT recipients required apheresis. In the 2002-3 group, TMA developed in 8 SOT (4 lung, 4 kidney) and 3 allo BMT recipients. TMA in the earlier group developed at a median of 6 mos post-SOT and 1.5 mos post-BMT; in the later group, 8 mos post-SOT and 1 mo post-BMT. One pt had seizures and intracranial hemorrhage during sirolimus-associated TMA after less severe TMA episodes with the other agents. The table shows the drug which pts were receiving when TMA developed (some pts had more than 1 episode). Of pts on sirolimus, 3 were also receiving tacrolimus concomitantly; the others were on no calcineurin inhibitors.

	Cyclosporine	Tacrolimus	Sirolimus
1995-2001 SOT	4	2	0
1995-2001 BMT	12	2	0
2002-2003 SOT	0	5	7
2002-2003 BMT	3	1	1

In the earlier group, 3/6 (50%) of SOT vs. 1/14 (7%) of BMT recipients survived whereas in the later group, 5/8 (62.5%) of SOT and 0/3 (0%) of BMT recipients survived. Concurrent or recent CMV infection occurred in 5/6 SOT (83%) in the earlier group, but only 1/8 SOT (12.5%) in the later group (p=0.029). All but 2 of the pts with sirolimus-associated TMA had levels at some point > 20 ng/ml. Conclusions: In the current era, sirolimus-associated TMA requiring apheresis is at least as common in SOT as that associated with calcineurin inhibitors, and may be severe. In both eras, survival in SOT pts with TMA is better than that of allo BMT recipients. CMV viremia was frequently associated with calcineurin inhibitor-associated TMA in SOT but not with sirolimus-associated TMA. Further study of risk factors for TMA will be of interest.

Abstract# 676 Poster Board #-Session: P132-II

IMPACT OF HLA-MATCHING AND PRESENSITIZATION ON THE INCIDENCE AND SEVERITY OF ACUTE AND CHRONIC INTESITNAL ALLOGRAFT REJECTION.

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Introduction: HLA donor/recipient matching is associated with a better graft outcome both in kidney and heart transplants, although the opposite is found in liver transplants. This study analyzes the histocompatability factors that affect the short and long term outcome of intestinal allografts. **Methods:** Tissue typing was done by standard lymphocyte cytotoxicity and PRA assessed. Graft rejection was diagnosed pathoclinically. Patient and allograft demographics and clinical variables were statistically analyzed for their association with pathologic alterations and outcomes. **Results:** Between May 1990 and March 2003, 216 patients (113 adults, 103 children) received 231 intestinal containing allografts. 102 were II, 89 LI and 40 MV (10 without liver). Immunosuppression advanced during this period from tacrolimus/steroids, to the addition of induction agents, to our latest steroid sparing preconditioning protocol. Crossmatch was positive in 19%. Statistically the only predictor of ACR was patient race, with black recipients more likely to reject. Predictors of chronic rejection were intestinal allografts without a composite liver (p=.002), lower number of HLA-B mismatches (p=.04), older donors (p=.02), black recipients (p=.002) and those who developed CMV (p=.004). Donor race (black) was borderline significant in predicting graft survival (p=.05) and significant for worse patient survival (black: p=.007). Younger recipients (p=.03) and those receiving organs from black donors (p=.03) appeared to be at higher risk for PTLD. Crossmatch, PRA > 5% and HLA-ABDR mismatch scores did not show any significance in this cohort.

Conclusion: Previously we reported a trend to increased frequency and severity of rejection with a positive crossmatch. Again no significance is achieved, but may be due to differential clinical and immunosuppressive therapies in this cohort. HLA mismatch has no bearing, except for an association with HLA-B and chronic rejection, although our relatively small numbers may impede analysis. Clinical and theraputic advances over the time period also confound analysis. However, we do not feel that high PRA, crossmatch status or HLA mismatch should alter donor graft utilization or patient selection.

Abstract# 677 Poster Board #-Session: P133-II

EFFECTS OF IMMUNIZATION ON HUMAN ALLOIMMUNE REPERTOIRES.

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Human alloimmunity may be influenced by environmental stimuli including immunizations. Such stimuli could nonspecifically enhance memory anti-donor immunity and/or specifically prime T or B cells with cross-reactivity to alloantigens. To assess the effects of immunization on alloimmunity in humans we performed serial assessments of peripheral T and B cell alloimmune responses in 2 cohorts of patients undergoing 2 different immunization regimens. 14 patients with adenocarcinomas were immunized 3 times over 3 mo with an experimental murine anti-idiotypic antibody administered in alum. In a second cohort, 12 normal student volunteers were immunized with the Hepatitis B vaccine (3 injections over 6 months). In both cohorts, blood samples were obtained prior to immunization, 1-2 months after the initial immunization and following the last immunization. Peripheral blood lymphocytes (PBLs) were isolated and tested against a panel of allogeneic splenic stimulator cells using an IFNg ELISPOT assay to assess the frequency of alloreactive effector/memory T cells in the peripheral blood. Plasma samples were evaluated for the presence or absence of anti-

HLA antibodies (Ab) using flow cytometry screening beads. Baseline studies demonstrated the presence of memory alloreactive T cell immunity (> 15 IFNg ELISPOTs per 300,000 PBLs) in 70% of the study patients. In each of the 2 cohorts, PBLs from 60% of individuals with baseline T cell alloreactivity showed a 1.5-3 fold increase in frequency by 1-3 months post-immunization. The frequency of alloreactive PBLs from concomitant control, nonimmunized volunteers, did not change during the study period. ~20% of subjects with no alloreactive PBLs at baseline developed new alloreactive PBLs by 1-3 months post-immunization. In addition, 2 of 14 adenocarcinoma patients had baseline positive anti-HLA Abs that were significantly increased following immunization. 4 of the remaining 12 patients developed new anti-HLA Abs during the immunization period. These data show that protective immunizations can have complex effects on human alloimmune repertoires. Both nonspecific activation/expansion of alloreactive memory immunity and cross-reactive priming of new alloreactive T and B cells can occur. The data further suggest that immunizations may affect the risk of rejecting a subsequently transplanted organ and bolster the argument for monitoring cellular and humoral alloimmunity in this setting.

Abstract# 678 Poster Board #-Session: P134-II

CAREGIVER BURDEN AS A PREDICTOR OF TRANSPLANT CANDIDATES' PSYCHOLOGICAL DISTRESS. James R. Rodrigue,[1] Gail L. Lisson,[1] Maher A. Baz,[2] James A. Hill,[2] John R. Wingard,[2] David R. Nelson.[2] *[1]Clinical & Health Psychology, University of Florida, Gainesville, FL; [2]Medicine, University of Florida, Gainesville, FL.*

Purpose: Primary research questions of this investigation included: (a) is the level of caregiving burden different among caregivers for heart, lung, liver, and bone marrow transplant patients, (b) does caregiver burden predict psychological distress within transplant candidates, and (c) do transplant patients' use of certain coping strategies buffer or facilitate the effects of caregiver burden on psychological health.

Methods: Methods included retrospective analyses of self-report measures collected from 117 transplant candidates and their designated primary caregivers during 2000-2001 pre-transplant psychological evaluations. Caregiver burden, state anxiety, methods of coping, optimism, and quality of life were measured by the Caregiver Strain Index, the State-Trait Personality Inventory, Brief COPE, the Life Orientation Test, and the SF-36 Health Survey, respectively.

Results: Univariate analyses showed that caregivers of patients awaiting bone marrow transplant reported statistically significant higher levels of caregiver burden than caregivers of liver and heart transplant candidates ($p \leq .05$). In addition, 11% of liver transplant caregivers, 16% of heart transplant caregivers, 20% of lung transplant caregivers, and 46% of bone marrow transplant caregivers reported clinically significant levels of caregiver strain to warrant further clinical evaluation and possible treatment. Regression analyses showed that caregiver burden was a significant predictor of transplant patients' state anxiety ($p \leq .05$) and quality of life ($p \leq .05$). Patients who had highly stressed caregivers had higher state anxiety and lower quality of life (mental health). Hierarchical regression analyses were conducted to further examine possible mediating effects. Patient coping strategies characterized by denial and behavioral disengagement mediated the relationship between caregiver burden and high anxiety, as well as between caregiver burden and lower quality of life. Patient coping strategies characterized by optimism and acceptance mediated the relationship between caregiver burden and anxiety and between caregiver burden and quality of life.

Conclusions: This study suggests that high levels of caregiver strain is associated with higher psychological distress and lower quality of life in transplant patients. Caregivers should also be a focus of evaluation during the pre-transplant period and interventions should be developed to facilitate their successful adaptation to transplantation.

Abstract# 679 Poster Board #-Session: P135-II

LONG TERM SUPPRESSION OF HLA EXPRESSION IN HUMAN CELLS BY LENTIVIRUS-MEDIATED GENE TRANSFER OF siRNA CASSETTES. James C. Cicciarelli,[2,3] Christopher Logg,[1] Kazuo Mizutani,[2] Noriyuki Kasahara,[1] Yuichi Iwaki,[2,3] David Cohen.[1] *[1]Department of Medicine, University ofCalifornia, Los Angeles (UCLA), Los Angeles, CA; [2]Deptartment of Urology, University of Southern California (USC), Los Angeles, CA; [3]Metic IC, Los Angeles, CA.*

To date, the primary strategies for avoiding rejection have been to minimize antigenic differences between donor and recipient by matching HLA and using potent immunosuppression. As an alternative approach, we propose to condition the graft cells by delivery of sequences encoding small interfering RNAs (siRNAs) targeted against HLA, thereby decreasing donor immunogenecity and recipient's immune response.

To this end, we have designed several siRNA sequences directed against non-polymorphic regions of HLA Class I, and identified U6 promoter-driven siRNA expression cassettes exhibiting the most potent inhibitory activity by direct transfection into human cell lines, followed by flow cytometric and Western blot analyses. Next, for long-term suppression of HLA expression, we have constructed and tested third-generation self-inactivating HIV-based lentivirus vectors for efficient and long-term gene delivery of these siRNA cassettes.

We have found that these lentivirus vectors, which readily infect quiescent non-dividing cells, are capable of highly efficient gene transfer to a wide variety of primary human cell types, including hematopoietic progenitor cells, differentiated epithelial cells, fibroblasts, myocytes, endothelial cells, and islet cells. Furthermore, permanent integration into the genome of the host cell is achieved. Currently we are conducting studies to determine the percent and durability of siRNA-mediated inhibition. Initial experiments, using beta 2 microglobulin siRNA sequences, we were able to obtain complete inhibition of expression of beta 2 microglobulin in a human cell line, but with less than 100 % efficiency; as shown by flow cytometry. We anticipate that lentivirus-mediated gene transfer of siRNA sequences will be useful for reduction of graft immunogenicity by treatment of donor, donor organs, and islet cell transplants.

KIDNEY IMMUNOSUPPRESSION: COMPLICATIONS

Abstract# 680 Poster Board #-Session: P136-II

INTERDEPENDENCE BETWEEN DRUG EXPOSURE, LYMPHOCYTE PROLIFERATION, TGF-β1 EXPRESSION AND ENDOTHELIN-1 PRODUCTION IN STABLE RENAL ALLOGRAFT RECIPIENTS TREATED WITH CYCLOSPORINE A OR TACROLIMUS. Johannes Waiser,[1] Torsten Boehler,[1] Michael Schneider,[1] Hans-Hellmut Neumayer,[1] Klemens Budde.[1] *[1]Dept. of Internal Medicine - Nephrology, University Hospital Charite, Campus Mitte, Berlin, Berlin, Germany.*

Purpose: Cyclosporine A (CsA) and tacrolimus inhibit lymphocyte proliferation via inhibition of calcineurin. In several systems, these compounds were shown to stimulate the production of profibrotic cytokines such as transforming growth factor-β (TGF-β) and endothelin (ET). Current evidence indicates that both cytokines play a role in the pathogenesis of chronic allograft nephropathy. Here, we investigated the interdependence between drug exposure, lymphocyte proliferation, TGF-β1 expression and ET-1 production in stable renal allograft recipients. Methods: Patients received double-immunosuppression consisting of either CyA + mycophenolate mofetil (MMF) (n = 34) or tacrolimus + MMF (n = 30). CyA (EMIT) and tacrolimus (IM_x) trough levels (both groups), as well as C2 levels (CyA group) were measured in whole blood. Simultaneously, lymphocyte proliferation was determined by MTT-test following incubation of peripheral blood mononuclear cells (PBMC) with anti-CD3 mAb (10 ng/mL). Additionally, TGF-β1 and ET-1 plasma levels were analyzed by ELISA. Results: The mean drug levels were 98.3 ± 32.8 ng/mL (C0) and 562.2 ± 171.3 ng/mL (C2) in the CyA group, and 9.1 ± 2.3 ng/mL (C0) in the tacrolimus group. Mean lymphocyte proliferation at C0 was significantly lower in the tacrolimus group as compared to the CyA group (0.26 ± 0.27 vs. 0.32 ± 0.24, $P < 0.05$). TGF-β1 plasma concentration at C0 was significantly higher in the tacrolimus group (18.8 ± 7.2 ng/mL vs. 11.1 ± 7.6 ng/mL, $P < 0.01$). In contrast, ET-1 plasma concentration was significantly higher in the CyA group (1.39 ± 4.2 fmol/mL vs. 0.84 ± 2.5 fmol/mL). In the CyA group, a negative correlation between drug levels and lymphocyte proliferation was found (C0: $r = -0.439$, $P < 0.05$; C2: $r = -0.769$, $P < 0.001$; AUC: $r = -0.677$, $P < 0.001$). Conclusions: Our results show that depending on the immunosuppressive maintenance regimen differences exist with respect to the effect on lymphocyte proliferation and the expression of profibrotic cytokines. Compared to tacrolimus treated patients, lymphocyte proliferation was less reduced in CyA treated patients. Additionally, lymphocyte proliferation nicely correlated with drug levels in this group. Concerning the expression of profibrotic cytokines, our results make clear that it may not be sufficient to investigate a single profibrotic cytokine, in order to estimate the profibrotic profile of immunosuppressive drugs.

Abstract# 681 Poster Board #-Session: P137-II

DOSE AND COST REDUCTION WITH CD3 GUIDED ANTI-THYMOCYTE GLOBULIN THERAPY IN KIDNEY AND LIVER TRANSPLANTATION. Maxine Lam,[1] Gary Wong,[1] Muhammad Zuberi,[1] Jennifer Harrison,[1] Sarah Nia,[1] Brenda Kisic,[1] Bassem Hamandi,[1] Florence Krakauer,[1] Edward Cole,[1] Gary Levy.[1] [1]*Multi-Organ Transplant, Toronto General Hospital, University Health Network, Toronto, ON, Canada.*

Background

Rabbit anti-thymocyte globulin (ATG) is commonly used for induction of immunosuppression and management of delayed graft function (DGF) in solid organ transplant recipients. ATG has traditionally been administered according to a fixed dose regimen for 7 to 10 days, with dose modifications for hematologic toxicity. Recently, two studies demonstrated that CD3 lymphocyte monitoring can be used to guide ATG dosing. This study was designed to examine whether implementation of a CD3 guided ATG dosing protocol would result in dose and cost reductions without compromising efficacy and safety compared to traditional dosing.

Methods

A CD3 guided ATG dosing protocol was developed in which patients received ATG 1.5mg/kg on the day of transplant with subsequent daily doses for CD3 counts exceeding 20 cells/mm³. Subjects included kidney and liver transplant patients receiving ATG for induction, as well as kidney transplant patients with DGF. Prospective enrolment occurred during the period of January to November 2003.

Kidney and liver transplant patients who received ATG according to the traditional dosing method for induction or DGF served as the control arm. Data was compiled through a retrospective chart review of patients transplanted during the period of September to December 2002.

Data for all patients was collected until the day of hospital discharge with a planned follow-up at one year. A sub-group analysis of kidney transplant patients will be performed at a future date to evaluate the efficacy of CD3 guided ATG dosing.

Results

Thirty-nine patients were enrolled in the control arm (17 kidney, 22 liver) while 12 patients received the intervention (10 kidney, 2 liver). Use of the CD3 guided ATG dosing strategy led to a 57% reduction in the mean total ATG dose per patient (704 mg *vs.* 302mg; p = 0.001) and a significant cost savings (mean cost per patient CDN$5531 *vs.* $2576; p = 0.001; includes cost of CD3 monitoring).

Subjects receiving CD3 guided ATG dosing experienced significantly less hematologic toxicity. There was no difference in the incidence of acute rejection, CMV or bacterial infection between the groups.

Conclusion

Implementation of a CD3 guided ATG dosing protocol may achieve dose reductions and cost savings compared to traditional dosing. Although this appears to be a promising approach, further analysis is required to fully assess the efficacy and safety of this protocol.

Abstract# 682 Poster Board #-Session: P138-II

MAINTENANCE IMMUNOSUPPRESSION IN SENSITIZED RECIPIENTS OF FIRST AND SECOND KIDNEY TRANSPLANTS FROM DECEASED DONORS. Wida S. Cherikh,[1] Alan Ting,[1] Adrian H. Cotterell,[2] H. M. Kauffman.[1] [1]*Research Department, UNOS, Richmond, VA;* [2]*Department of Surgery, Virginia Commonwealth University, Richmond, VA.*

Purpose. Association of discharge immunosuppressive regimens using cyclosporine (CYA), tacrolimus (TAC), azathioprine (AZA) and mycophenolate mofetil (MMF) on graft survival among sensitized first and second kidney transplant recipients from deceased donors was determined.

Methods. We included sensitized recipients (peak PRA>9%) of first and second kidney transplant recipients from the UNOS/OPTN database during 1/1/95-12/31/00 (N=9,674). Recipients were categorized into one of the following discharge regimens: CYA+AZA, CYA+MMF, TAC+AZA, TAC+MMF. Records with survival of >750 days were censored for comparable follow-up among the regimens. A multivariate Cox model was used to determine the effect of drug regimen on time to graft loss in first and second transplants, adjusting for other variables. Results are presented as relative risk (RR) of graft loss and p-value.

Results. The table shows that as compared to CYA+AZA, TAC+MMF was associated with a significantly reduced risk of graft loss in first transplants (p=0.037). For second transplants, both TAC+AZA and TAC+MMF had an increased risk of graft loss (p=0.042 and p=0.115, respectively). When comparing second versus first transplants, TAC+AZA and TAC+MMF had an increased risk of graft loss (p=0.034 and p=0.097, respectively).

	1st TX			2nd TX			2nd vs. 1st TX	
REGIMEN	N	RR	P	N	RR	P	RR	P
CYA+AZA	1809	1.00	-	326	1.00		0.865	0.428
CYA+MMF	3,762	0.885	0.104	808	0.993	0.965	0.971	0.859
TAC+AZA	193	0.747	0.116	75	1.671	0.042	1.935	0.034
TAC+MMF	1,764	0.827	0.037	937	1.267	0.115	1.326	0.097

One-year mean serum creatinine was higher in CYA-based than TAC-based regimens for first and second transplants (data not shown).

Conclusions. Among sensitized first kidney transplant recipients, TAC+MMF was associated with significantly improved graft survival. None of the four maintenance immunosuppressive drug regimens had a statistically significantly reduced risk of graft loss in sensitized second kidney transplants, although CYA-based regimens were associated with comparable graft survival to that of first transplant kidney recipients. Use of strategies to reduce B-cell lymphocyte activity may be desirable for improving transplant outcome in sensitized recipients.

Abstract# 683 Poster Board #-Session: P139-II

SIROLIMUS-BASED THERAPY FOLLOWING EARLY CYCLOSPORINE WITHDRAWAL RESULTED IN SUPERIOR RENAL ALLOGRAFT SURVIVAL AT 48 MONTHS COMPARED WITH CONTINUOUS COMBINED SIROLIMUS AND CYCLOSPORINE. Josep M. Campistol,[1] Henri Kreis, Rainer Oberbauer, Alfredo Mota, Hany Riad, Jeremy Chapman, Giovanni Stallone, Juan Carlos Ruiz, Giuseppe Nanni, James T. Burke, Martine Gioud-Paquet, the Rapamune Maintenance Regimen Study Group. [1]*Hospital Clinic i Provincial, Barcelona, Spain.*

Background: This trial tested whether withdrawing cyclosporine (CsA) from a sirolimus (Rapamune®, SRL)-CsA-steroid (ST) regimen would affect graft survival. Originally planned for 36 months, the duration was amended to 60 months after review of the 12-month results.

Methods: 430 eligible patients receiving SRL-CsA-ST were randomly assigned (1:1) at 3 months ± 2 weeks to remain on triple therapy (SRL-CsA-ST group), or to have CsA withdrawn and SRL trough concentrations increased (SRL-ST group). Graft survival was determined by the log-rank test, excluding loss to follow-up as an event (11.6% vs 7.0%, SRL-CsA-ST vs SRL-ST) and by censoring these patients from the time they were lost to follow-up.

Results: At 48 months, graft survival was significantly better after CsA withdrawal, either when including death with a functioning graft as an event (84.1% vs 91.5%, p =0.024) or when excluding it (90.5% vs 96.1, p = 0.025). No significant differences were observed in the incidence of death (7.9% vs 4.7%) or biopsy-proven acute rejection after randomization (7.0% vs 10.2%, SRL-CsA-ST vs SRL-ST, respectively). Calculated GFR, including values from discontinued patients and setting GFR to 0 for functional graft loss, was significantly higher (44.5 vs 58.3 mL/min, p<0.001) with CsA withdrawal. Mean arterial blood pressure (101.0 vs 97.6 mm Hg, p = 0.046) and hemoglobin (126.4 vs 135.6 g/L, p = 0.031) were also significantly better with SRL-ST. Lipid parameters were not significantly different. Hypertension, creatinine increase, CsA toxicity, abnormal kidney function, toxic nephropathy, edema, hyperuricemia, gingival hyperplasia, and herpes zoster were reported significantly more often in patients continuing on CsA. Abnormal liver function tests, hypokalemia, thrombocytopenia, joint disorder, abnormal healing, and ileus were reported more frequently with SRL-ST. Based on the growing difference in favor of SRL-ST therapy, the protocol was amended to discontinue SRL-CsA-ST patients from assigned therapy. All patients will be followed through 60 months.

Conclusion: Eliminating CsA from a SRL-CsA-ST regimen at 3 months rapidly improved renal function and ultimately resulted in better graft survival.

Abstract# 684 Poster Board #-Session: P140-II

COMPARATIVE ANALYSIS OF RENAL FUNCTION IN PATIENTS WITH CADAVERIC KIDNEY TRANSPLANTS TREATED WITH TACROLIMUS (TaC) OR CYCLOSPORIN (CsA). Miguel Gonzalez-Molina,[1] Jose M. Morales, Roberto Marcen, Domingo Del Castillo, Jose M. Grinyo, Salvador Gil-Vernet, Federico Oppenheimer, Jose M. Campistol, Luis Capdevila, Ildefonso Lampreave, Francisco Valdes, Amado Andres, Fernando Anaya, Fernando Escuin, Manuel Arias, Luis Pallardo (Spanish Renal Forum). [1]*Department of Nephrology, Hospital Universitario Carlos Haya, Malaga, Spain.*

The introduction of TaC for renal transplant (RT) patients and its comparison with CsA have led to a great debate. Serum creatinine levels during the first six postransplant months are the best predictor of long-term graft survival. This prospective study analyzed renal function with two immunosuppression regimens involving TaC or CsA, with mycophenolate mofetil (MMF) and prednisone (P).

Methods. A total of 2203 cadaveric RT patients were followed from January 2000 to December 2002 at 13 hospitals; 1538 treated with TaC+MMF+P and 665 with CsA+MMF+P. Adjusted ANOVA and Mantel-Haenzel test were done for the age and sex of the donor and age, sex and weight of the recipient.

Results. At six months post-RT patients treated with TaC were receiving a lower dose of MMF (1019.28±380.63 vs 1441.00±712.90 mgs; p=0.0005) and had significantly lower serum creatinine in the following groups: total (1.54±0.64 vs 1.77±0.93 mg/dL; p=0.0005), with acute rejection (1.79± 0.76 vs 2.31±1.26 mg/dL; p=0.0005) and recipients from donors younger than 55 years (1.47±0.56 vs 1.66±0.96 mg/dL; p=0.0005). The difference in recipients from donors older than 55 years was not significant (1.82±0.82 vs 1.89±0.88 mg/dL). The percentage of patients with serum creatinine

lower than 1.5 mg/dL was higher in those treated with TaC in the same three groups: total (OR 0.49; 95% CI 0.40-0.60; p=0.0005), with acute rejection (OR 0.52, 95% CI 0.34-0.78, p=0.001) and in recipients from donors younger than 55 years (OR 0.52, 95% CI 0.41-0.68; p=0.0005). The difference in recipients from donors older than 55 years was not significant (OR 0.9, 95% CI 0.64-1.26). Mortality was lower in patients treated with TaC (OR 2.06, 95% CI 1.28-3.31; p=0.002).

Conclusions. RT patients treated with TaC have a better renal function and less mortality than those receiving CsA.

Abstract# 685 Poster Board #-Session: P141-II

EFFECT OF IMMUNOSUPPRESSANTS ON TWO YEAR KIDNEY TRANSPLANT SURVIVAL IN THE RECENT ERA. Barbara A. Bresnahan,[1] Wida Cherikh,[2] Yulin Cheng,[2] Maureen A. McBride,[2] Nauman Siddiqi,[1] Sundaram Hariharan.[1] *[1]Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]Department of Biostatistics, United Network of Organ Sharing, Richmond, VA.*

There has been a progressive increase in kidney half-life with each transplant year. During the last 10 years there have been several newer medications added to the immunosupressive regimen. The current study analyzed the effect of different immunosuppressants on two year kidney graft survival.

Methods: All adult kidney transplants (N=70,724) from 1995-2001 with at least 8 days survival and follow-up>764 days reported to UNOS/OPTN were analyzed by discharge immunosuppression (CSA+Aza, CSA+MMF, CSA alone, TAC+Aza, TAC+MMF, TAC alone). Multivariate Cox modeling was performed to calculate hazard ratios for graft loss using recipient and donor demographics, transplant (immunologic and non-immunologic) and post-transplant variables (including transplant year and DGF).

Results: Since 1995 the relative risk for graft loss in all treatment groups has continued to decline. The usual donor and recipient variables including black recipient, pre-Tx dialysis, older donor, DGF, and diabetes were associated with a higher relative risk of graft loss.

	MMF-based regimens	p-value	TAC-based regimens	p-value
Relative risk graft loss	0.837 [0.796, 0.881]	<0.0001	0.995 [0.994, 1.049]	0.8623
Relative risk graft loss (DWFG)	0.775 [0.726, 0.827]	<0.0001	1.073 [1.003, 1.148]	0.0408

After correcting for various variables the relative risk for graft loss was not different in CSA vs TAC treated patients. If censored for death with a fuctioning graft (DWFG), there was a minimally increased risk for graft loss in the TAC-based regimens (p=0.0408). There was a marked improvement in the relative risk for graft failure with the use of MMF-based regimens (p<0.0001).

Conclusions: With each transplant year there has been a decrease risk of graft failure in all treatment groups. There is no difference in two year graft survival in TAC vs CSA treated patients. Use of MMF markedly decreased the relative risk for graft loss.

Abstract# 686 Poster Board #-Session: P142-II

USE OF SIROLIMUS AS INITIAL THERAPY AFTER RENAL TRANSPLANTATION : PRELIMINARY RESULTS OF A RANDOMIZED PILOT STUDY IN PATIENT RECEIVING MARGINAL KIDNEYS. Eric Thervet,[1] Antoine Durrbach,[2] Lionel Rostaing,[2] Nacéra Ouali,[2] Philippe Wolf,[2] Claire Pouteil-Noble,[2] Michele Kessler,[2] Béatrice Viron.[2] *[1]Service de Nephrologie, Hopital Saint-Louis, Paris, France; [2]MK Study Group, France.*

Sirolimus (SRL) is a non-nephrotoxic drug, but recent concerns have emerged about its influence on graft function recovery and its use in recipients of marginal kidney (MK). The aims of this pilot study were to evaluate the renal function, the incidence and severity of delayed graft function (DGF) and slow graft function (SGF) in patients receiving MK and treated by SRL or cyclosporine (CsA).

Methods: In this prospective study, 72 patients were randomized to receive SRL from day 0 (15 mg twice then 10 mg/d, targeted C0 10-20) or CsA from day 6 (4 mg/kg/d, targeted C0 100-300 or C2 800-1200) and a 7 days course of polyclonal antibodies, MMF and steroids. MK was defined as the presence of at least 2 of the following donor characteristics: age > 60 yrs, cold ischemia time > 30 h, creatinine > 150 µmol/L, instable hemodynamic status, cardio or cerebrovascular past medical history or cause of death. DGF was defined as the need of a hemodialysis session and SGF as a serum creatinine > 250 µmol/L on day 7. GFR was measured using the Cockroft formula. We report here the preliminary results of 43 patients at 6 months.

Results. At month 6, 64 % and 90 % of patients were still receiving SRL and CsA respectively (p=0.07). Treatment cessation for adverse events was more frequent in SRL (p=0.008).

DGF duration was 31 days in SRL vs. 12 days in CsA. GFR in ITT and observed data analysis were respectively 41 and 53 ml/min in SRL vs. 41 and 48.5 ml/min in CsA.

Conclusion. These preliminary results do not support the use of SRL in recipients of marginal kidney.

	SRL	CsA	p
Patient survival (%)	100	100	1
Graft survival (%)	82	100	0.09
DGF (%)	19	24	1
SGF	71	67	0.5
BPAR (%)	30	20	0.7

Abstract# 687 Poster Board #-Session: P143-II

TACROLIMUS DOSE ADJUSTMENT AND POST-TRANSPLANT DIABETES MELLITUS IN US RENAL TRANSPLANTATION. Mark A. Schnitzler,[1] Karen L. Hardinger,[2] Daniel C. Brennan.[3] *[1]Washington University; [2]St. Louis College of Pharmacy, St. Louis, MO.*

The use of tacrolimus compared to cyclosporine has been associated with approximately twice the rate of post-transplant diabetes mellitus (PTDM). Tacrolimus dose reduction has been suspected to lessen the risk of developing PTDM. We sought to evaluate these conjectures in a large immunosuppression prescription data base in non-diabetic recipients of renal transplants.

METHODS: Data were drawn from the United States Renal Data System (USRDS) data base. The diagnosis of PTDM was identified with ICD-9 codes, and immunosuppression regimens were identified with prescription payments in Medicare billing records supplied by the USRDS. Recipients of first, single organ renal transplants between 1995 and 1998 with no evidence of diabetes mellitus prior to 30 days post-transplantation were included in the analysis if a calcineurin inhibitor prescription payment was recorded by 30 days post-transplant. Multivariate, time-varying techniques were used to estimate the time dependent risks of PTDM. The study endpoint was the diagnosis of diabetes. Subjects were censored from analysis at the time of their last recorded immunosuppression prescription, graft failure or death.

RESULTS: 6,715 patients were prescribed cyclosporine and 1,421 patients were prescribed tacrolimus by 30 days post-transplant were studied. Patients prescribed tacrolimus compared to cyclosporine were associated with a 75% (P=0.0008) increased risk of PTDM. Conversion to tacrolimus in patients initially treated with cyclosporine was associated with a 74% (P<0.0001) increased risk of PTDM. The magnitude of tacrolimus or cyclosporine dose reductions were not associated with the risk of developing PTDM.

CONCLUSION: Tacrolimus based immunosuppression was associated with increased risk of PTDM relative to cyclosporine both when used as the initial immunosuppressant and when used in conversion. This risk did not decline with dose adjustments. We can not support the conjecture that tacrolimus reduction lessens the risk of PTDM.

Abstract# 688 Poster Board #-Session: P144-II

POSTTRANSPLANT DIABETES MELLITUS IN AFRICAN AMERICAN KIDNEY TRANSPLANT PATIENTS ON TACROLIMUS AND SIROLIMUS: EARLY ONSET AND RESISTANCE TO STEROID WITHDRAWAL. Kenneth A. Bodziak,[1] William Weiss,[1] Joshua J. Augustine,[1] Thomas C. Knauss,[1] Donald E. Hricik.[1] *[1]Medicine, University Hospitals of Cleveland, Cleveland, OH.*

African American kidney transplant recipients (AAs) are at high risk for developing posttransplant diabetes mellitus (PTDM), especially when treated with steroids and tacrolimus. We compared the incidence and timing of PTDM in 60 consecutive AAs treated with prednisone, tacrolimus (target trough levels, 5-8 ng/ml), and sirolimus (target trough levels, 10-15 ng/ml) to those of 19 AAs transplanted in a earlier era and treated with comparable doses of prednisone, tacrolimus (target levels 8-12 ng/ml), and mycophenolate mofetil (MMF). The incidence of PTDM, defined as the need for insulin or oral hypoglycemic agents for more than one month, was 32% in each group despite a trend toward lower trough tacrolimus levels in the sirolimus-treated AAs. The onset of PTDM was 3±3.7 months in sirolimus-treated patients and 11.0±8.6 months in MMF-treated patients (p=0.003). Eight patients in the sirolimus-treated group were withdrawn from prednisone between 3 and 5 months after transplantation. With follow-up after cessation of steroids ranging from 12 to 37 months, all 8 patients remained on treatment for PTDM, consisting of insulin (n=2), an oral agent (n=4) or both (n=2). One patient presented with diabetic ketoacidosis prior to steroid withdrawal and had a C-peptide concentration of 0.2 ng/ml (normal range, 0.5 to 3.0 ng/ml), suggesting new onset of type 1 diabetes mellitus. C-peptide levels measured in 6 of the other 7 patients were either normal (n=4) or frankly elevated (n=2). We conclude that use of prednisone, tacrolimus and sirolimus in AAs is associated with a variant of PTDM that occurs relatively early after kidney transplantation and is resistant to withdrawal of steroids. The variable C-peptide levels in our patients suggest heterogeneous pathophysiologic mechanisms, ranging from irreversible islet cell toxicity to a state of insulin resistance.

Abstract# 689 Poster Board #-Session: P145-II

INCIDENCE AND RISK FACTORS FOR POSTTRANSPLANT DIABETES MELLITUS (PTDM) IN RENAL ALLOGRAFT RECIPIENTS IN SPAIN. A PROSPECTIVE AND MULTICENTER STUDY. Roberto Marcen, Jose M. Morales, Domingo Del Castillo, Miguel Gonzalez-Molina, Federico Oppenheimer, Jose M. Campistol, Jose M. Grinyo, Salvador Gil-Vernet, Luis Capdevilla, Ildefonso Lampreave, Francisco Valdes, Amado Andres, Fernando Anaya, Fernando Escuin, Manuel Arias, Luis Pallardo. *Nephrology, Hospital Ramon y Cajal, Madrid, Spain.*

New onset diabetes mellitus is an important complication after renal transplantation with increasing incidence in the last years due to the new immunosuppressive agents. It has been considered a risk factor of mortality by cardiovascular diseases. However, its incidence and risk factors are not definitively established. The purpose of the study was to investigate the incidence and risk factors of PTDM in a Mediterranean country. Methods. From a prospective and multicenter database of thirteen hospitals in Spain focusing on cardiovascular risk factors, 2012 non-diabetic renal allograft recipients transplanted from January 2000 to December 2002 were included. Diabetes was diagnosed following the criteria of the American Diabetes Association. 1423 patients were treated with Tacrolimus, Mycophenolate mofetil (MMF) and steroids and 589 with Cyclosporine-ME (CsA), MMF and steroids.

Results. At 6 months after transplantation 176 patients (8.75%) developed new onset diabetes. Compared to patients without diabetes, patients with PTDM were older (53.7±12.4 vs 48.4±13.6 years; p=0.000), with higher body mass index (BMI) (26.7±4.8 vs 24.7±4.2 kg/m2; p=0.000). There were no differences on sex distribution or immunosuppressive treatment (7.1% on CsA-MMF vs 8.8% on Tacro-MMF; p=0.527) or doses and levels of Tacro or CsA between diabetics and no diabetic. There were 28 out of 115 diabetic patients on Tacro-MMF (24.3%) and 11 out of 31 on CsA-MMF (26.2%) on treatment with insulin (p=0.977).

By multivariate logistic regression analysis, when all patients were included in the model, only older age (>55 years) was associated with PTDM (OR 1.73; 95%CI 1.21-2.47; p=0.002). In patients younger than 55 years, PTDM was associated with high BMI (OR 1.98; 95%CI 1.57-2.49; p=0.000). We did not find any factor associated to PTDM in recipients older than 55 years.

Conclusions. The incidence of PTDM at six months in immunosuppressive protocols including MMF is 8% in Spain and it was associated with older age and increased BMI. Notably, immunosuppression with Tacrolimus did not increase the risk of PTDM compared with Cyclosporine.

Abstract# 690 Poster Board #-Session: P146-II

ELEVATED URIC ACID LEVELS PRECEDE MEASURABLE CHANGES IN GFR OR BLOOD PRESSURE IN RENAL TRANSPLANT PATIENTS TREATED WITH TACROLIMUS AS COMPARED TO SIROLIMUS. A. S. Mahale,[1] S. C. Textor,[1] M. AbuAttieh,[1] H. A. Khamash,[1] S. J. Taler,[1] N. L. Driscoll,[1] J. E. Augustine,[1] T. S. Larson,[1] J. M. Gloor,[1] M. D. Griffin,[1] T. R. Schwab,[1] F. G. Cosio,[1] M. D. Stegall.[4] *[1]Transplant Center, Mayo Clinic, Rochester, MN.*

Introduction: The cause of renal allograft dysfunction following renal transplantation in tacrolimus(FK506)-based regimens is likely multifactorial. Uric acid has been recognized in experimental models as a potential vascular toxin & may be a contributing factor. We thus sought to compare serum uric acid (UA) levels & renal function in patients receiving a FK506-based regimen with those receiving a calcineurin-inhibitor free regimen.

Study Design: We examined UA, creatinine, GFR (iothalamate clearance) & BP measurements in 87 renal transplant recipients who had been randomized at the time of transplant to either FK506 or SLR in combination with Cellcept & Prednisone. Laboratory & clinical parameters were obtained at 4 & 12 mths post transplant. Ambulatory BP monitoring (ABP) was obtained at 12 mths. Student's t-test was used for statistical comparison.

Conclusion: Despite similar levels of renal function & BP, UA levels were significantly higher in patients treated with FK506 compared to SLR. These results indicate an early & progressive increase in UA before measurable changes in GFR are evident. These data are consistent with the hypothesis that subsequent differences in GFR between FK506 & SLR based regimens may in part reflect UA mediated vascular injury to the allograft.

Laboratory & clinical results in the 2 groups at 12 mths post-transplant & UA at 4 & 12 mths

	SLR(n=19)	FK506(n=68)	P value
Age (yrs)	51.5 ± 3.2	51.3 ± 1.7	0.96
Weight (kg)	81.7 ± 5.2	81.8 ± 2.5	0.98
BSA (m²)	1.92 ± 0.07	1.93 ± 0.03	0.92
Systolic BP (mm Hg)	121.7 ± 4.4	122.7 ± 2.1	0.84
Diastolic BP (mm Hg)	67.2 ± 3.0	74.1 ± 1.5	0.05
Awake average systolic BP (mm Hg)	140.9 ± 3.2	135.3 ± 1.8	0.13
Awake average diastolic BP (mm Hg)	74.4 ± 2.6	79.1 ± 1.4	0.12
Diuretics (%)	31.6	20.6	0.92
S Creatinine (mg/dl)	1.49 ± 0.09	1.48 ± 0.05	0.95
Iothalamate clearance (ml/min)	51.9 ± 4.3	55.9 ± 2.5	0.43
GFR by Cockroft-Gault equation (ml/min)	64.5 ± 5.4	66.3 ± 2.9	0.78
UA (4 mths)*	4.44 ± 0.38	6.18 ± 0.19*	0.0001
UA (12mths)*	5.22 ± 0.46	6.70± 0.25*	0.006

*UA levels were significantly higher in the FK506 group at both 4 and 12 mths. UA levels were also statistically higher at 12 mths compared to 4 mths in both groups (p=0.036 SLR group; p=0.02 FK506 group).

Abstract# 691 Poster Board #-Session: P147-II

LONG-TERM EXPERIENCE WITH THYMOGLOBULIN INDUCTION: MALIGNANCY AND OPPORTUNITISTIC INFECTION. Agnes Lo,[1] Nicole Walker,[1] M. Francesca Egidi,[2] Hosein-Shokouh Amiri,[3] Santiago Vera,[3] Nosratollah Nezakatgoo,[3] A. Osama Gaber.[3] *[1]Pharmacy, University of Tennessee, Memphis, TN; [2]Nephrology, University of Tennessee, Memphis, TN; [3]Surgery, University of Tennessee, Memphis, TN.*

Introduction: Thymoglobulin (Thymo) has been routinely used for induction in renal transplantation. The purpose of this logitundinal study is to determine the incidence of malignancies and opportunistic infections in a cohort of cadaveric renal transplant recipients receiving Thymo induction.

Methods: Observational study of 275 cadaveric renal transplant recipients transplanted between 01/01/1998 to 12/31/2000. The endpoints were the incidences of posttransplant lymphoproliferative disorder, malignancies, and opportunistic infections.

Results: The cohort consisted of 275 patients, 66% African-Americans, 56% male and a mean age of 44 years. The mean follow-up was 3 years. Of the 275 patients observed, 197 received Thymo for induction and 77 did not (30 daclizumab, 30 no antibody, 12 OKT3, 5 Atgam). There were no differences in demographics and incidences of delayed graft function between the two groups. Maintenance immunosuppressive regimens were similar between the two groups with majority of the patients receiving tacrolimus, mycophenolate mofetil, and steroids. The total accumulative dose of Thymo was 7.0 ± 3.6 mg/kg. There were no differences in patient survival, 92% in the Thymo group and 87% in the No-Thymo group, p=0.16 (log-rank). However, graft survival rate was significantly higher in the Thymo group (75%) than in the No-Thymo group (62%), p=0.04 (log-rank). Thymo was also associated with a lower incidence of acute rejection (13%) than the No-Thymo group (29%), p<0.01. There were no significant differences in serum creatinine levels at 3 years between the two groups 1.7 ± 0.8 mg/dL and 1.8 ± 0.9 mg/dL in the Thymo and No-Thymo group, respectively. There was only one case of PTLD, in the Thymo group. The incidence of malignancies was very low, 0.04% in the Thymo group and 0.03% in the No-Thymo group. The incidences of opportunistic infections were similar between the two groups.

Incidences of opportunisitic infections

	Thymoglobulin (n=197)	No-Thymoglobulin (n=77)
CMV	6%	11%
HSV	2%	4%
VZV	2%	3%
Polyoma	5%	5%
Parovirus	5%	0%
Invasive fungal disease	2%	6%
Toxoplasmosis	0.5%	2%

p=NS for all variables

Conclusions: Thymo induction is associated with a very low incidence of posttransplant malignancies and opportunistic infections in renal transplant recipients. Moreover, Thymo induction was associated with a lower incidence of acute rejection and improvements in graft survival.

Abstract# 692 Poster Board #-Session: P148-II

CHARACTERIZATION OF THE HEMATOLOGIC EFFECTS OF CAMPATH IN RENAL TRANSPLANT RECIPIENTS. James E. Hartle,[1] Kamal Chater,[2] Sayeed K. Malek,[3] Evan R. Norfolk,[1] Michael S. Schwartzman,[1] Taher M. Yahya,[1] Santosh Potdar.[3] *[1]Nephrology, Geisinger Medical Center, Danville, PA; [2]Internal Medicine, Geisinger Medical Center, Danville, PA; [3]Transplant Surgery, Geisinger Medical Center, Danville, PA.*

Campath (Alemtuzumab) has recently begun to be utilized for induction therapy in renal transplantation. Although Campath has been shown to have significant effects on hematologic parameters when it has been used for treatment of hematologic malignancies, its effect with dosage regimens used in renal transplantaion has been poorly defined. We began utilizing Campath for induction therapy in renal transplantation in May 2003. The dosage of Campath used was 30 mg IV given pre-operatively. The patients subsequently were treated with maintenance therapy of tacrolimus or mycophenolate mofetil. In addition all patients were treated with a regimen of rapidly tapering prednisone. We found that 18/20 (90%) of the patients experienced a decrease in their platelet count, with the lowest platelet count noted of 61,000 cells/microliter. The average time to development of thrombocytopenia was 1.1 days post-op. The average duration of thrombocytopenia was 14.5 days (range 2-85 days). In addition to the thrombocytopenia, absolute lymphopenia (<1000 lymphocytes/microliter) was noted in 20/20 (100%). The median time to development of lymphopenia was 1 day post-op. The range of nadir lymphocyte counts was 0-140. To date no patient has returned to a lymphocyte count greater than 1000 (longest duration of follow-up 154 days). In contrast absolute neutrapenia (<1000) did not occur in any patient. Furthermore there was no association of the hematologic abnormalities encountered with any of the maintenance immunosuppression used.

In summary Campath induction therapy for renal transplantation can result in significant and prolonged thrombocytopenia and lymphopenia. Such hematologic suppression may necessitate alteration in other medications typically utilized in the post-transplant period.

Abstract# 693 Poster Board #-Session: P149-II

SIDE EFFECTS AND DAILY LIFE FUNCTION IN KIDNEY TRANSPLANT RECIPIENTS AT 1 TO 3 YEARS POSTTRANSPLANT: A REPORT FROM THE PATIENT OUTCOMES REGISTRY FOR TRANSPLANT EFFECTS ON LIFE (PORTEL). Donna Hathaway,[1] the PORTEL Study Group. [1]*College of Nursing, University of Tennessee Health Science Center, Memphis, TN.*

Background: In a previous cross-sectional study, we evaluated the impact of immunosuppressive regimen and time since transplant on side effect profile and quality of life (QOL) in kidney transplant recipients. In this study, we examine treatment-related side effects and QOL in the first year posttransplant and prospectively evaluate a cohort of patients with three-year follow-up data.

Methods: Solid organ recipients 16 years of age or older with functioning grafts were eligible to participate in PORTEL regardless of immunosuppressive regimen or time since transplant. The PORTEL survey obtained information on demographics, clinical outcomes, medications, side effects as measured by the Memphis Survey and QOL as measured by the SF-12 Mental Component Summary (MCS) and Physical Component Summary (PCS). Patients completed surveys every 6 months.

Results: The study population included 185 first-time kidney transplant recipients within 12 months of transplant. The average age was 47, 55% were male and the majority were Caucasian (62%) followed by African American (14%). Immunosuppressive medications included prednisone (91%), mycophenylate mofetil (MMF) (76%), tacrolimus (50%) and cyclosporine (41%). In the first year posttransplant, patients reported poor physical QOL. Patients also reported the most problems in the life/role domain, which includes activities of daily living and the miscellaneous domain, which captures cosmetic and lifestyle variables such as weight gain, hair growth and sexual problems. When grouped by calcineurin inhibitor, there were no differences in demographics, rejection rates and physical QOL between treatment groups. Compared to patients on cyclosporine, patients on tacrolimus reported less severe problems in the life/role (mean 5.6 vs. 8.0, $p<0.05$) and miscellaneous (mean 7.9 vs. 11.1, $p<0.05$) domains. Differences in the miscellaneous domain were sustained after controlling for use of prednisone and MMF. Higher doses of prednisone (>10 mg/day) were associated with significantly worse physical QOL scores ($p<0.001$) regardless of other medication combinations. Longitudinal data to 30 months posttransplant were available for 19 patients. Physical QOL and life/role function improved, while mental QOL remained stable throughout the follow-up period.

Conclusion: Treatment-related side effects impact daily activities and various lifestyle variables in the first year posttransplant. Physical QOL and adaptation to life/role function improve with time.

Abstract# 694 Poster Board #-Session: P150-II

OVER-IMMUNOSUPPRESSION AFTER HLA-IDENTICAL LIVING-RELATED KIDNEY TRANSPLANTATION. Nicole M. van Besouw,[1] Jeroen H. Gerrits,[1] Saskia M. Postma,[1] Jacqueline Rischen,[1] Jacqueline van de Wetering,[1] Lenard M. B. Vaessen,[1] Barbara J. van der Mast,[1] Willem Weimar.[1] [1]*Internal Medicine - Transplantation, Erasmus MC, University Medical Center Rotterdam, Rotterdam, ZH, Netherlands.*

HLA-identical living-related kidney transplant patients may still receive standard doses of immunosuppression. We wondered why these patients should be exposed to the adverse effects of immunosuppression any longer.

We tapered HLA-identical living-related renal transplant patients on azathioprine (AZA) in combination with prednisone to half of their AZA dose and 5-10 mg/day prednison. We questioned whether the *in vivo* load of immunosuppression influenced their donor-specific T-cell reactivity, defined as the reactivity against minor histocompatibility antigens (mHag's).

Patients (n=15) who were at least 2 years (median 4.3 years, range: 2.3-15.2) after transplantation, were reduced from 100% AZA (median: 1.7 mg/kg AZA, range 1.0-2.2) in two steps to 50% of their AZA dose (median 0.7 mg/kg AZA, range: 0.5-1.1). The reactivity against mHag's was measured by IFN-γ Elispot-assay as published recently *(Transplantation, 2003)*, and the reactivity before and after tapering was compared with the reactivity at 3 months after HLA-identical living-related kidney transplantation (n=16).

Three months after transplantation, we found a frequency of donor-specific IFN-γ producing cells in the range of 5 to 115/10^6 PBMC (median: 30/10^6 PBMC). At least 2 years after transplantation, before reduction of immunosuppression, the frequency of IFN-γ producing cells was significantly lower (median 0/10^6 PBMC, range 0-320) ($p=0.04$). Tapering of immunosuppression did not affect the frequency (median 5/10^6 PBMC, range 0-540), and was still lower than in the early period after transplantation ($p=0.03$). These patients did not suffer from acute rejection after tapering immunosuppression.

From these results we conclude that HLA-identical living-related kidney transplant recipients can safely be reduced to 50% of their AZA dose without affecting the immune response. Our Elispot data indicate that these patients remain over-immunosuppressed, and that the immunosuppression could be reduced further or even stopped.

Abstract# 695 Poster Board #-Session: P151-II

PROSPECTIVE STUDY ON LATE CONSEQUENCES OF SUBCLINICAL NON-COMPLIANCE WITH IMMUNOSUPPRESSIVE THERAPY IN RENAL TRANSPLANT PATIENTS. Hans Vlaminck,[1] Bart Maes,[1] Yves Vanrenterghem.[1] [1]*Dept. of Nephrology and Renal Transplantation, University Hospitals Leuven, B-3000, Leuven, Belgium.*

Background: In this prospective study we compared the incidence of late acute rejections (acute rejection more than one year post transplantation) and changes in serum creatinine over time between compliers and non-compliers with immunosuppressive therapy more than 1 year post transplantation and explored the relative contribution of non-compliance and other known risk-factors in the occurence of late acute rejection episodes.

Methods: Using a prospective design, 146 adult renal transplant recipients (56% males; median age 47 years, IQR: 19) varying in time post transplantation (median 4 years; range: 1-18 years) were followed during a five-year period. Patients were interviewed at inclusion in the study regarding their intake of immunosuppressive medication and categorized as non-compliers if they admitted to have skipped immunosuppressive medication on a regular basis during the previous 12 months. The occurrence of a late acute rejection during the 5-year follow-up period was recorded.

Results: The sample consisted of 22.6% non-compliers of which 21.2% experienced a late acute rejection compared to 8% in the group of compliers at 5 years post-inclusion ($p<0.05$). Kaplan Meier survival analysis showed a decreased rejection free time in non-compliers compared to compliers ($p=0.03$). Non-compliant patients had a 3.2 higher risk of late acute rejections (Cox regression analysis, $p=0.005$). Non-compliers experienced a higher increase in serum creatinine over time (Linear Mixed Models, $p<0.001$).

Conclusions: Non-compliance in renal transplant patients more than 1-year post transplantation is associated with an increased risk for late acute rejection and a higher increase in serum creatinine during the following 5 years.

KIDNEY IMMUNOSUPPRESSION: NEW CHALLENGES

Abstract# 696 Poster Board #-Session: P152-II

RISK FACTORS FOR GRAFT FAILURE IN PATIENTS CONVERTED TO SIROLIMUS WITH CALCINEURIN INHIBITOR MINIMIZATION. David J. Taber,[1] Jeffrey Rogers,[2] Elizabeth E. Ashcraft,[1] G. Mark Baillie,[1,2] Angello Lin,[2] Prabhakar K. Baliga,[2] Kenneth D. Chavin,[2] P. R. Rajagopalan.[2] [1]*Department of Pharmacy Services, Medical University of South Carolina, Charleston, SC;* [2]*Division of Transplant Surgery, Medical University of South Carolina, Charleston, SC.*

Conversion of patients to sirolimus (SRL) with subsequent reduction in calcineurin inhibitor (CI) exposure has become an attractive option for adult kidney transplant (KTX) recipients with chronic allograft nephropathy. The aim of this study was to determine the risk factors associated with graft failure in KTX patients who were converted to SRL with CI minimization. METHODS: This was a retrospective chart review of all adult kidney transplant recipients who were converted to SRL while being maintained on CIs at lower concentrations. Data collection included baseline demographics and transplant characteristics as well as patient outcomes. Cox regression analysis was performed to determine what risk factors were independently and significantly associated with graft failure. RESULTS: A total of 124 patients were included in this analysis. Patients were transplanted between 11/87 and 1/03, and were converted to SRL between 10/99 and 5/03. Average follow-up from time of transplant was 1678±1093 and average follow-up from time of SRL conversion was 815±327 days. Table 1 displays the odds-ratio of developing graft failure for various characteristics.

Odds-Ratio For Graft Failure

Variable	Odds-Ratio	95% Confidence Interval	P-value
Non-Therapeutic SRL	4.00	1.3 - 11.8	**0.01**
Age	0.98	0.94 - 1.02	0.31
African-American Ethnicity	4.61	1.3 - 16.3	**0.02**
HLA Mismatch	1.20	0.9 - 1.6	0.20
PRA >20%	3.31	1.3 - 8.4	**0.01**
Mean CI Level	1.0	0.9 - 1.1	0.61
Cadaveric Donor	1.41	0.4 - 5.6	0.63
No Induction Therapy	1.6	0.7 - 3.6	0.30
Average SrCr at SRL conversion	1.1	0.9 - 1.3	0.37

The characteristics that were independently associated with graft outcomes were SRL concentration, African-Americans, and PRA >20%. CONCLUSION: Average SRL concentration appears to be an important factor in determining graft outcomes in patients converted to SRL with CI minimization.

Abstract# 697 Poster Board #-Session: P153-II

ERYTHROPOIESIS AFTER KIDNEY TRANSPLANTATION: COMPARISON OF SIROLIMUS TO MYCOPHENOLATE MOFETIL. Joshua J. Augustine,[1] Thomas C. Knauss,[1] Kenneth A. Bodziak,[1] Christopher T. Siegel,[2] James A. Schulak,[2] Donald E. Hricik.[1] *[1]Medicine, University Hospitals of Cleveland, Cleveland, OH; [2]Surgery, University Hospitals of Cleveland, Cleveland, OH.*

Anemia and erythrocytosis are common after kidney transplantation. Antiproliferative agents such as mycophenolate mofetil (MMF) may play a role in the pathogenesis of anemia. The influence of sirolimus on posttransplant erythropoeisis was examined in 214 kidney or kidney-pancreas (KP) recipients transplanted between 1999 and 2002 and treated either with sirolimus-(N=87) or MMF-based (N=127) therapy. We excluded patients who either 1) lost their grafts or 2) changed their antiproliferative drug during the first 12 mos. All patients received either cyclosporine (CsA) or tacrolimus (FK). Anemia was defined as hemoglobin (Hb) <12 g/dL in women and <13 g/dL in men. Posttransplant erythrocytosis (PTE) was defined as hematocrit >51% at any time. At 6 mos, the prevalence of anemia was 42% in MMF patients vs 57% in sirolimus patients (p=0.024); at 12 mos, the prevalence was 31% with MMF and 57% with sirolimus (p<0.001). At 12 mos, Hb concentration was 13.5±2 g/dL in MMF patients and 12.1±2 g/dL in sirolimus patients (p<0.0001). The incidence of PTE was 19% in the MMF group and 8% in the sirolimus group (P=0.027). By comparison to the MMF group, the sirolimus group included more black patients (74% vs 17%, p<0.001) and had higher rates of acute rejection (21% vs 8%, p=0.006) and higher creatinine levels at 12 mos (1.8±.7 vs 1.5±.5, p< 0.0001). Multiple linear regression was used to examine the influence of sirolimus, age, gender, race, donor age, donor source, KP transplant, CsA vs FK, acute rejection, use of angiotensin inhibitors, and serum creatinine on Hb concentration at 12 mos. Significant independent correlates of lower Hb levels included older recipient age (p<0.0001), female gender (p<0.0001), use of sirolimus (p<0.0001), and higher creatinine (p=0.002). Use of angiotensin inhibitors also correlated positively with Hb at 12 mos (p=0.033) when all 214 patients were analyzed. However, when 30 patients with PTE were excluded, this correlation was eliminated, reflecting the use of angiotensin inhibitors for treatment of PTE. Sirolimus remained a significant correlate of lower (Hb) at 12 mos when PTE patients were excluded (p=0.006). Using logistic regression, older age (p=0.006), female gender (p=0.004), creatinine (p<0.05) and use of sirolimus (p<0.05) all correlated negatively with the presence of PTE. Conclusions: compared to treatment with MMF, treatment with sirolimus is associated with a higher prevalence of anemia, lower Hb levels, and a lower incidence of PTE.

Abstract# 698 Poster Board #-Session: P154-II

CUTANEOUS ADVERSE EVENTS IN RENAL TRANSPLANT RECIPIENTS ON SIROLIMUS BASE THERAPY. Emmanuel Mahe,[1] Emmanuel Morelon,[2] Sophie Lechaton,[2] Rafik Mansouri,[2] Marie-France Mamzer,[2] Yves De Prost,[1] Christine Bodemer,[1] Henri Kreis.[2] *[1]Dermatology, Necker Hospital, Paris, France; [2]Renal Transplantation, Necker Hospital, Paris, France.*

Side effects of Sirolimus, the last immunosuppressive drug introduced in organ transplantation, are mainly dyslipidemia, diarrhea, anemia, thrombopenia, arthralgia, lymhoceles and wound healing problems. A few cutaneous events have also been mentioned such as acne, edemas, aphtosis and skin infections. However their frequency and relationship to sirolimus therapy are still unknown.

We conducted a phase-IV study to evaluate the frequency and the severity of skin, hairs, nails, and mucous cutaneous adverse events in renal transplant recipients (RTR) on sirolimus base therapy in a single renal transplantation center in France.

Eighty consecutive RTR on sirolimus base therapy (60% of male; middle age, 48 y; median duration of graft, 2.2 y) have been evaluated. Sirolimus was used as first line therapy from the time of transplantation in 45% of patients and switched from CNI to sirolimus in 55% of cases. Median duration of sirolimus treatment was 12 months (range: 0.75-84 months).

On average 99 % of patients complained of 7 cutaneous adverse events each. The most frequent cutaneous adverse events were pilosebaceous apparatus involvement, observed mostly in male (acne-like eruption (46%), scalp folliculitis (26%), hidradenitis suppurativa (12%)), edematous phenomenon (chronic edema (55%) acute and recurrent edema (15%)), mucous membrane disorders (aphtosis (60%), epistaxis (60%), chronic gingivitis (20%) and chronic fissure of the lips (11%)) and nail disorders (chronic onychopathy (74%), periungueal infections (16%)). The imputation of sirolimus was considered probable for these four groups of symptoms as they have appeared on sirolimus therapy, have either an unusual aspect or a higher incidence than what is usually observed on calcineurin inhibitor therapy. In addition, they always disappeared after sirolimus withdrawal. Twenty-five percents of patients complained of serious cutaneous adverse events leading to SRL cessation in 6%.

Conclusion: Skin disorders are frequent in RTR. However, it is probable that their frequency has increased since the introduction of sirolimus therapy. They are a frequent reason for withdrawing sirolimus, either because of their severity or more often because of their social and functional consequences.

Abstract# 699 Poster Board #-Session: P155-II

INDUCTION WITH BASILIXIMAB (SIMULECT) IN ELDERLY RECIPIENTS OF KIDNEY TRANSPLANTS ALLOWS IMPROVED SAFETY, REDUCED REJECTION, AND LOWER COST WHEN COMPARED TO ANTI-LYMPHOCYTE GLOBULIN (ATGAM) AND MUROMONAB (OKT-3). Michael Heifets,[1] Mohammad I. Saeed,[2] Michael J. Moritz,[2] Debra Sierka,[3] Susan Stabler,[3] Mitten H. Parikh,[2] Mysore S. Anil Kumar.[2] *[1]Nephrology/Transplantation; [2]Surgery/Transplantation, Drexel Univ. College of Medicine; [3]Pharmacy/Transplantation, Hahnemann Univ.Hospital/Tenet, Philadelphia, PA.*

The choice of induction immunosuppression for kidney transplantation in elderly recipients is dictated by consideration of infection risk, as well as efficacy, in prevention of acute rejection, thus allowing to reduce the subsequent maintenance immunosuppression and its attendant long-term side effect profile. We present data on 183 elderly kidney transplant recipients who were older than 60 at the time of transplant (mean 66±5 yrs) over the last 12 years. These patients received induction with either anti-lymphocyte globulin (ATGAM), muromonab (OKT-3), basiliximab (Simulect), and basiliximab, followed by steroid free maintenance immunosuppression. We compared incidence of delayed graft function (DGF), acute rejection (AR), side effects, patient/graft survival and costs of immunosuppression. Differences between groups were tested for significance using chi-square. Results, as shown below, indicate lower AR and DGF rates in both basiliximab groups *vs* ATGAM *vs* muromonab. Complete steroid avoidance did not result in increased AR rates. Basiliximab based induction was free of side effects, typically encountered when polyclonal, or monoclonal, antibodies are used, such as the need for central line, complications thereof, and first dose reactions.

	ATGAM	OKT-3	Basiliximab	Basiliximab, no steroids
n	29	45	40	69
Primary nonfunction	2 (6.9%)	2 (4.4%)	1 (2.5%)	3 (4.3%)
Delayed graft function	9 (31%)	14 (31%)	7 (17.5%)**	10 (14.5%)***
Acute rejection	14 (48%)	16 (35.6%)*	14 (35%)**	25 (36.2%)***

*sig difference Atgam vs OKT-3 group, chi-square p < 0.05; ** sig difference basiliximab vs ATGAM, p<0.005; ***sig difference basiliximab/no steroids vs ATGAM vs OKT-3, p<0.005.

One year patient[*graft*] survival rates were 83%[*80%*] for ATGAM and 90-93% [*88-90%*] for all other groups without reaching significant differences between groups. Death with functioning graft was the most common cause of graft loss. Post-transplant hospital stay was 7±3 days for both basiliximab groups, 11±3 days for muromonab group and 15±6days for ATGAM group. Cost of induction therapy was $2400±100 for basiliximab, $6000±1600 for muromonab and $9000±2200 for ATGAM. We conclude that basiliximab is preferrable agent for induction in the elderly kidney transplant recipients. Additionally, basiliximab eliminates the need for steroid use in maintenance immunosuppression.

Abstract# 700 Poster Board #-Session: P156-II

ETHNIC VARIATIONS IN POLYMORPHISMS WITHIN GENES INVOLVED IN RESPONSE TO IMMUNOSUPPRESSIVE AGENTS. Faieza J. Qasim,[2] Mohammed R. Bazrafshani,[1] Kay V. Poulton.[1] *[1]Transplantaion Laboratory, Manchester Royal Infirmary, Manchester, United Kingdom; [2]Dept Renal Medicine, Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: We have adopted a systematic approach to identify polymorphic variants in genes which may influence individual responses to immunosuppressive therapy. We have selected genes encoding products involved in the absorption, action or metabolism of the most commonly used immunosuppressive agents (including cyclosporin, tacrolimus and mycophenolate mofetil). Assays have been devised for genotyping polymorphic variants of the selected genes. Priority has been given to genotyping genetic variants known to exert a functional effect on the encoded gene product. In addition to this, all non-synonymous polymorphic variants, and single nucleotide polymorphisms within the promotor region of the gene were also tested.

Objective: The objective of this study was to assess the population distribution of variant alleles within the following genes: MDR-1, FKBP12 and IMPDH-1.

Methods: In our centre, the most prevalent ethnic groups are UK Caucasoids, and Asian (Indo-Pakistani). 100 individuals from each of these populations were genotyped in this study. Using SNpShot, PCR-SSP and PCR-RFLP based methods, we analysed eight polymorphisms in MDR-1, two polymorphisms in FKBP12 and five polymorphisms in IMPDH-1. Haplotype analysis was performed on data obtained to determine the significance of any linkage across each gene.

Results: Two polymorphisms of the MDR-1 gene in exon 22 (C3435T) and exon 26 (silent) were significantly linked in UK subjects. C3435T has been associated with low levels of expression of P-glycoprotein. There was also significant variation in the distribution of C3435T alleles between Caucasoid and Asian populations, with the T allele at this position having a higher frequency in Asian populations (p=0.05). Alleles of both FKBP12, and all five IMPDH-1 markers were present at high frequency in our populations with no significant variation between Caucasoids and Asians.

Conclusion: The lack of variation in genotypes for FKBP12 and IMPDH-1 suggest that although these variants may influence individual drug responses, they are unlikely to account for adverse effects noted commonly within specific ethnic groups. The ethnic variation in genotypes observed for MDR-1 suggests that C3435T variant may be a potential candidate for causing adverse reactions observed more commonly in patients of Asian origin.

Abstract# 701 Poster Board #-Session: P157-II

THREE YEAR RESULTS OF A RANDOMIZED STUDY COMPARING AS INDUCTION TREATMENT SIMULECT® AND THYMOGLOBULINE®. Matthias Buchler,[1] Lilia Benfatma,[2] Patrice Lepogamp,[3] Franck Bridoux,[4] Yann Lemeur,[5] Olivier Toupance,[6] Sophie Caillard,[7] Christiane Mousson,[8] Bruno Hurault de Ligny,[9] Jean F. Marliere,[1] Yvon Lebranchu.[1] *[1]Nephrology, CHU Bretonneau, Tours, France; [2]Nephrology, CHU Rouen, Rouen, France; [3]Nephrology, CHU Rennes, Rennes, France; [4]Nephrology, CHU Poitiers, Poitiers, France; [5]Nephrology, CHU Limoges, Limoges, France; [6]Nephrology, CHU Reims, Reims, France; [7]Nephrology, CHU Dijon, Dijon, France; [8]Nephrology, CHU Strasbourg, Strasbourg, France; [9]Nephrology, CHU Caen, Caen, France.*

The long-term efficacy of induction therapy with either a monoclonal anti RIL2 receptor antibody (Simulect®) or with polyclonal anti-lymphocyte antibodies (Thymoglobuline®) is still a matter of debate. In a multicenter study including 99 renal graft recipients at low immunological risk (PRA<30%), 49 patients were randomized to receive Simulect® (Group S) and 50 patients were treated with Thymoglobuline® (Group T). Long term immunosuppression included ciclosporin (Neoral®), mycophenolate mofetil (Cellcept®) and corticosteroids, which were progressively withdrawn between months 6 and 9. We previously reported comparable one year patient and graft survival between Groups S and T (98% vs 100% and 94% vs 96%, respectively). The incidence of biopsy-proven rejection was low in both groups (8%). We report here the 3 year patient and graft survival as well as renal function in the study population.

No further graft loss occurred between one year and 3 years in either group but one patient died having lost the graft within the first year (group T), giving 3 year patient and graft survival in groups S and T of 98% vs 98% and 94% vs 96%, respectively (p=ns). The daily dose of Neoral® (207 ± 53.1 mg/day vs 201± 53.8 mg/day) and Cellcept® (1827 ± 293 mg/day vs 1884 ± 334 mg/day) was not different between the two groups at three years and only 21.6% and 15.8% of the patients were still on steroids in groups S and T respectively, (p=ns). Mean serum creatinine at 3 years was 137 ± 48 mmol/L in group S and 138 ± 40 mmol/L in group T. Proteinuria higher than 1 g/day occurred in 3 patients of group S and 4 patients of group T. No case of lymphoma was reported.

Our results show that induction therapy with Thymoglobuline® or Simulect® combined with Neoral®, Cellcept® and steroids gives excellent and similar three year patient and graft survival, with good renal function in non-hyperimmunized patients receiving their first cadaveric renal allograft.

Abstract# 702 Poster Board #-Session: P158-II

A SINGLE CENTER EXPERIENCE WITH STEROID-SPARING IMMUNOSUPPRESSION IN PANCREAS AND RENAL TRANSPLANTATION: THYMOGLOBULIN® INDUCTION WITH DELAYED USE OF RAPAMUNE® AND PROGRAF®. R. Brian Stevens,[1] Jean Botha,[1] Wendy Grant,[1] Gerald Groggel,[2] James Lane,[2] Lucile Wrenshall.[1] *[1]Department of Surgery, University of Nebraska Medical Center, Omaha, NE; [2]Department of Medicine, University of Nebraska Medical Center, Omaha, NE.*

Although Rapamune® (sirolimus) has proven to be a potent immunosuppressive agent and is used in many steroid-free or -sparing regimens, reports of impaired wound healing, abscesses and increased lymphocele rates have dampened the enthusiasm for its use. We hypothesized that induction with Thymoglobulin ®, elimination of a sirolimus loading dose and in selected cases a 3-4 week window of sirolimus avoidance would minimize the incidence of these complications without increased risk of early rejection or graft loss.

We now report our results using this approach in primary kidney (KTA) and pancreas (PxTx) transplants performed from August 2001-November 2003. These transplants involved use of the following steroid-sparing immunosuppression protocol: 1. Thymoglobulin induction ® (4 doses RATG QOD at 1.5 mg/kg) with solumedrol 1mg/kg q6hrs x 2 with the first 2-3 doses, 2. Sirolimus 5 mg/day adjusted to reach blood levels of 8-12 ng/ml, 3. FK-506 3 mg BID adjusted to reach blood levels of 6-8 ng/ml. KTA patients with ATN receive a maximum of 7 mg/kg RATG over 14 days, sirolimus and FK506 are started before the last dose. PxTx and obese KTA patients are placed on MMF and FK-506 for the first 3 weeks, after which sirolimus is started at 5 mg/day and therapeutic levels are reached within a week, MMF is then discontinued. Rejection episodes are based on biopsy results.

With this approach we have performed 129 transplants: KTA=110 [50% LRD] and PxTx =19. All transplants have at least 30 days follow-up with an average follow-up of just over 12 months. Our results are summarized in the table below. We had no episodes of PTLD. All patients with rejection, once treated, were returned to steroid-free maintenance immunosuppression.

In conclusion sirolimus can be safely used in steroid-sparing regimens with acceptable rates of patient and graft loss, rejection, wound complications, lymphoceles, and ureteral/pancreatic-duodenal anastomotic leaks. Our data compares favorably to reports of others using standard steroid based immunosuppression with or without lytic induction.

A SINGLE CENTER EXPERIENCE WITH STEROID-SPARING IMMUNOSUPPRESSION

	Death	Graft Loss	Rejection	Wound Complications	Lymphocele	Leaks	CMV/BK infections
KTA	0.9% (n=1)*	2.7% (n=3)**	5.4% (n=6)	8.2% (n=9)	3.6% (n=3)	0.9% (n=1)	3.6% (n=4)
PxTx	0%	0%	5.3% (n=1)	15.8% (n=3)	5.3% (n=1)	0%	0%

* MI greater than 30 days post-transplant ; ** Recurrent disease (n=2)

Abstract# 703 Poster Board #-Session: P159-II

RENAL GRAFT SURVIVAL AND CALCINEURIN INHIBITOR. Robert S. Woodward,[1] Andrea Kutinova,[1] Mark A. Schnitzler,[2] Daniel C. Brennan.[3] *[1]HMP, UNH, Durham, NH; [2]HAP, Washington Univ, St Louis, MO; [3]Medicine, Washington Univ, St Louis, MO.*

Purpose: When tacrolimus has been evaluated as a post renal transplant maintenance immunosuppression agent, it has been associated with fewer acute rejections than cyclosporine (Vincenti et al, Transplantation, 2002) and improved graft survival when compared to no tacrolimus (Kasiske et al, Am J Transplant, 2003). We among others have linked tacrolimus to greater risk of new onset diabetes mellitus (NODM) (Woodward, Am J Transplant, 2003). The current study is a head-to-head comparison of graft survival among nondiabetic renal transplant recipients initially immunosuppressed with the two most popular calcineurin inhibitors, tacrolimus (FK) and microemulsion cyclosporine (CsA).

Methods: Following Kasiske et al, we examined data provided by the United States Renal Data System (USRDS) on all first, single-organ, renal transplants which occurred during the years 1996 to 2000. Importantly, we then limited the data to the patients for whom CsA (n=8698) or for whom FK (n=2658) was the initial maintenance immunosuppression. “Both” or “neither” were excluded. We used Cox Proportional Hazards regressions to estimate the FK-related relative risk of graft failure in models controlling for other significant donor, recipient, and transplant characteristics. Intermediate outcome variables such as diabetes, acute rejection, and delayed graft function were excluded to focus attention on the net relationship between immunosuppression agent and graft failure.

Results: In the model controlling for significant donor, recipient, and transplant characteristics, and when compared to microemulsion cyclosporine, tacrolimus had equivalent graft failure rates (hazard ratio = 1.02, p=0.762). Significant factors affecting graft failure included: age of the recipient (age: hazard ratio = 0.96, p<0.0001; age squared hr = 1.054, p<0.0001), recipient’s race (black: hr = 1.32, p<0.0001), donor’s gender (male: hr = 0.83, p=0.0006), the number of HLA mismatches, mycophenolate mofetil (hr = 0.77, p=0.0009), and azathioprine (hr = 0.81, p=0.033), cold ischemia time (hr = 1.01, p=0.0007), and panel reactive antibody status (hr = 1.50, p=0.004).

Conclusions: While tacrolimus is being used with increasing frequency, analyses of the USRDS data show no net advantage in the ultimate transplantation outcome, graft survival.

Abstract# 704 Poster Board #-Session: P160-II

A NEW APPROACH TO DESENSITIZATION OF POSITIVE T- AND B LYMPHOCYTE CROSS MATCH RECIPIENTS: KIDNEY TRANSPLANTATION ACROSS HLA BARRIERS FROM LIVING DONORS. Khalid Al Meshari,[1] Khalid Al Shaibani,[1] Ibrahim Al Ahmadi,[1] Ahmed Chaballot,[1] Khaled Hamawi,[1] Abdulghani Tabakhi,[2] Samhar Al Akash,[3] Hazem El Gamal,[1] Syed Raza,[1] Kesavamurthy Mohan,[1] Ahmad Al Jedai,[1] Arla Manser.[1] *[1]Renal Transplant Program, King Faisal Specialist Hospital and Research Centre, Riyadh, Central Province, Saudi Arabia; [2]Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Central Province, Saudi Arabia; [3]Department of Pediatrics, King Faisal Specialist Hospital and Research Centre, Riyadh, Central Province, Saudi Arabia.*

We report our experience with desensitization and the renal transplant outcome in seven highly sensitized individuals with positive T & B cross-match (XM) (group I) and in three individuals with positive T XM (group II). Desensitization is defined as abrogation of positive T-cell IgG XM by complement dependent cytotoxicity (CDC) and flow cytometry (FC). All transplants were from living donors.

Desensitization protocol for group I consisted of: (1) pre-transplant protein A immunoadsorption (IA), and intravenous immunoglobulin (IVIG); (2) peri-transplant induction with Thymoglobulin® and Rituximab®; (3) post-transplant quadruple therapy with Tacrolimus, Rapamycin, Mycophenolate Mofitil, and Prednisone. Desensitization protocol for group II was similar to group I without IA.

All but one patient in group I were females. All but one patient in group I were successfully desensitized and received kidneys from their historically positive XM living donors. All transplanted patients were alive with functioning grafts at the last follow up period:

	Age Year	FU Months	Cr µmoL/L	AMR♠	SCR♣	PB♦
GI (N=6)	42 (33-56)	9 (1-14)	106 (89-149)	1	0	NL (5/6)
GII (N=3)	51 (42-56)	2 (1-4)	103 (92-116)	1	NA	NA

♠AMR: Antibody-mediated rejection
♣SCR: subclinical rejection
♦PB: protocol biopsy at three and six months

Conclusions:

Desensitization and subsequent living donor kidney transplantation can be performed successfully in highly sensitized positive T & B XM recipients after desensitization according to the above protocols. Renal allograft functional and histological parameters at six months suggest that these grafts are likely to have an excellent long-term prognosis.

Abstract# 705 Poster Board #-Session: P161-II

BASILIXIMAB (SIMULECT) WITH CICLOSPORIN (NEORAL) AS A STRATEGY FOR STEROID AVOIDANCE IN RENAL TRANSPLANTATION. Neil R. Parrott,[1] Abdel Q. Hammad,[2] Christopher J. E. Watson,[3] Peter J. A. Lodge,[4] Christopher Andrews.[5] *[1]Renal Transplant Unit, Manchester Royal Infirmary, Manchester, United Kingdom; [2]Renal Transplant Unit, Royal Liverpool University Hospital, Liverpool, United Kingdom; [3]Department of Surgery, Addenbrooke's Hospital, Cambridge, Cambridgeshire, United Kingdom; [4]Department of Organ Transplantation, St James' University Hospital, Leeds, West Yorkshire, United Kingdom; [5]Biostatistics, Novartis Pharmaceuticals UK Ltd, Frimley, Surrey, United Kingdom.*

Historically, ciclosporin monotherapy has produced excellent long-term graft and patient survival rates when used as either initial or maintenance (>1 yr) immunosuppression. Conversely, addition of steroids results in a dose-related reduction in survival figures. However, amongst patients started on Neoral alone, rejection rates are relatively high and fewer than 50% remain steroid-free in the long-term. In order to investigate the utility of CD25 antibody (anti-IL-2R) as a strategy for avoidance of steroids or other additional immunosuppression, we conducted a prospective, multicentre, randomised, double-blind, placebo controlled, 12 month study of basiliximab induction on 108 kidney transplants receiving ciclosporin for microemulsion (Neoral) monotherapy. Patients were randomised pretransplant to receive a two dose course of basiliximab (n=52) or placebo (n=56).

Requirement for oral steroids at any time in the study was lower in the basiliximab group (33% vs 61%; P=0.004). Maintenance steroid use was lower with basiliximab than placebo at 6 months (26% vs 60%; P<0.001) and at end of study (25% vs 61%; P<0.001). More basiliximab patients than placebo patients were still maintained on Neoral monotherapy at 6 months (52% vs 29%; P=0.018) and at the end of study (46% vs 27%; P=0.046). 73% of the basiliximab group and 61% of the placebo group continued with Neoral as sole agent or with adjuncts until the end of study. The main reasons for changing from Neoral monotherapy were acute rejection and delayed graft function. Rejection occurred in 29% basiliximab patients and 43% placebo patients (P=0.17). One year graft and patient survival were 88% and 98% for basiliximab and 88% and 96% for placebo. The mean and median values for serum creatinine were consistently lower in the basiliximab group at every timepoint in the study: at 12 months median creatinines were 141 vs 164 µmol/l for the basiliximab and placebo groups respectively (P=0.55)

Conclusion. This is the first reported study of basiliximab induction with Neoral monotherapy immunosuppression. This strategy of Simulect induction significantly reduced the need for added maintenance immunosuppression allowing approximately 50% of the patients to be maintained on Neoral monotherapy, and 75% to be maintained on steroid-free, tailored immunosuppression at 1 year post transplant.

Abstract# 706 Poster Board #-Session: P162-II

A PROSPECTIVE PILOT STUDY OF EARLY CORTICOSTEROID ELIMINATION UNDER MODERN IMMUNOSUPPRESSION IN PATIENTS AT HIGH IMMUNOLOGIC RISK: ONE-YEAR RESULTS. R. R. Alloway,[1] J. Trofe,[1] R. E. Boardman,[1] C. C. Rogers,[1] M. Kidd,[1] J. F. Buell,[1] M. J. Hanaway,[1] R. Munda,[1] J. W. Alexander,[1] M. J. Thomas,[1] P. Roy-Chaudhury,[1] M. A. Cardi,[2] J. Austin,[2] S. Goel,[2] S. Safdar,[2] S. Huang,[2] E. S. Woodle.[1] *[1]Transplantation, University of Cincinnati, Cincinnati, OH; [2]Transplantation, The Christ Hospital, Cincinnati, OH.*

Early corticosteroid elimination (ECE) in high immunologic risk (HIR) renal Tx patients has been avoided due to previously published reports of increased risk of rejection. However ECE in HIR may be possible with newer IS agents (tacrolimus, sirolimus, MMF) particularly with T cell depleting antibody induction. Therefore, we have conducted the first prospective study of ECE in patients at risk for rejection. **Methods**: 25 pts were enrolled prospectively in an IRB approved HIPAA compliant protocol. IS consisted methylprednisolone 7 day taper, tacrolimus(target level 4-8ng/ml), sirolimus(target level 8-12 ng/ml), and MMF(2 gm/day). Induction with daclizumab 2mg/kg on POD 0, 14 was administered to the first 10 patients, then changed to thymoglobulin on POD 0 and 2 and daclizumab on POD 14. Recipient inclusion criteria were repeat Tx or pts with peak PRA≥25%. Data was analyzed for acute rejection (AR), graft loss, and death. **Results**: 25 pts with median followup of 402 (range 42-720) days(d) were analyzed. Recipient demographic characteristics were mean age 42yo, 40% AA, 28% male, 68% repeat Tx, 68% cad. Pretransplant immunologic markers revealed 36% with a peak flow PRA >25%, and a median 3 HLA AB MM and 1 HLA DR MM. Median time to therapeutic tacrolimus and sirolimus levels was 4 and 11 days respectively. 72% of the pts are currently CS free, 36% have experienced biopsy proven CI toxicity with 1 requiring CI discontinuation due to HUS, and two pts have required sirolimus discontinuation. The median MMF dose is 1.25(range 0.5-3g/d). The pts experienced rates of AR, graft survival, and pt survival of 40%, 88% and 96% respectively. Graft loses were due to patient death (infectious), chronic allograft nephropathy, and recurrent FSGS. Six of 10 pts (60%) with daclizumab induction alone experienced AR, but AR rates fell to 27% when Thymoglobulin was introduced, (p-value=0.1). Mean serum creatinines at 1, 3, 6, and 12 months are 1.5mg/dL, respectively. Four patients have a SrCr >2.0. **Conclusions**: Pts at immunologic risk for rejection can successfully undergo ECE in combination with Thymoglobulin, tacrolimus, sirolimus, and mycophenolate mofetil with acceptable rates of AR, graft and patient survival. This observation supports previous observations that Thymoglobulin reduces the risk of AR in steroid free regimens.

Abstract# 707 Poster Board #-Session: P163-II

ANEMIA IN RENAL TRANSPLANT RECIPIENTS ON SIROLIMUS BASE THERAPY FOR CHRONIC ALLOGRAFT NEPHROPATHY. Olivier Thaunat,[1] Emmanuel Morelon,[1] Sophie Lechaton,[1] Rafik Mansouri,[1] Marie-France Mamzer,[1] Marie-Noelle Peraldi,[1] Henri Kreis.[1] *[1]Renal Transplantation, Necker Hospital, Paris, France.*

Thrombocytopenia is the most frequent hematologic disorder attributed to sirolimus (SRL). Anemia and leucopenia are also frequently observed after renal transplantation in patients (pts) on SRL base therapy. However, SRL responsibility for anemia has never been demonstrated because of its usual use in combination with other myelotoxic drugs such as mycophenolate mofetil or azathioprine. We report 8 cases of anemia in stable transplant renal recipients related to SRL therapy.

8 renal transplant pts with biopsy-proven chronic allograft nephropathy (CAN) have been switched from calcineurin inhibitor to SRL base therapy to avoid nephrotoxicity. Corticosteroids dosage were not modified. Azathioprine was withdrawn at the time of SRL introduction to avoid myelotoxicity in 7/8 pts. SRL trough level target was 12-25 ng/ml (HPLC). All pts had stable renal function before the switch (mean calculated creatinine clearance was 41 ±12 mL/min), and none of them were given EPO. Before the switch mean hemoglobin level was 12.2 ± 1.2g/dl, mean white cell count 5087±2100 / mm3 and mean platelets count 225000 ±83000 / mm3.

Mean duration of SRL therapy before anemia lowest level was 11.5 ± 3 weeks. The lowest mean level of hemoglobin was 8.2 ± 1.34 g/dl. Biological features of the anemia are summarized in table 1. Anemia was associated with SRL-related interstitial pneumonitis in 3/ 8 patients. Platelet and leucocyte counts were also slightly decreased (mean platelet count 163 000 ±66000/mm3, mean leucocyte count 3800 ±1200/mm3). Anemia improved in all patients and resolved in 7 patients within 10.5 ±4.7 weeks after SRL withdrawal. This favorable outcome occurred despite the reintroduction of Aza (1 pts) or MMF (2 pts) and without using EPO therapy. Finally we observed a positive rechallenge test in one patient.

Conclusion: SRL induces microcytic aregenerative anemia without iron deficiency similar to inflammation-induced anemia. Withdrawal of SRL lead to rapid improvement and complete resolution of symptoms.

Table 1 : Biological features of sirolimus-induced anemia

	Mean ±SD
Serum iron level (mmol/L)	5.9±3.7
Serum ferritin level (µg/L)	644±322
Mean corpuscular volume (femtoliter)	81.4±2.7
Serum haptoglobin (g/L)	2.1±1.2
Reticulocyte count (cells/mm3)	51 600±22600
Serum LDH	536±104
CRP (mg/L)	15±11
Schizocytes	Absent

Abstract# 708 Poster Board #-Session: P164-II

LONG TERM RESULTS OF SINGLE PERIOPERATIVE HIGH DOSE OF ATG AS INDUCTION IN KIDNEY TRANSPLANTATION. R. Samsel,[1] A. Chmura,[1] G. Korczak-Kowalska,[1] Z. Wlodarczyk,[2] J. Pliszczynski,[1] L. Adadynski,[1] D. Wasiak,[1] B. Lagiewska,[1] M. Glyda,[3] J. Wyzgal,[1] T. Cieciura,[1] M. Durlik,[1] L. Paczek,[1] W. Rowinski.[1] *[1]Transplantation Institute, Medical University of Warsaw, Warsaw, Poland; [2]University Dept. of Transplantation, Medical Univesity Bydgoszcz, Bydgoszcz, Poland; [3]Transplantation Dept., County Hospital, Poznan, Poland.*

The rationale for perioperative administration of mono- or polyclonal antibodies used as induction therapy in organ transplantation is based on the fact that activation of the host immune system begins immediately on revascularization. Generally these immunosuppressive antibodies are given for the first 7-10 days postoperatively. The aim of this study was to asses the safety and efficacy of high single dose (9mg/kg) ATG Fresenius S given immediately before revascularization to kidney allografts recipients receiving triple drug immunosuppresion (Neoral, steroids and Cellcept which was substituted by azathioprine 4 months after transplantation). Seventy-nine, adult first cadaveric kidney recipients were included into the study. Patients were randomised to receive ATG or not. There were no differences between two groups regarding donor factors, degree of sensitisation, no of mismatches and cold ischemia time. No serious side effects or serious adverse events connected to ATG administration were observed.5 years follow up results are shown.

Induction Therapy with high single dose of ATG seems to be safe and efficacious in kidney transplantation.

	ATG(+) N=39	CONTROL N=40
Number of patients with delayed graft function	16 (41.02%)	20(50%)
Number of patients with rejections	9	16
Total number of rejection episodes	12	26
steroid resistant rejections	4	6
5 years of graft survival	71.8%	65%

Abstract# 709 Poster Board #-Session: P165-II

COMPARISON OF RAPID STEROID ELIMINATION WITH BASILIXIMAB INDUCTION VS STANDARD IMMUNOSUPPRESSION (MMF,TACROLIMUS AND STEROIDS) IN RECIPIENTS OF LIVE-DONOR RENAL ALLOGRAFTS. Luis Campos, Anne M. Weiland, Benjamin Philosophe, Eugene J. Schweitzer, Clarence E. Foster, Stephen T. Bartlett. *Department of Surgery, University of Maryland, Baltimore, MD.*

The exclusion of corticoid steroids from chronic immunosuppressive regimens could avoid their long term secondary effects of glucose intolerance, dysmorphism, osteoporosis and alteration of the endogenous production of suprarenal hormones. Nonetheless, the immunosuppressive effect and safety of steroids is well known. They are easy to prescribe and follow; are inexpensive and thus widely used by clinicians. In this study we question the long term need for steroids in recipients of live-donor renal grafts. **Patients and methods:** In this retrospective analysis we compared a standard regimen (**SR**) of MMF(2g/day), tacrolimus (levels of 10-12 ng/ml) and prednisone VS a Rapid Steroid Elimination Protocol (**RSEP**) consisting of i.v. solumedrol (day 0,1 and 2 only), basiliximab (20 mg on day 0 and 4), MMF (2g/day) and tacrolimus (levels of 10-12 ng/ml). Study group RSEP n=69, 2:1 male/female ratio, age(mean)= 49.9. Control group SR n=72, 1:1 male/female ratio, age (mean)= 49 years. The groups were statistically indistinct regarding HLA and DR mismatches, ESRD diagnosis, comorbidities, related vs unrelated grafts and PRA%. Follow up was > then one year. Results:

Results:

Group	Patient Survival (%)	Graft Survival (%)	Rejection (%)	Days to Rejection	Creatinine (mean)	Mean Length Stay (d)	Deaths (n)
RSEP (n=69)	94.29	95.7	21.7	325 (+/- 33)	1.38	7.2 (+/- 3.9)	2
SR (n= 72)	97.22	95.8	20.8	818 (+/- 41)	1.57	10 (+/- 21.7)	2

At one year 16 patients on the RSEP group had been placed on chronic steroids: 2 patients had recieved a pancreas after kidney transplant; 14 had biopsy proven acute cellular rejection. The RSEP group had 2 deaths, both with functioning grafts, from CVA. One graft was lost from recurrent FSGS. The SR group had 2 deaths, both due to sepsis. One graft was lost due to recurrent FSGS. **Conclusions:** Both groups of patients that were placed on chronic immunosuppressive regimens with or without steroids exhibited similar graft and patient survival at one year, with comparable rejection rates and serum creatinine levels. This suggests that after brief induction with i.v steroids and basiliximab, 77% of the recipients of live donor renal allografts do not require chronic steroids. Further follow up will answer if the steroid free group is susceptible to late (>2 years) rejection episodes and the effect of steroids (or no steroids) on chronic allograft nephropathy.

Abstract# 710 Poster Board #-Session: P166-II

EVALUATION OF BONE MINERAL DENSITY AFTER RENAL TRANSPLANTATION ON LOW-DOSE STEROID IMMUNOSUPPRESSION. Darrin Willingham,[1] Martin L. Mai,[1] Nasimul Ahsan,[1] Peter Fitzpatrick,[1] Thomas A. Gonwa.[1] *[1]Department of Transplantation, Mayo Clinic Jacksonville, Jacksonville, FL.*

Background: High dose maintenance prednisone (> 9 mg/day) and cumulative prednisone exposure have been identified as risk factors for increased bone loss after renal transplantation (RT). At our program, the maintenance prednisone dose in new RT recipients was reduced from 10 mg to 5 mg a day in all patients transplanted after January 1, 2002.We report the first year bone mineral density (BMD) results in RT recipients who received maintenance prednisone at 10 mg a day (historical control) and 5 mg a day. **Methods:** All patients who received RT from April, 2000 (start of the program) to November 30, 2002 and had pre-transplant and one-year BMD (measured by DEXA imaging) were retrospectively reviewed. **Results:** 54 patients were treated with predisone 10 mg a day and 51 with prednisone 5 mg a day. The majority of these patients also received tacrolimus and mycophenolate mofetil. The table displays the percent change in BMD from pre-transplant to one-year post-transplant measured at the femoral neck and the lumbar spine.

Percent Change BMD - Pre-transplant to One-Year Post

% change BMD	Pred 10 mg	Pred 5 mg	p value
Femoral neck	-3.61	-1.37	p=0.10
Lumbar spine	0.11	0.40	p=0.93

Percent change in BMD from pre-transplant to one-year post-transplant measured on all patients in the lumbar spine was 0.25 and in the femoral neck -2.54. The next table shows the number of patients with osteoporosis or osteopenia in the femoral neck and lumbar spine at one-year post-transplant.

Number of patients with osteoporosis or osteopenia

Location	**Osteoporosis** Pred 10 (n)	**Osteoporosis** Pred 5 (n)	**Osteopenia** Pred 10 (n)	**Osteopenia** Pred 5 (n)
Femoral neck	10 (54)	8 (51)	24 (54)	24 (51)
Lumbar spine	4 (54)	1 (51)	20 (54)	16 (51)

There was no significant difference in parathyroid hormone levels pre-transplant or at one-year post-transplant between the prednisone groups. **Conclusions:** Reducing maintenance prednisone dose from 10 mg to 5 mg a day did not significantly reduce bone loss at one-year post-transplant. The number of patients with osteoporosis or osteopenia at one-year post-transplant was similar between the groups. Bone loss continues to be a problem after RT on newer immunosuppression protocols.

Abstract# 711 Poster Board #-Session: P167-II

EFFECT OF STATINS AND BISPHOSPONATES ON POST TRANSPLANT BONE DISEASE IN KIDNEY AND KIDNEY/ PANCREAS TRANSPLANT RECIPIENTS. Elena Slavcheva,[1] Charles McCuskey,[2] Gregory Jaffers.[1] *[1]Transplantation, TAMU Scott and White, Temple, TX; [2]Emergency Medicine, TAMU Scott and White, Temple, TX.*

Statins are associated with increased bone mineral density (BMD), in the general population and in transplant recipients. We examined the effect of statin therapy on BMD in transplant recipients treated with a bisphosphonate (BisP) for established osteopenia or osteoporosis. BMD was measured using a Lunar DPX-L bone mineral densitometry unit (Lunar, Co.; Madison, WI) (DEXA). Statins were prescribed for hyperlipidemia and BisP were prescribed for osteoporosis defined as T-score ≤ -2.5 (WHO criteria) and for osteopenia (T ≤ -1.5) based on intial DEXA at the treating physicians discretion. The standard immunosuppressive regimen included cysclosporin, Cellcept and steroids with steroid taper to 5 mg a day maintenance dose by 3-6 months post transplant. Oral calcium and vitamin D were administered routinely. Baseline DEXAs were analyzed for the effect of six months or more of statin therapy on initial BMD. Follow-up DEXA scans were analyzed for the effect BisP with or without statin therapy on lumbar spine (LS) and femoral neck (FN) BMD. Means were compared using a t-test or kruskal wallis as indicated. Categorical variables were compared with Chi-square or Fisher's exact test.

A total of 439 DEXAs in 133 patients were analyzed. The demographic characteristics did not differ among the studied groups. The initial mean BMD of the LS was 1.206 g/cm^2 in the statin (S+) vs 1.205 g/cm^2 in the no statin (S-) group (P=NS) with mean T-scores of -0.1 and -0.2 respectively. The initial mean BMD at the FN was 0.865 g/cm^2 in (S+) and 0.863 g/cm^2 in (S-) (P=NS) with mean T-scores of -1.3 and -1.2 respectively. In 306 consecutively paired DEXA scans (first to second, second to third, etc.) BisP therapy resulted in improvement of BMD in FN of +2.0% vs 0.1% in nonBisP (P<0.03). There was a trend towards improvement in LS BMD of +1.4% vs -0.15% (P=NS) in BisP vs. nonBisP. When analyzed in regard to statin therapy in patients treated with BisP LS BMD changed by +0.38% on statin vs. +2.3% not on statin (P=NS) and FN BMD changed by +1.94% in BisP and statin vs. +2.13 % BisP not on statin(P=NS). A similar effect was shown over time on BMD of statin vs. no statin therapy in nonBisP treated patients.

BisP therapy improves BMD in FN in transplant recipients with continued improvement documented by multiple serial scans. Statin therapy did not predict a higher BMD on the initial DEXA scan nor did statins enhance the effect of BisP on post transplant bone disease in our population.

Abstract# 712 Poster Board #-Session: P168-II

LUMBAR BONE MINERAL DENSITY (BMD) AFTER KIDNEY TRANSPLANTATION. A PROSPECTIVE STUDY. Roberto Marcen, Julio Pascual, Carmen Caballero, Jose L. Teruel, Juan J. Villafruela, Javier Ocaña, Maria T. Tenorio, Cristina Galeano, Francisco J. Burgos, Joaquin Ortuño. *Nephrology, Hospital Ramon y Cajal, Madrid, Spain.*

Osteopenia expressed by low BMD is a frequent complication after kidney transplantation and appears early after the procedure. Most of the data available are cross-sectional studies or with short-term follow-up. The purpose of the present study was to investigate prospectively the evolution of lumbar BMD on a population of renal transplant on low-dose steroids.

Methods: In 65 patients with functioning graft, 15 on treatment with Cyclosporin (CsA) and 50 with Tacrolimus, serum biochemical markers of bone metabolism and BMD at the lumbar vertebrae L2-L4 and in the femoral neck were prospectively evaluated in at least four serial examinations (at transplant, 1y, 2y and 3y thereafter).

Results: At the time of transplantation, BMD in L2-L4 was similar to that of the general population of matched age and sex (z-score=-0.421±1.276). SCr was 1.57±0.46 mg/dl, intact PTH levels were 89±79 pg/ml, 6 patients had values >250 pg/ml. During the follow-up, the mean of BMD in L2-L4 did not change from baseline to 3years (? BMD, +2.187±11.147%). However, according to the variations in L2-L4 BMD, patients could be divided in three groups: 20 patients without variation in BMD, 20 with decreased BMD (? BMD, -7.45± 4.70%) and 35 with increased BMD (? BMD, +11.75±10.28 %). Decreased BMD appeared in the first posttransplant year and remained stable until 3y (baseline 0.964±0.164; 1y 0.904±0.151; 2y 0.900±0.148 and 3y 0.886±0.140 gm/cm2; p <0.001). Increased BMD was maintained along the follow-up (baseline 0.860±0.176; 1y 0.901±0.161; 2y 0.923±0.174 and 3y 0.954±0.178 gm/cm2; p <0.001). There was a parallel increment in femoral neck BMD (baseline 0.712±0.144; 1y 0.749±0.119; 2y 0.763±0.214 and 3y 0.826±0.184 gm/cm2; p <0.001). The last two groups were different in the baseline BMD of L2-L4(p<0.05). The PTH levels were lower in the group with decreased BMD(160±164 vs 111±110 pg/ml; p=0.213) but the differences did not reach statistical significance. No patient was treated with calcium supplements or vitamin D. BMD losses were not associated to gender, time on dialysis, steroid doses or inmunosuppression.

Conclusions: About one third of the transplanted patients showed significant BMD losses during the first year. We could not identify the risk factors of this complication but low PTH levels could be implicated. Our results do not support long-term administration of antiosteoporotic therapy.

Abstract# 713 Poster Board #-Session: P169-II

THE SIGNIFICANCE OF PROTOCOL BIOPSIES IN THE MODERN IMMUNOSUPPRESSIVE ERA; WHAT FOR? Mark R. Laftavi,[1] Rabie N. Stephan,[1] Barbara K. Stefanick,[2] Romesh Kohli,[1] Andrea Rubino,[1] Mary Applegate,[1] Hayley Guzowski,[1] Fadi Y. Dagher,[1] Oleh G. Pankewycz.[1] *[1]Division of Transplant, Buffalo General Hospital, Kaleida Health, Buffalo, NY; [2]Pathology, Buffalo General Hospital, Kaleida Health, Buffalo, NY.*

Despite a significant improvement in the short term survival of kidney transplants, long-term allograft survival remains limited by Chronic Allograft Nephropathy (CAN). CAN may be due to several immunological and non-immunological factors. Two important causes of CAN are (1) subclinical rejection resulting from suboptimal immunosuppression and (2) chronic calcineurin inhibitor (CNI) toxicity. Protocol biopsies are an attractive tool for the detection of subclinical rejection and drug toxicity. The aim of this retrospective study was to evaluate the usefulness of protocol biopsies in the modern immunosuppressant era.

Patient and Method: From July 2001 to October 2003, 193 patients (pts.) received kidney transplants (ktx) in our center. 53 pts. underwent a protocol biopsy at 1, 6 and 12 months post transplant.

Patient Demographics

Age	Male	AA	Diabetics	CIT
50.4 ± 12.9	32 (60%)	9 (17%)	19 (36%)	18.1 ± 6.8

All pts received 3-5 doses of Thymoglobulin (average total dose 3.4 ± 1.2 mg/kg) and tacrolimus (TAC), mycophenolate mofetil (MMF) and steroids (250mg/iv POD 0 and 125 mg/iv POD 1, 30 mg/po POD2). Steroids were tapered to zero in 26 pts in one week. 27 pts continued prednisone 5 mg/day indefinitely.

Results: A total of 91 protocol biopsies were performed. One pt (1%) demonstrated grade 2A rejection at one month biopsy which was treated with steroid bolus therapy. Ten biopsies (10%) showed borderline rejection. Five biopsies (5%) revealed tacrolimus toxicity and one pt showed cholesterol emboli. At one month, 2 pts (5%) showed minimal fibrosis and 2 pts (5%) demonstrated mild fibrosis. At 6 months, 1 pt. (4%) had minimal fibrosis, 4 pts. (15%) showed mild and one pt. (4%) revealed moderate fibrosis. At one-year biopsies, only one pt with borderline subclinical rejection at 3 months biopsy, the amount of fibrosis increased from mild to moderate. In the rest of the biopsies the amount of fibrosis remained unchanged.

Conclusion: When using thymoglobulin induction in combination with TAC and MMF with or without steroids, the rate of subclinical rejection is very rare (1%). Protocol biopsies in the modern immunosuppressant era may not be necessary to detect subclinical rejection. However, protocol biopsies may provide valuable information for monitoring fibrosis (CAN) and chronic drug toxicity and may prove useful in directing modifications of immunosuppression.

Abstract# 714 Poster Board #-Session: P170-II

PARTICIPATION IN CLINICAL STUDIES DOES NOT ALTER OUTCOMES AFTER LIVING DONOR KIDNEY TRANSPLANTATION. Todd V. Brennan,[1] Catherine K. Chang,[1] Stephen J. Tomlanovich,[1] Alan Bostrom,[2] JoAnn K. Zlatunich,[1] Flavio Vincenti,[1] Sandy Feng.[1] *[1]Department of Surgery, Division of Transplantation, University of California San Francisco, San Francisco, CA; [2]Department of Epidemiology & Biostatistics, University of California San Francisco, San Francisco, CA.*

Clinical trials to assess novel therapies are essential to advance organ transplantation (tx). It is unknown whether participation in clinical trials impacts upon tx outcomes. **Methods:** Our cohort comprised of 373 unsensitized adults undergoing primary living donor kidney transplantation (LDKT) between 1997–2001, divided into study (SPs) and non-study patients (NSPs). Recipient and donor demographics, tx characteristics, and frequency of clinic visits, readmissions, graft ultrasounds and biopsies during the 1st post-tx year were compared using Fisher's exact, Chi-squared, and Mann-Whitney tests. Patient and graft survival and rejection within 1yr of tx were determined by Kaplan-Meier analysis. One-yr creatinine (Cr), Cr clearance, and delta-Cr (1 yr - 6 mo Cr) were compared using the Mann-Whitney test. **Results:** Nearly 1/3 (122 of 373) of all LDKT recipients were enrolled in studies. Compared to NSPs, SPs tended to be male (p=0.001) and less well matched with their donor (p=0.003) (Table 1). During the 1st post-tx year, SPs had more clinic visits (p=0.02) and readmissions (p=0.01), but did not undergo more graft ultrasounds or biopsies. SPs had higher 1 yr Cr compared to NSPs (p=0.002) but there were no significant differences in Cr clearance (p=0.06) or delta-Cr (p=0.38) (Table 2). Finally, patient survival, graft survival and rejection during the 1st post-tx year were entirely comparable for SPs and NSPs (Table 2). **Conclusions:** A substantial proportion of our adult LDKT recipients participate in clinical trials. SPs, who frequently receive novel therapies, enjoy comparable outcomes to NSPs receiving full-dose standard immunosuppression. Participation resulted in a modest intenstification of post-tx follow-up but did not increase biopsy frequency. These results should encourage tx centers to vigorously approach tx candidates to participate in clinical trials.

Table 1. Demographics of LDKT: NSP vs. SP

CHARACTERISTIC	NSP	SP	P-VALUE*
R Age (yrs)	44.1 ± 13.6	46.1 ± 13.3	0.17 (MW)
R Gender (%M)	54.2	72.1	**0.001** (FE)
R Ethnicity			0.15 (FE)
R Disease			0.57 (CS)
R Dialysis Mode			0.17 (FE)
D Age (yrs)	41.6 ± 10.8	42.9 ± 10.3	0.24 (MW)
D Gender (%M)	33.9	34.4	0.36 (FE)
D Ethnicity			0.13 (FE)
Laparoscopic procurement (%)	21.5	27.0	0.24 (FE)
Tx Year			0.36 (FE)
D-R Unrelated (%)	26.3	32.8	0.22 (FE)
HLA Mismatch			**0.003** (CS)
0 Mismatch # (%)	40 (17.1)	3 (2.6)	
≤3 Mismatches # (%)	161 (68.8)	65 (57.0)	
WIT (minutes)	32.1 ± 9.2	31.2 ± 8.2	0.59 (MW)

*MW=Mann-Whitney, FE=Fisher's exact, CS=chi-squared

Table 2. Outcomes of LDKT: NSP vs. SP

OUTCOME	NSP	SP	P-VALUE*
One yr Cr (mg/dL)	1.45 ± 0.60	1.59 ± 0.54	**0.002** (MW)
One yr Cr Clearance (ml/min)	68.2 ± 22.5	63.0 ± 18.3	0.062 (MW)
Delta Cr (mg/dL)	0.003 ± 0.59	0.061 ± 0.46	0.38 (MW)
Clinic Visits**	13.6 ± 5.9	14.9 ± 5.4	**0.03** (MW)
Readmissions**	0.67 ± 1.20	0.99 ± 1.45	**0.01** (MW)
Allograft Ultrasounds**	1.10 ± 1.44	1.06 ± 1.46	0.67 (MW)
Allograft Biopsies**	0.48 ± 0.76	0.54 ± 0.76	0.36 (MW)
5 yr Patient Survival (%)	94.0	91.8	0.43 (LR)
5 yr Graft Survival (%)	87.0	90.2	0.92 (LR)
5 yr Graft Survival^ (%)	90.8	96.1	0.41 (LR)
Acute rejection** (%)	21.1	23.0	0.69 (FE)

*MW=Mann-Whitney, FE=Fisher's exact, LR=log-rank
**Within 1 yr of transplantation
^Death censored

Abstract# 715 Poster Board #-Session: P171-II

IMPACT OF DACLIZUMAB AND LOW DOSE CYCLOSPORINE IN COMBINATION WITH MYCOPHENOLATE MOFETIL AND STEROIDS ON RENAL FUNCTION AFTER KIDNEY TRANSPLANTATION. Josef Fangmann,[1] Wolfgang Arns,[2] Hans Marti,[3] Klemens Budde,[4] Hans Neumayer,[4] Tobias Beckurts,[5] Juergen Floege,[7] Raimund Margreiter,[8] Johann Hauss.[1] *[1]Chirurgie, Universitaet, Leipzig, Germany; [2]Chirurgie, Universitaet, Koeln-Merheim, Germany; [3]Nephrologie, Inselspital, Bern, Switzerland; [4]Med.Klinik V, Charite, Berlin, Germany; [5]Chirurgie, Universitaet, Koeln, Germany; [6]Innere Med.VI, Universitaet, Koeln, Germany; [7]Nephrologie, Universitaet, Aachen, Germany; [8]Chirurgie, Universitaet, Innsbruck, Austria.*

Background: The purpose of this study is to determine whether Daclizumab (Dac) induction in combination with Mycophenolate Mofetil (MMF) enables a low dose Cyclosporine (CsA) regimen. Safety, efficacy and impact on early renal function of this regimen are compared to standard immunosuppression (standard-dose CsA and MMF).

Methods: This multicenter international, prospective, randomized study was conducted in 14 centers in 3 European countries. Patients were randomized into two groups. Standard group: CsA trough levels: 150-250 ng/ml with MMF (2g/day). Dac group: 2 mg/kg Dac first dose, followed by 4 additional doses of 1 mg/kg every 2 weeks, MMF (2g/day), and low dose CsA which was defined as 50% of the standard trough levels in the individual centers ranging from 75 to 125 ng/ml. Steroids were tapered identically in both groups. A total of 156 patients were enrolled in the study. Only data from 121 patients with a follow-up of at least 3 months are presented in this interim analysis.

Results: 59 patients were analyzed in the Dac group and 62 in the standard group. Baseline characteristics were similar for both groups. CsA levels in the Dac group at day 7, 28, and 95 were reduced to 53%, 65%, and 68%, respectively, compared to the standard group and thus did not reach exactly the intended reduction of 50%. Patient survival at 3 months was similar in both groups with 98% and 100% in the Dac and in the standard group, respectively. After three months follow-up 5 grafts were lost in the standard group versus 1 graft lost in the Dac group. The incidence of biopsy proven acute rejection episodes was 7% following Dac induction compared to 27% in the standard group (p=0.0035). Mean creatinine values were 1.5 ± 0.5 mg/dl in the Dac group versus 2.0 ± 1.4 mg/dl in the standard group at week 12. There were no differences in the incidence of either serious adverse events or major infections.

Conclusion: Daclizumab induction in combination with MMF is a safe and efficacious regimen in kidney transplantation that allows a profound primary sparing of CsA. There is a tendency towards a better renal function after 3 months in the Dac group. With respect to graft survival and acute rejection episodes it is superior to standard-dose CsA in combination with MMF.

Abstract# 716 Poster Board #-Session: P172-II

ELDERLY GRAFT RECIPIENTS DO NOT SHOW SIGNS OF "RELATIVE IMMUNODEFICIENCY". G. Bold,[1] P. Nickel,[1] F. Presber,[2] D. Bitti,[2] J. Juergensen,[1] Ch. Rosenberger,[1] N. Eibl,[1] U. Frei,[1] H.-D. Volk,[2] P. Reinke.[1] *[1]Dept. of Nephrology and Intensive Care; [2]Dept. of Medical Immunology, Charite, Berlin, Germany.*

To improve the use of older donor kidneys we participate in the ESP where kidneys are allocated locally to older recipients with short cold ischaemia time regardless of HLA compatibility. As decreasing immunity in elderly patients has been reported, initially, we used a calcineurin-free immunosuppressive protocol. Because of numerous rejections, we switched to a Basiliximab/ FK506/ steroid-based protocol with good results. In order to learn more about the immune system in the elderly recipients, we compared several immune functions pre and post Tx in ESP and in the younger patients allocated by the regular allocation programms (ETKAS).

The results of 11 ESP (67.4 yr.; donors: 67.6 yr.) were compared with 17 ETKAS patients (51.1 yr; donors: 34.2 yr.). The acute rejection rate,1-year graft and patient survival were comparable in both groups.

We determined the numbers of T, B and NK cells as well as monocytic HLA-DR/CD86 expression by flow cytometry pre-Tx and in the follow-up post Tx. The secretion of IFN-g/IL-4 and TNF-a/IL-1b was measured 24 hr after Con A and LPS stimulation, respectively. HCMV-specific and allospezific IFN-g producing T cell frequencies were determined by flow cytometry and by ELISPOT Assay, respectively.

CD4+ T cell counts and monocytic HLA-DR expression were significantly higher in the ESP patients in comparison with ETKAS. We found no significant differences between the groups regarding CD8+ T, NK and B cell counts as well as LPS induced TNF-a/IL-1b production before transplantation. Both groups showed also comparable Con A induced IFN-g memory T cell response and comparable frequencies of donor-specific and CMV specific IFN-g producing T cells pre Tx. In the early phase post Tx both groups showed a similar T lymphopenia. The recovery of T cells, particularly of CD4 + T cells, as well as recovery of ConA induced IFN-g T cell response post Tx was reduced in the ESP patients. LPS induced TNF-a secretion and monocytic HLA-DR/CD86 expression after Tx were comparable between the groups.

Elderly graft recipients do not show signs of "relative immunodeficiency" compared with younger graft recipients pre Tx. A protocol based on Basiliximab/FK506/steroid turned out to be succesful in preventing early post Tx complications. Despite powerful prevention of acute rejection and delayed recovery of T cell function, ESP patients on this immunosuppressive protocol had no enhanced risk for infections. Our data support a powerful initial immunosuppression in ESP patients.

Abstract# 717 Poster Board #-Session: P173-II

USE OF LOW-DOSE TACROLIMUS, DACLIZUMAB, MYCOPHENOLATE MOFETIL AND STEROIDS IN NON HEART BEATING RENAL TRANSPLANTS. María Marques,[1] Ana Sanchez-Fructuoso,[1] Dolores Prats,[1] Jose Conesa,[1] Natalia Ridao,[1] Julia Blanco,[2] Alberto Barrientos.[1] *[1]Nephrology, Hospital Clínico San Carlos, Madrid, Spain; [2]Pathology, Hospital Clínico San Carlos, Madrid, Spain.*

In non-heart beating donor (NHBD) kidney transplants, it is desirable to reduce the calcineurin antagonist dose to avoid deleterious effects on the kidney grafts, which show a high incidence of acute tubular necrosis. The present study was designed to examine whether a therapy regime based on anti-CD25 monoclonal antibody induction plus mycophenolate mofetil will allow the use of low-dose tacrolimus in the immediate post-transplant period, without increasing the number of acute rejection episodes.

Methods: 177 consecutive NHBD renal transplant recipients were treated as follows: Group I (*N*=21), cyclosporine (8 mg/kg/day) plus azathioprine plus steroids; Group II (*N*=65), low-dose cyclosporine (5 mg/kg/day) plus mycophenolate mofetil plus steroids; Group III (*N*=17), low-dose tacrolimus (0.1 mg/kg/day) plus mycophenolate mofetil plus steroids; and Group IV (*N*=69), daclizumab plus low-dose tacrolimus (0.1 mg/kg/day) plus mycophenolate mofetil plus steroids. 21+65+17+69=172

Results: The incidence of DGF was 76.2% in group I, 72.3% in group II, 76.5% in group III, and 42% in group IV (p=0.000). The proportions of rejection-free patients were 76.2%, 46.2%, 35.3% and 71% respectively for the four groups (p<0.001). Vascular rejection (Banff grade II or III) rates were 19%, 30.8%, 52.9% and 18.8%, respectively (p=0.025). Graft survival rates were similar in each group (95.0%, 95.4%, 94.1% and 97.1% respectively at 2 years; p=0.97). Patient survival was worse in group I.

Conclusions: The combined use of daclizumab, low-dose tacrolimus, mycophenolate mofetil, and steroids seems to be effective at lowering the incidence of delayed graft function in NHBD kidney transplant recipients, with no negative repercussions on acute rejection.

Abstract# 718 Poster Board #-Session: P174-II

CARDIOVASCULAR RISK FACTOR PROFILE IN WOMEN RECIPIENTS OF RENAL TRANSPLANT: DIFFERENCES BETWEEN TACROLIMUS (TAC) AND CYCLOSPORIN A (CSA).

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Background: Renal transplantation is the preferred modality of therapy for women suffering from end-stage renal disease. The leading cause of death in women is cardiovascular disease (CVD). The role of calcineurin inhibitors in the cardiovascular risk profile in women recipients of renal transplant has not been well established. The goal of this study was to investigate the differences in cardiovascular risk factors associated with the use of TAC or CSA in a cohort of women recipients of a renal-only transplant. **Methods:** Between January 1994 and December 1998, 254 adult women received a renal transplant at our center. 163 (64.2%) patients received CSA and 91 (35.8%) received a TAC-based regime. Drug availability and patient preferences dictated the use of either agent. This was an intention-to-treat design. The baseline characteristics of the groups were not different in regard to age, body mass index, type of donor (deceased vs. living), duration of dialysis, hypertension, hypercholesterolemia, history of diabetes, smoking, or previous cardiovascular events. Prednisone dose was not different between the groups. **Results:** At 1 year post transplant, patient (TAC 99% vs. CSA 98%, p=0.9) and graft survival (TAC 98% vs. CSA 92%, p=0.2) were not statistically different. Other 1-year associations are shown in the table. By univariate and multivariable analyses, the cardiovascular event-free survival at 5 years (CSA 85.5% vs. TAC 82.1%, p = 0.18) was not statistically significant. **Conclusion:** Compared to CSA, TAC was associated with significantly reduced incidence of hyperlipidemia and diastolic hypertension. The impact of post-transplant diabetes on CVD must to be taken into account as well. Larger cohorts and longer follow up are needed to evaluate whether the differences in the cardiovascular risk profile between CSA and TAC will be translated into significant changes in the incidence of cardiovascular events in women following renal transplantation.

	TAC	CSA	p-value
BMI (kg/m²)	27.9 ± 0.6	27.5 ± 0.5	0.7
Systolic BP (mmHg)	134 ± 2.0	133 ± 1.3	0.8
Diastolic BP (mmHg)	79 ± 1.2	82 ± 0.8	0.022
Hypertension	56.3%	61.2%	0.5
Creatinine (mg/dl)	1.3 ± 0.04	1.3 ± 0.04	0.4
Total Cholesterol (mg/dl)	190 ± 3.4	220 ± 3.6	<0.001
Hypercholesterolemia	42%	68.1%	<0.001
Patients on lipid-lowering	26%	40%	0.047

Abstract# 719 Poster Board #-Session: P175-II

MMF DOSE MODIFICATION FOLLOWING GI COMPLICATIONS IN RENAL TRANSPLANTATION.

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We have shown that the combination of GI complications and MMF discontinuation during the first year following renal transplantation are associated with later graft failure. It was not clear in that analysis if MMF withdrawal occurred after GI complications and we could not examine MMF dose reductions. Here we examine the association with graft failure of MMF dose reduction or withdrawal following a GI complication.

METHODS: Patient records for all adult renal transplant recipients were drawn from the United States Renal Data System (USRDS) registry database between 1995 and 2000. Diagnoses of GI complications were drawn from ICD-9 codes and MMF prescriptions were drawn from payment records contained in Medicare billing data supplied by the USRDS. Patients were included in the analysis if a diagnosis of a GI complication was recorded following transplantation and prescription records indicated MMF at the time of GI diagnosis. The study interval began with the GI diagnosis and ended at graft failure with censoring at three-years post-transplant, last expected follow-up record, and last recorded immunosuppression payment. Associations were estimated using multivariate time varying methods.

RESULTS: Outcomes of 3,324 renal transplant recipients with a GI diagnosis were examined. Patients prescribed an MMF dose reduction were associated with an increased hazard of graft failure. The relative hazard of graft failure was 2.2 (P = 0.03) during intervals of dose reduction less than 50% and 2.5 (P = 0.003) during intervals of dose reduction greater than 50%, referenced against a relative hazard of 1.0 which would indicate no association between dose reduction and graft failure. The relative hazard of graft failure increased further to 3.9 (P < 0.0001) during intervals of MMF discontinuation.

CONCLUSION: MMF dose reduction and withdrawal following the diagnosis of a GI complication are associated with considerably heightened risk of graft failure in renal transplant recipients. Patients prescribed MMF dose modifications following GI complications should be managed with great care.

Abstract# 720 Poster Board #-Session: P176-II

LONG-TERM FUNCTION OF RENAL ALLOGRAFT WITH THE USE OF RAPAMUNE AND TACROLIMUS COMBINATION.

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Background: The combination of SRL and TRL has proven to be effective therapy in renal transplantation. However, most published series are limited by small sample size and short term follow-up. We report the largest single center series using this combination with a long term follow-up of at least one year. **Patients:** A retrospective analysis of all transplants performed between January 2000 and December 2002 that were maintained on such combination therapy was done. Two hundred and seventy patients (176 males and 94 females, mean age 46 years) received deceased (DD; 60%) or living donor (LD; 40%) allografts. Sixty patients (22%) were African-American, and 39 (14%) were recipients of a second or subsequent transplants. The average cold ischemia time for DD grafts was 28:36 hours, and of these grafts, 91 (56%) experienced delayed graft function. Induction therapy was used in 96% of DD recipients vs 54% of LD recipients. **Results:** Patient and graft survival data are shown in (table I). In the DD recipients, 15 grafts (5.5%) were lost due to primary non-function. The one year acute rejection rate was not significantly different in the DD vs LD groups (14.7% vs 12%). Average serum creatinine levels at one and two years were 2 and 1.9 mg/dl, and 1.6 and 1.9 mg/dl, in the DD and LD recipients, respectively. Various complications (with incidence of) occurred: lymphocele (6%), superficial wound infection (7%), wound dehiscence (6%), deep vein thrombosis (7%), ureteric obstruction/damage (5%), de novo transplant diabetes mellitus (17%), and verious post-transplant malignancies (mainly skin cancers, 8%). Other complications included fungal (n=9), and viral (n=13) infections and hemolytic uremic syndrome (HUS) developed in 3 patients. In 29 patients, SRL, and in 11 others, TRL was discontinued. **Conclusion:** The combination of TRL/SRL is equally effective in DD and LD renal transplant recipients. Excellent creatinine values were reached at 2 years, despite the high rate of DGF in the DD recipients.

	DD(n=163)	LD(n=107)
Acute rejection rate	14.7%	12%
1 year pt survival	89%	97%
2 year pt survival	84%	94%
3 year pt survival	83%	94%
1 year graft survival	87%	93%
2 year graft survival	82%	90%
3 year graft survival	82%	88%

Abstract# 721 Poster Board #-Session: P177-II

EFFECT OF STEROID AVOIDANCE ON EARLY GRAFT FUNCTION AFTER KIDNEY TRANSPLANTATION.

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Purpose: Steroid free immunosuppression regimens are currently used in clinical practice. The purpose of this study is to evaluate the effect of glucocorticoids on immediate renal allograft function after kidney transplantation. **Methods:** From April 2002 to February 2003, ten patients were enrolled in a steroid free immunosuppressive regimen pilot study (tacrolimus, mycophenolate mofetil, and prolonged course of daclizumab). The immediate renal allograft function (daily serum creatinine levels from post-operative day 1 to 6) were compared to 12 case controlled patients in the same time interval (treatment with tacrolimus, mycophenolate mofetil, and standard steroid taper which consisted of methylprednisolone 125mg in the OR, then 100mg, 80mg, 60mg, 40mg, 30mg, 20mg daily followed by a prednisone taper. Statistical analysis included Mann-Whitney U-Test was performed. **Results:** In the study period, there were 10 cadaveric and 12 living donor kidney transplants performed. In the cadaveric group, 6 were treated with steroids and 4 were not, and there were 6 patients in each treatment strata in the living donor group. The combined groups' serum creatinine (mg/dL) with and without steroid treatment can be seen in Table 1. The serum creatinine is generally lower in the steroid treatment group and reaches statistical significance by the fourth post-operative day and remains as such afterwards. There was only one patient with biopsy proven acute rejection on post-operative day 8 in the steroid-free living donor group. **Conclusion:** In this study, complete steroid avoidance was associated with a slower decline of the serum creatinine after kidney transplantation. Anti-inflammatory effects of steroids may help minimize the ischemia-reperfusion injury. Peri-operative steroid utilization may thus be advantageous over steroid avoidance in that regard.

	Serum creatinine after kidney transplantation		
Post-operative day	Steroid (n=12) median [range]	No steroids (n=10) median [range]	p value
1	7.9 [4.6-13.3]	9.3 [5.4-14.2]	0.46
2	6.8 [2.1-10.1]	7.9 [3.7-16.6]	0.32
3	3.9 [1.4-10.8]	5.2 [1.8-17.6]	0.36
4	2.5 [1.1-5.8]	5.4 [2.6-17.6]	0.04
5	2.2 [1.0-5.7]	6.1 [1.7-17.9]	0.007
6	2.1 [1.0-5.0]	6.6 [2.4-13.6]	0.004

LIVER TRANSPLANTATION: DISEASE RECURRENCE

Abstract# 722 **Poster Board #-Session: P178-II**

MAINTENANCE OF EXCELLENT PATIENT SURVIVAL AFTER LIVER TRANSPLANTATION FOR CHRONIC HEPATITIS C IN A SINGLE CENTER DURING THREE ERAS OF IMMUNOSUPPRESSION CHARACTERIZED BY WIDE VARIATION IN USE OF OKT3. Lisa M. Forman,[1] Igal Kam,[2] Gregory T. Everson.[1] [1]*Medicine, University of Colorado School of Medicine, Denver, CO;* [2]*Surgery, University of Colorado School of Medicine, Denver, CO.*

Background: Recurrent hepatitis C (HCV) after liver transplantation may accelerate hepatic fibrosis and lead to graft loss and patient death. This accelerated natural history is likely related to immunosuppression, particularly OKT3 and Solumedrol pulse therapy. Recent studies have suggested faster disease progression and inferior survival with recent years, partly because of stronger immunosuppression. Since 1988, we have had three distinct eras of immunosuppression with marked differences in utilization of OKT3.

Specific Aim: 1. Compare patient survival for patients transplanted for HCV during three distinct eras in immunosuppression in our center. 2. Determine whether prevalence of OKT3 usage during these eras adversely impacts survival.

Methods: Using data from our center's Liver Transplant Database, we performed a retrospective cohort study of 261 HCV Ab-positive patients out of a total of 799 transplants between the years 1988-2003. Three eras of immunosuppression were identified: I, cyclosporine (CSA)-based with late steroid withdrawal (SW) (after 1 yr) from 11/1/88 to 8/8/95; II, very early SW (14d) with sequential assignment to either CSA or tacrolimus (TAC) with or without mycophenolate mofetil (MMF) from 8/9/95 until 1/28/00; and III, very early SW (3d), sirolimus (RAPA)-based with sequential assignment to either CSA or TAC from 1/29/00 to 4/1/03. Patient and graft survival were estimated using the Kaplan-Meier method with comparison between groups using the log-rank test.

Results: Demographics of patients with HCV, including age, donor age, BMI, race, and sex were similar between eras. Patient survival was similar between eras:

Era	Pts with HCV (% of total tx'd)	% Pts Rx with OKT3 (% induction)	1 yr Pt Survival (95% CI)	3 yr Pt Survival (95% CI)
I	54 (22)	70.3 (3.7)	89.1 (79.0-99.3)	82.6 (69.4-95.8)
II	100 (38.3)	39.0 (13)	88.9 (81.0-95.9)	83.8 (75.3-92.4)
III	107 (47.4)	3.7 (0)	92.7 (86.9-98.4)	85.0 (74.4-95.6)

Within each era, the percentage and pattern of graft loss was similar between HCV-positive and HCV-negative recipients. Causes of death were similar in the three eras.

Conclusion: In three separate eras of different immunosuppression regimens, our use of OKT3 varied from 70.3% to 3.7% without any adverse effect on patient survival. This result implies that specific immunosuppressive regimens or OKT3 per se may not be a dominant factor affecting outcome in the HCV-positive transplant recipient.

Abstract# 724 **Poster Board #-Session: P180-II**

LIVING DONOR LIVER TRANSPLANTATION (LDLT) IS SAFE AND EFFECTIVE FOR HEPATITIS C RECIPIENTS. A. Fahmy,[1] C. A. O'Mahony,[1] H. Kaul,[1] G. R. Morgan,[1] D. John,[1] T. Diflo,[1] L. Teperman.[1] [1]*Transplant Surgery, NYU Medical Center, New York, NY.*

Hepatitis C associated cirrhosis is the most common indication for liver transplantation. Recurrence following transplantation is universal. Recently published data suggests that HCV recurs earlier and is more severe in LDLT recipients than in cadaveric recipients (CAD). AIM: To compare HCV recurrence rates in adult right lobe LDLT vs. CAD recipients. METHODS: Retrospective analysis of HCV infected patients who underwent liver transplantation from 9/99 to 8/03. 71 patients underwent LDLT in this period, 39 with HCV related cirrhosis. Patients enrolled in HCV-related clinical trials, and those who died within 30 days of surgery were excluded. Data from 33 LDLT and 52 CAD recipients transplanted during the same period were analyzed. Groups were matched for age and sex. Immunosuppression consisted of low dose steroids and tacrolimus. The diagnosis of recurrent HCV was based on histologic findings. Biopsies were performed for clinical indicaton (increased AST/ALT/GGT) and not on a protocol basis. RESULTS: Average MELD scores were significantly lower in LDLT recipients. Mean follow-up was 20.6 months in LDLT and 19.3 months in the CAD group. One or more rejection episodes were observed in 10 (30.3%) patients in the LDLT group and 10 (19.2%) in the CAD group (p=0.20). Rejections were treated with steroids as per our protocol. Histologic recurrence of HCV occurred in 19 (57.6%) patients in the LDLT group and 32 (61.5%) of the CAD recipients (p=0.72). Average time to recurrence was 163 days in LDLT compared to 186 days in the CAD group (p=0.70). In the LDLT group, biopsies in 8 (24.2%) patients showed recurrence with progression to portal fibrosis while 10 (19.2%) patients in the CAD group demonstrated fibrosis (p=0.58). Two LDLT recipients developed a late fibrosing cholestatic hepatitis, both at 14 months. One patient in the CAD group developed an early fibrosing cholestatic hepatitis. Recurrent hepatitis C led to graft loss in 1 (3%) LDLT recipient and 4 (7.7%) CAD recipients (p=0.37). CONCLUSION: In our study, there was no significant difference between the number of HCV recurrences or the time to recurrence between LDLT and CAD recipients. Although it appears that portal fibrosis occurs more in the LDLT group, the difference was not statistically significant. Graft loss due to hepatitis was more common in cadaveric recipients. While this study demonstrates that HCV recurrence rates are similar in LDLT and CAD recipients a large prospective trial needs to be undertaken to study this problem.

Abstract# 725 **Poster Board #-Session: P181-II**

IMPACT OF HLA COMPATIBILITIES ON OUTCOME IN HCV POSITIVE PATIENTS AFTER ORTHOTOPIC LIVER TRANSPLANTATION. Ulf P. Neumann,[1] Marcus Bahra,[1] Jan M. Langrehr,[1] Peter Neuhaus.[1] [1]*Dept. of Surgery, Charité, Virchow Clinic, Berlin, Germany.*

Orthotopic liver transplantation (OLT) for end-stage hepatitis-C-virus (HCV) infection is commonly complicated by recurrence of HCV and a significant number of patients will develop severe graft hepatitis after OLT. However, the relevance of HLA-matching in the recurrence of HCV is still under discussion. In this study we investigated the effect of HLA-compatibilities on outcome and fibrosis progression of HCV positive patients after OLT.

In a retrospective analysis 165 liver transplants in HCV positive patients with complete donor/recipient HLA typing were reviewed for recurrence of HCV and outcome after OLT. Follow up ranged from 1 to 158 months (median=63,3 months). Immunosuppression consisted of either CsA based quadruple induction therapy including ATG or an IL2- receptor antagonist or with Tacrolimus. Protocol liver biopsies were performed after 1-, 3-, 5-, 7-, and 10 years and staged according to the METAVIR scoring system.

The overall 1-, 5-, and 10 years graft survival figures were 81,8%, 69,11 and 62%, respectively. There was no correlation between number of HLA-compatibilies and graft survival in the study population. The number of rejection episodes was significantly increased in patients with less HLA compatibilities ($p<0.05$). In contrast to this the fibrosis progression was signicantly faster in patients with 1 or more HLA compatibilities when compared to patients with no HLA compatibility.

In conclusion, HLA-matching does not influence graft survival in patients after OLT for end-stage HCV infection. However, despite less rejection episodes the fibrosis progression was increased in patients with more HLA compatibilities within the first year after OLT.

Abstract# 726 **Poster Board #-Session: P182-II**

AN ANALYSIS OF HEPATITIS C VIRUS REINFECTION IN LIVER TRANSPLANTS PERFORMED USING EXTENDED CRITERIA DONOR ALLOGRAFTS. M. Gupta, M. J. Hanaway, T. D. Merchen, J. F. Buell, M. Alonzo, M. J. Thomas, E. S. Woodle, S. M. Rudich. *Division of Transplant Surgery, University of Cincinnati, Cincinnati, OH.*

Cirrhosis due to hepatitis C virus (HCV) is a leading indication for orthotopic liver transplantation (OLT). Following OLT for HCV, as many as 80 to 90% of all allografts become re-infected. As many transplant programs are utilizing extended criteria donor (ECD) livers, the impact of this practice on recurrent HCV infection remains unknown. **PURPOSE**: To analyze rates of HCV re-infection in OLT recipients receiving ECD livers compared to a control group of standard criteria donor (SCD) liver allografts. **METHODS**: All OLTs performed between Jan 2002-Oct 2003 were categorized as having received either an ECD or SCD liver at our center. ECD grafts were defined as having any one or more of the following: donor age > 60 yrs, biopsy exhibiting >40% macrovesicular and/or >75% microvesicular steatosis, any donor down time (±CPR), requirement of 2 or more pressors within 12 hours of procurement, or liver function studies > 5 x upper limit of normal. All recipients underwent liver biopsy at 90 days or earlier, if there was an appreciable rise in baseline liver function tests (LFTs). **RESULTS**: The two groups (ECD and SCD liver recipients) were similar in regards to: MELD score (match and lab), gender, age, and graft ischemic (total and warm) times. Ninety OLTs were performed during the study period: 42 ECD livers and 48 SCD livers were transplanted. HCV-related cirrhosis accounted for 24/42 (57.1%) of the OLTs in the ECD group and 20/48 (41.7%) of the OLTs in the SCD group (p=0.14). Biopsy-proven recurrent HCV infection was noted in 21 (23.3%) of all allografts. The ECD group was found to have a 33.3% recurrence rate (8/24) while in the SCD group a recurrence rate of 65% (13/48) was noted (p=0.04). No significant differences were observed between the groups in LTFs of patients requiring biopsies. **CONCLUSION**: The aggressive use of extended criteria donor hepatic allografts was noted not to lead to an increased rate of HCV re-infection in the recipient allograft. Indeed, there was a statistically significant increase in HCV re-infection in those recipients receiving standard criteria donor livers. This suggests that perhaps a "stressed donor allograft" is protected from viral re-infection.

Abstract# 727 Poster Board #-Session: P183-II

RELAVANCE OF RIBAVIRIN CONCENTRATION IN PLASMA/ BLOOD IN POST LIVER TRANSPLANT PATIENTS INFECTED WITH HEPATITIS C VIRUS(HCV). Ashok Jain,[1] Raman Venkataramanan,[3] Bijan Eghtesad,[2] Shakil Obaid,[2] Randeep Kashyap,[1] Amadeo Marcos,[2] John Fung.[2] *[1]Surgery, University of Rochester, Rochester, NY; [2]Surgery, University of Pittsburgh, Pittsburgh, PA; [3]Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA.*

Introduction: Hepatitis C viral infection (HCV) is the commonest indication for liver transplantation (LTx). Recurrence of HCV is uniform after transplantation. Combination of pegylated interferon (peg-INF) with Ribavirin, which is not without the side effects, has been used as anti HCV treatment. It has been recommended that patients with renal dysfunction should receive lower doses of Ribavirin in order to reduce the incidence of hemolysis.

Aim of the present study is to examine the role of measurement of plasma and whole blood concentration of Ribavirin to determine this relationship to hemolysis, renal dysfunction, and treatment response.

Material: The left over 34-blood sample drawn for tacrolimus level from 23 post LTx patients (who were on Ribavirin) were used to estimate Ribavirin concentration in plasma (all samples) and blood (17 samples) by HPLC. Patient's clinical history and concurrent medications were evaluated.

Results: There was a wide variation in the Ribavirin concentration ranging from 1.8 µ/ l to 122.1 µ/l with mean plasma concentration of 48.4± 10.2 µ/l. There was a close correlation between the plasma and whole blood Ribavirin level (mean plasma level 42.7± 21.8 µ/l; mean blood level 37.5 ± 17.3 µ/l.) No correlation was found in the response rate (biochemical, histogical or HCV viral load), hemolysis, renal function or length of Ribavirin treatment on subsequent estimations. However the concentrations were higher when subjects were on *antiretroviral* agents (5 samples mean 87.3± 39.4 µ/l) and lower when patients were on *proton pump inhibitors* (11 samples mean 29.8±20.3 µ/l).

Conclusion: Despite of the fact that Ribavirin is highly bound to red blood cells, the concentration in whole blood and plasma were similar. Higher concentrations were observed with concurrent use of anti retroviral agents and lower with proton pump inhibiter. The toxicity to the concentration could not be established in this pilot study. It appears that Ribavirin, as a parent compound may not be responsible for therapeutic effect or hemolysis. It is possible that its phosphorelated metabolite of Ribavirin may be determining factor to response rate and toxicity. Further more studies are needed to elucidate the role Ribavirin with peg-INF in recurrent HCV post liver transplantation.

Abstract# 728 Poster Board #-Session: P184-II

HEPATITIS C RECURRENCE (HCVR) IN LIVER TRANSPLANT RECIPIENTS (OLT) UNDER MYCOPHENOLATE MOFETIL (MMF) IMMUNOSUPPRESSION (IS): A LONG-TERM FOLLOW UP. Carlos G. Fasola,[1] George J. Netto,[1] Edmund Q. Sanchez,[1] Shrinath Chinnakotla,[1] Marlon F. Levy,[1] Robert M. Goldstein,[1] Goran B. Klintmalm.[1] *[1]Transplantation, Baylor University Medical Center, Dallas, TX.*

Purpose: To assess the incidence of HCVR 5 years (y.) post OLT in patients (pt) treated (Rx) with MMF for different periods.

Methods: We have previously shown that HCV-OLT have a low incidence of HCVR at 1- and 2-year of follow up (f/up) when Rx with MMF in a dose- and a time-dependent fashion. However, it is not known for how long should HCV-OLT remain on MMF, since its availability in the market is relatively recent. MMF was used as rescue agent for steroid-resistant acute rejection (SR-ACR) or as a renal-sparing drug in cases of CSA nephrotoxicity. HCV-OLT 1993-1997 were divided into 3 groups by the type of IS received: No-MMF (n=61): cyclosporine (CSA) + steroids (Pred); MMF < 3 y. (n=10): CSA + MMF + Pred; MMF ≥ 5 y.(n=8): CSA + MMF + Pred. CSA doses were adjusted to keep Rx levels. Pred. was taper down to 5-10 mg after year 1. MMF pt kept an average of 1 g/day. No-MMF pt were controls (no SR-ACR) from same era. HCVR was defined by graft fibrosis (stage:0-4) as per Batts and Ludwig; fibrosis progression was defined by the increase in at least 1 stage over 5-year f/up. Protocol f/up included: liver histology and liver tests at years 1, 2 and 5. Multiple parameters were studied: demographics, ICU / Hospital stay, ACR, bacterial and CMV infections, etc. Chi² - test used for table analyses. A p value < 0.05 = significant. Results: No statistical differences were found for other parameters studied. The table shows significant histologic findings:

HEPATITIS C RECURRENCE AT FIVE YEARS

	No - MMF	MMF < 3 Years	MMF≥5 Years
FIBROSIS STAGE[1]	n (%)	n (%)	n (%)
None (Stage 0)	20 (32%)	0 (0%)	3 (37%)
Moderate (Stages 1-2)	23 (26%)	4 (40%)	5 (63%)
Severe (Stages 3-4)	20 (32%)	6 (60%)	0 (0%)
TOTAL	63 (100%)	10 (100%)	8 (100%)

	No-MMF	MMF < 3 Years	MMF ≥5 Years
FIBROSIS PROGRESSION[2]	n (%)	n (%)	n (%)
Absent	39 (64%)	2 (20%)	6 (75%)
Present	22 (36%)	8 (80%)	2 (25%)
TOTAL	61 (100%)	10 (100%)	8 (100%)

p=0.049[1]; p=0.020[2]

Conclusions: Patients kept on MMF at all times presented lesser HCVR and lesser HCVR progression at 5-y and their graft function (not shown) was similar to controls who did not have SR-ACR or nephrotoxicity. Patient on MMF for < 3 years had higher incidences of HCVR and HCVR progression. We conclude from this limited initial experience that MMF is an important component of HCV-OLT IS and pt should remain on it for long terms.

Abstract# 729 Poster Board #-Session: P185-II

HCV ASSOCIATED CHOLESTASIS RELATED TO BILIARY COMPLICATIONS FOLLOWING DECEASED AND LIVING DONOR LIVER TRANSPLANTATION: POOR OUTCOMES DESPITE THERAPY. Paul J. Gaglio,[1] Elizabeth C. Verna,[1] Alex Novogrudsky,[1] Jay Lefkowitch,[1] Milan Kinkhabwala,[1] Jean C. Emond,[1] Robert S. Brown.[1] *[1]Center for Liver Disease and Transplantation, Columbia University P & S, New York, NY.*

Introduction:Cholestatic Hepatitis C (CHC), a severe variant of recurrent HCV following transplantation is defined by a serum total bilirubin greater than 10mg/dl, liver biopsy consistent with portal expansion and ductular proliferation, and the absence of extra-hepatic biliary tract obstruction. However, HCV associated cholestasis with similar histologic and laboratory findings may occur in the presence of extra-hepatic biliary obstruction. The incidence and natural history of this phenomenon remains incompletely described, particularly when comparing deceased (DD) and living donor (LD) liver transplant recipients.

Methods: A retrospective analysis of all 102 (71 DD and 31 LD) HCV infected patients transplanted at our center between 7/99 and 6/03 was performed. The incidence of extrahepatic biliary obstruction (EBO) defined as any anastamotic or ampullary stricture requiring ERCP or PTC placed stent or surgical revision was determined, and the evolution of HCV in these patients was assessed. HCV associated cholestasis was defined as a total bilirubin greater than 10mg/dl, with similar histologic findings as CHC.

Results: The overall incidence of EBO was 23%, 19% in DD and 32% in LD (p = NS). When comparing the incidence of HCV with cholestasis in patients with EBO, 42% of patients developed this complication while 58% did not (p = ns). There was no difference in the rate of HCV associated cholestasis associated with EBO when comparing LD to DD. In patients who developed HCV with cholestasis in the setting of EBO, outcomes were poor; graft failure and death occurred in 60%, 30% had severe cholestasis despite therapy, and 10% recovered following biliary stenting and therapy with Interferon and Ribavirin

Conclusions: 1) HCV recurrence with cholestasis may occur in patients with extrahepatic biliary obstruction post transplant. 2) This syndrome has identical laboratory findings and similar histopathology to cholestatic hepatitis C, a syndrome which occurs in the absence of biliary obstruction. 3) HCV associated cholestasis in the setting of extrahepatic biliary obstruction portends a poor prognosis, despite attempts to relieve biliary obstruction and treat HCV.

	DD (71 pts)	LD (31 pts)	p value
Extrahepatic Biliary Obstruction	19%	32%	NS
With HCV/cholestasis	36%	50%	NS
w/o HCV/cholestasis	64%	50%	NS

Abstract# 730 Poster Board #-Session: P186-II

EFFICACY AND SAFETY OF INDUCTION THERAPY WITH ANTITHYMOCYTE GLOBULINS (ATG) IN HEPATITIS C VIRUS POSITIVE LIVER TRANSPLANT PATIENTS: COMPARISON WITH ANTI-CD25 INDUCTION THERAPY. Nassim Kamar,[1] Karine Sandres-Saune,[1] Bertrand Suc,[1] David Ribes,[1] Jean Sebastien Borde,[1] Olivier Cointault,[1] Karl Barange,[1] Dominique Durand,[1] Janik Selves,[1] Lionel Rostaing.[1] *[1]Mutiorgan Transplant Unit, CHU Rangueil, Toulouse, France.*

Hepatitis C virus (HCV) reinfection is the rule following orthotopic liver transplantation (OLT) for HCV-related cirrhosis. In this retrospective study we compared the efficacy, the safety and the overt HCV recurrence rate following induction therapy using either ATG or anti-CD25 monoclonal antibodies (basiliximab or dacluzimab) in combination with steroids and tracolimus. We included 31 consecutive OLT patients ; those who died before day 30 were excluded from that study. They were transplanted between 01-99 and 12-02. The patients were follow-up until day 180. Overt HCV recurrence was defined as an increase in liver enzymes with histological evidence of HCV-related lesions, in the absence of acute rejection. There were 20 men, 11 women of a mean age of 57 ± 6 years. All were HCV RNA positive at the time of OLT ; genotype 1(1b) was found in 74 % (64.5 %) of patients. The first 16 patients received ATG induction (Group I) (1 mg/kg/d for 3 consecutive days, then adapted to TCD2(+) lymphocyte count [to be less than 50/mm3] and to tacrolimus trough levels). The following 15 patients (group II) received induction therapy with either basiliximab (20 mg twice at day 1 and 4) or dacluzimab (2 mg/kg at day 1 and 1 mg/kg at day 8). Tacrolimus was started on average at day 1. Steroids were started per op. The rate of acute rejection was similar both in group I (37.5 %) and in group II (20%) (p=ns). The rate of bacterial (50% in group I vs. 40% in group II, p=ns), viral, i.e. cytomegalovirus (25% in group I vs. 33% in group II, p=ns), and fungal (18.75 in group I vs. 26.6% in group II, p=ns) infections was similar in both groups. Renal function as well as hematological parameters were

similar in both groups during the study period. Liver enzyme levels, i.e. aspartate and alamine aminotransferase, gamma glutamyl transpeptidase and bilirubin were similar in both groups from day 8 until day 180. Overt HCV recurrence occured in 56.25 % in group I and in 80 % in group II (p = ns). HCV RNA viral load was not statistically different during follow up between the 2 groups ; however there was an increase from baseline values.

We conclude that induction therapy with ATG in OLT HCV(+)/RNA (+) recipients is efficient, safe as compared to induction with anti-CD25 antibodies, particularly with respect to HCV recurrence and overall infections.

Abstract# 731 Poster Board #-Session: P187-II

STEROID-FREE IMMUNOSUPRESSION RESULTS IN DECREASED HCV RECURRENCE IN LIVER TRANSPLANT RECIPIENTS. Nahel Elias,[1] Martin Hertl,[1] Tatsuo Kawai,[1] Dicken S. C. Ko,[1] Michael Thiim,[2] Raymond T. Chung,[2] A. Benedict Cosimi.[1] *[1]Transplantation Unit, Massachusetts General Hospital, Boston, MA; [2]Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA.*

HCV recurrence after liver transplantation is a serious complication. We investigated whether steroid-free immunosupression with Thymoglobulin® induction reduces HCV recurrence following liver transplantation (OLT). **METHODS:** We enrolled twelve patients between 11/02 – 10/03. 36 prior OLT recipients with HCV cirrhosis served as control. The immunosuppressive protocol consisted of: Thymoglobulin® 1.5 mg/kg/d on days 0-3, Azathioprine (AZT) 100 mg/d for 3 months, Cyclosporine (CyA) starting on day 4 (to maintain levels of 200-300ng/ml). **RESULTS:** After a mean follow-up of 8 months, nine patients are currently on single-drug-regimen (CyA or Tacrolimus). Six patients had abnormal liver function tests (LFT) due to: alcoholic hepatitis; HCV recurrence; biliary complication (2 pts); rejection (now on AZT and Tacrolimus); and cholestasis of unknown origin (HCV or rejection, now on Tacrolimus only). All but the first patient had biopsy-proven diagnoses. The first three patients have normalized their LFT; the last three are improving. Six patients have undetectable HCV-RNA by PCR after completing 3-6 months of anti-HCV therapy (pegylated interferon and Ribavirin) and five have moderate to high HCV-RNA and are not tolerating anti-HCV therapy due to severe side effects. One patient is in the early post-operative period and has not started anti-HCV therapy yet. In the steroid-free group the incidence of high viral load (HCV RNA > 100,000 IU/mL) was lower than the control group [42% vs. 85%] (Fig. A), and the incidence of rejection- or HCV-related liver dysfunction episodes [25% vs. 50%] was also lower (Fig. B).

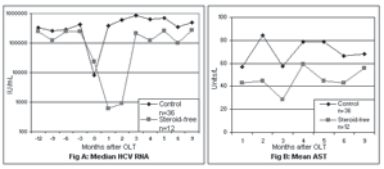


Fig A: Median HCV RNA Fig B: Mean AST

CONCLUSIONS: Steroid-free immunosupression with Thymoglobulin® induction in OLT for HCV cirrhosis leads to low incidence of rejection, lower viral replication and decreased HCV recurrence rate in the allografts.

Abstract# 732 Poster Board #-Session: P188-II

ACUTE REJECTION OR RECURRENT HEPATITIS C: CAN LIVER BIOPSY MAKE THE DIAGNOSIS? Ryan McTaggart,[1] Linda Ferrell,[2] Charles Lassman,[3] Alan Bostrom,[4] Peter Bacchetti,[4] Norah Terrault,[1] John Roberts,[1] Sandy Feng.[1] *[1]Department of Surgery, Division of Transplantation, University of California San Francisco, San Francisco, CA; [2]Department of Pathology, University of California San Francisco, San Francisco, CA; [3]Department of Pathology, Dumont-UCLA Transplant Center, Los Angeles, CA; [4]Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA.*

The diagnosis of acute rejection (AR) versus recurrent hepatitis C (R-HCV) after liver transplantation (LTx) is important since treatment of either may result in reciprocal consequences. We aimed to assess the reliability of liver biopsy (bx) to discriminate AR from R-HCV. **Methods:** Two LTx pathologists from high-volume centers reviewed 38 blinded bxs obtained from HCV+ recipients between 4 wks and 6mos after LTx: 20 bxs were from 1993-94 (Era1) and 18 were from 2000-01 (Era 2). Historical diagnoses were mild or moderate AR, R-HCV, or both. Each pathologist scored their present-day readings for AR and R-HCV: 1=Unlikely; 2=Possible; 3=Probable; 4=Likely. An experienced clinician scored both present-day and historical readings. Using the clinician's scores, interrater agreement and agreement with historical diagnoses were measured using weighted kappa (wK) statistics. Frequencies of exact (ExAg) and relative (RelAg) agreement (difference=0 or 1) were determined. **Results:** There were 33 bxs for cause and 5 protocol bxs. The clinician was an excellent interpreter of the present-day readings of both pathologists (wK P1=0.80; wK P2=0.72)(Table 1a). Interrater agreement is modest for AR (wK = 0.18) and good for R-HCV (wK=0.33)(Table 1b). Comparison of present-day and Era1 readings showed poor agreement for both AR (wK=0.02) and R-HCV (wK=-0.05). Comparison of present-day and Era2 readings showed poor agreement for AR (wK=-0.03) and modest agreement for R-HCV (wK=0.18)(Table 1c). **Conclusions:** Today, 2 LTx pathologists reading early bxs after LTx for HCV exhibit fair agreement on the diagnoses of AR and R-HCV. However, their present-day readings correlate poorly with historical readings suggesting that interpretation of histologic findings diagnostic for AR and R-HCV have evolved over time. Liver bx is not a reliable mechanism to discriminate AR from R-HCV.

TABLE 1a: CLINICIAN VERSUS PATHOLOGISTS 1 AND 2

Diagnosis	Pair	wKappa	RelAg (%)	ExAg (%)
Overall	C vs. P1	0.800 (0.71-0.88)	99	75
	C vs. P2	0.721 (0.62-0.81)	97	66

TABLE 1b: PATHOLOGIST 1 VERSUS PATHOLOGIST 2

Diagnosis	Pair	wKappa	RelAg (%)	ExAg (%)
AR	P1 vs. P2	0.182 (-0.01-0.37)	68	34
R-HCV	P1 vs. P2	0.331 (0.12-0.54)	76	58

TABLE 1c: PRESENT-DAY READINGS VERSUS HISTORICAL READINGS

Diagnosis	Pair	wKappa	RelAg (%)	ExAg (%)
AR	P1P2 vs. Era 1	0.021 (-0.14-0.19)	48	28
	P1P2 vs. Era 2	-0.037 (-0.27-0.19)	55	25
R-HCV	P1P2 vs. Era 1	-0.054 (-0.26-0.15)	58	20
	P1P2 vs. Era 2	0.184 (-0.05-0.42)	75	31

Abstract# 733 Poster Board #-Session: P189-II

HISTOLOGIC EVALUATION OF HEPATITIS C RECURRENCE AND ACUTE CELLULAR REJECTION FOLLOWING OLTX: A PRELIMINARY REPORT ON THE HEPATITIS C THREE TRIAL. George J. Netto, the Hepatitis C Three Trial Group. *Pathology, Baylor University Medical Ctr, Dallas, Tx.*

Purpose: The primary objectives of the Hepatitis C three trial are to compare the rates of hepatitis C (HCV) recurrence, acute cellular rejection (ACR), and treatment failure (death, graft loss or more than one dose of corticosteroid for presumptive rejection) among three immunosuppressive treatment regimens.

Design: The trial is an open-label, prospective, randomized multicenter study involving adult patients receiving OLTX for end stage HCV liver disease randomized to three Rx Arms. Arm 1: tacrolimus (TAC) and steroids (Pred); arm 2: TAC, mycophenolate mofetil (MMF) and Pred and arm 3: TAC, MMF and daclizumab (steroid free arm). A total of 312 pts from 17 institutions will be randomized at a ratio of 1:1: 2. Per protocol, liver biopsies are obtained at days 1, 90, 365 and 730. All biopsies are reviewed by a central pathologist. HCV recurrence is evaluated according to Batts and Ludwig schema (*Batts et al. Am J Surg Pathol 1995: p1409*). ACR is evaluated using the 1997 Banff schema (*Demetris et al. Hepatology 1997: p658*). Primary composite end points are defined as a recurrent HCV of stage ≥ 2 during the first year or grade ≥ 3 at any point post transplantation and or an ACR of Banff global grade ≥ 2 with RAI score ≥ 4. The current abstract summarizes the histologic findings of 150 biopsies obtained from 74 pts with a F/U range of (6 –380 days) post OLTX. All hepatitis C recurrences and rejections were biopsy diagnosed. This submission is based on the data safety monitoring board from November in order not to violate the trial.

Results:

HCV recurrence: Histologic HCV recurrence as defined above was established in 20/74 pts (27%). 13 of the HCV recurrences were diagnosed on or before day 90 post OLTX biopsy (18 % three months recurrence rate). The shortest and longest durations to HCV recurrence were 37 and 260 days post OLTX respectively.

ACR: Histologic ACR as defined above was established in 6/74 pts (8%). Only one pt had severe ACR (Banff global grade 3). The RAI scores range was 4-7.The earliest and latest ACR occurred on days 3 and 48 respectively.

Conclusions: At three months post OLTX, 18 % HCV recurrence rate and 8% ACR incidence is histologically demonstrated in 74 pts with complete data and histology reported to the study center in the Hepatitis C three trial. Accrual is expected to be complete by March 2004. An updated report on histologic findings in all pts completing three months F/U will be presented.

Abstract# 734 Poster Board #-Session: P190-II

HYPOGAMMAGLOBULINEMIA IN LIVER TRANSPLANT PATIENTS: INCIDENCE, TIMING, RISK FACTORS, AND OUTCOMES. Shira Doron,[1] Robin Ruthazer,[2] Arthur Rabson,[3] Barbara G. Werner,[4] David R. Snydman.[1] *[1]Division of Infectious Diseases, Tufts-New England Medical Center, Boston, MA; [2]Division of Clinical Care Research, Tufts-New England Medical Center, Boston, MA; [3]Department of Pathology, Tufts-New England Medical Center, Boston, MA; [4]Massachusetts State Laboratory Institute, Jamaica Plain, MA.*

Background Recent studies in heart and lung transplantation have reported rates of hypogammaglobulinemia (HG) as frequent as 70% and an association with development of infection. In an effort to describe the incidence, timing, risk factors, and outcomes of HG in the liver transplant patient population, we analyzed IGG levels from serum collected during a randomized trial. **Methods** The original study, designed to determine the impact of CMV IG vs. placebo on mortality and infectious outcomes, enrolled 146 patients who underwent liver transplantation between December 1987 and June 1990. Frozen serum samples from post-transplant weeks 0,4,8,12,16,24 and 32 were thawed and analyzed. Patients with HG (defined as an IGG level below 560 mg/dl) were compared to those with normal IGG levels for clinical factors associated with HG such as underlying diagnosis, number of rejection episodes and amount of immunosuppressive medication received. Comparison was also made for principal study outcomes which included CMV infection, invasive fungal disease, bacteremia and one- and five-year mortality. Patients who received CMV IG were compared to those who received placebo for development of HG. **Results** Serum from 112 patients was available for study. A total of 29 (26%) patients met our definition of HG at least once during 8 months of post-transplant follow-up. Of the patients with HG, 14 had it at baseline sampling. Of the 15 patients who developed HG later in the post-transplant course, the median time to HG was 30 days post-transplantation. There were no differences between patients with and without HG in the prevalence of the various clinical factors examined. There was no difference between patients who received CMV IG and patients who received placebo in the rate of development of HG. Patients with HG did not experience subsequent higher rates of infectious outcomes when compared to patients without HG. However, patients with HG did experience higher one-year (31% versus 14%) and five–year (52% versus 28%) mortality compared to those without HG ($p<0.05$). **Conclusion** HG occurs in a significant proportion of liver transplant recipients. It is associated with increased mortality up to five years post-transplant. Our results do not support the association of HG with infectious outcomes reported by other investigators. Risk factors associated with HG in liver transplant patients need to be further elucidated. Supported by a grant from MedImmune and NIH grants M01RR00054 and R10 DK31389.

Abstract# 735 Poster Board #-Session: P191-II

VALGANCICLOVIR FOR CMV PROPHYLAXIS IN LIVER TRANSPLANT RECIPIENTS: AN INITIAL EXPERIENCE. Suzanne C. Berkman,[1] Elizabeth Ashcraft,[2] G. Mark Baillie,[2] David Taber,[2] Angello Lin,[1] Jeffrey Rogers,[1] P. Baliga,[1] Kenneth D. Chavin.[1] *[1]Department of Surgery, Div. of Transplant Surgery, Medical University of South Carolina, Charleston, SC; [2]Department of Pharmacy Services, Medical University of South Carolina, Charleston, SC.*

Valganciclovir (VGC) has not been approved for CMV prophylaxis in liver transplant recipients. The PV16000 compared oral ganciclovir to valganciclovir for primary prophylaxis for CMV and showed liver transplant patients who took valganciclovir had a statistically significant increased incidence of tissue invasive CMV disease in comparison to oral ganciclovir. We sought to determine the safety and efficacy of VGC for prophylaxis in our liver transplant patients. METHODS: We performed a chart review of all adult liver transplant recipients from October 2001 to June 2003. Patients with D+/R-, antilymphocyte antibody treated acute rejection, plasmapharesis, or clinical justification were classified as primary prophylaxis. Secondary prophylaxis patients were treated with VGC after a documented CMV infection. CMV infection was defined as CMV pp65+ with symptoms of infection or a biopsy positive for CMV. RESULTS: 93 patients were assesed, 24 were identified as receieving primary (n=18) or secondary (n=6) prophylaxis. Primary prophylaxis included patients with D+/R- (n=11), anti-lymphocyte antibody treated acute rejection, (n=3) plasmapheresis (n=2), or clinical judgement (2).

Table 1: VGC Results

	Mean VGC Duration (days)	Mean Follow-Up post-VGC (days)	CMV Infection	Tissue Invasive CMV
Primary	119±84 (range: 30-395)	410±176 (range: 123-637)	0 (0%)	1 (5.5%)
Secondary	158±102 (range: 5-306)	261±66 (range: 203-335)	0 (0%)	0 (0%)

Three patients had a temporary VGC discontinuation (6±1.5 days) for thrombocytopenia or leukopenia. An allergic reaction, D-/R- serostatus, and physician discretion resulted in the three permanent discontinuations. The only episode of tissue invasive disease proved to be a ganciclovir-resistant strain probably due to subtherapeutic dosing. CONCLUSION: VGC appears safe and effective for primary and secondary CMV prophylaxis in liver transplant patients with appropriate dosing.

Abstract# 736 Poster Board #-Session: P192-II

PROPHYLAXIS OF CMV INFECTION IN ADULT LIVER TRANSPLANT PATIENTS USING VALGANCICLOVIR (VALCYTE). Michael R. Marvin,[1] Maureen B. Burke-Davis,[1] Marcelo E. Facciuto,[1] Leona Kim-Schluger,[2] David C. Wolf,[2] Patricia A. Sheiner.[1] *[1]Surgery, New York Medical College - Westchester Medical Center, Valhalla, NY; [2]Medicine, New York Medical College - Westchester Medical Center, Valhalla, NY.*

Background: Cytomegalovirus infection is a common infection after solid organ transplantation occurring in from 25-85% of patients depending on prophylactic strategies and immunosuppressive protocols. Valganciclovir (VG) is a relatively new antiviral medication that can be administered once daily, and is indicated for cardiac, pancreas and renal transplantation. Little data is available regarding its effectiveness after liver transplantation (LT). **Methods:** We retrospectively reviewed the charts of all patients undergoing LT from 11/2001-9/2003. Variables included type of immunosuppression, incidence of rejection, donor and recipient CMV status, time to development of CMV, and diagnosis. Prophylaxis against CMV included oral VG (900mg once daily) for 3 months followed by acyclovir (200 mg twice daily). Symptomatic CMV infection was diagnosed by the pp65 antigen assay. Immunosuppression consisted of tacrolimus or cyclosporine, MMF, and steroids. Approximately 50% of the patients also received daclizumab (1-2 mg/kg intraoperatively and on post-operative day #4). **Results:** A total of 71 LTs were performed. 21 LTs were excluded from analysis because of either death or re-LTs within 60 days (n=14), non-use of VG (n=4), or lack of known pre-transplant CMV status (n=3). A total of 50 LTs in 49 patients were analyzed. Seventy percent of all patients were CMV + prior to LT. The overall incidence of CMV infection was 14% (n=7) and is shown divided by donor/recipient CMV status in the table below.

Incidence of CMV Infection

Group	CMV (+) n, (%)	CMV (-) n, (%)
D-/R-	1 (25)	3 (75)
D+/R-	3 (27)	8 (73)
D-/R+	2 (17)	10 (83)
D+/R+	1 (4)	22 (96)

D=donor CMV status, R=Recipient CMV status, CMV (+)=CMV infection, CMV (-)=No CMV infection.

Median time from LT to development of CMV was 146 days. At the time of diagnosis only one of the seven patients was on VG; the others were on acyclovir. Treatment of CMV was with VG (900 mg orally, twice daily)(n=4), intravenous ganciclovir (n=2), or decrease in steroids (n=1). Patients who experience rejection had greater than twice the incidence of CMV($p=0.22$). Daclizumab did not influence CMV infection. **Conclusion:** Once daily oral VG is effective in the prevention of CMV infection after LT, and leads to similar rates of CMV post-LT when compared with published series. The low rate of CMV infection post-LT in pre-operative CMV + patients suggests that eliminating prophylaxis in this group may be safe.

Abstract# 737 Poster Board #-Session: P193-II

VANCOMYCIN-RESISTANT ENTEROCOCCUS IN LIVER TRANSPLANT RECIPIENTS*: A DRAMATIC IMPROVEMENT IN OUTCOMES WITH NEW ANTI-VRE ANTIBIOTICS*. Jordana L. Soule,[1,2] Ali J. Olyaei,[1,2] Ann M. H. Busch,[1,2] Jonathan M. Schwartz,[3] Hugo R. Rosen,[3] John M. Ham,[1,2] Susan L. Orloff.[1,2] *[1]Department of Surgery, Division of Transplantation, Oregon Health & Science University, Portland, OR; [2]Department of Surgery, Division of Transplantation, Portland VA Medical Center, Portland, OR; [3]Department of Medicine, Division of Hepatology, Oregon Health & Science University, Portland, OR.*

BACKGROUND: *Enterococcus species* are among the most frequent causes of bacterial infection after liver transplantation. Studies have reported a strong association with Vancomycin-resistant *Enterococcus* (VRE) colonization and infection and mortality in liver transplant recipients. With the advent of effective antibiotic therapy against VRE, the outcomes of VRE-positive liver transplant recipients are unknown. The aim of this study is to prove that the use of anti-VRE antibiotics results in a significant improvement in the outcomes of VRE-positive liver transplant recipients.

METHODS: Retrospective single center case review comparing outcomes in liver transplant recipients in March 2000 through September 2003 during the anti-VRE antibiotic era (Group A) to those from October 1994 through September 1998 when there was no effective anti-VRE therapy (Group B). VRE-positive liver transplant recipients were identified by infection control database. All patients were reviewed via liver transplant databases and Medical Records.

RESULTS: The overall mortality rate for VRE-negative Group A was 15%. A total of 36 clinical isolates of VRE were identified in 15 liver transplant recipients; 12 male/3 female. Two recipients had a positive urine isolate prior to transplantation (4 and 21 days). The 1 year mortality rate in VRE-positive Group A patients was 13% (vs 28% VRE-positive Group B); these deaths were due to sepsis. The 1-year mortality rate in Group A VRE-**infected** patients was 33% (vs 82% VRE-**infected** Group B). Characteristics common among VRE-positive patients were: prior vancomycin use (80%), invasive procedures post-transplantation (80%), fungal infection (53%) and previous vancomycin-sensitive enterococcus infection (33%).

CONCLUSION: Although previous outcomes in VRE-positive liver transplant recipients were devastating, the recent advent of anti-VRE antibiotics has improved mortality remarkably. The use of these new antibiotics will change the future approach to liver transplantation of VRE-positive patients.

Abstract# 738 Poster Board #-Session: P194-II

RETROSPECTIVE EVALUATION OF SURGICAL PROPHYLAXIS AND BACTERIAL COMPLICATIONS AFTER LIVER TRANSPLANTATION. E. Clark Drake,[1] Benjamin T. Duhart, Jr.,[1] A. Osama Gaber,[2] Naseem Amarshi,[1] M. Hosein Shokouh-Amiri,[2] Timothy H. Self,[1] John M. Norwood.[3] *[1]Department of Pharmacy, University of Tennessee Health Science Center, Memphis, TN; [2]Division of Transplant Surgery, Department of Surgery, University of Tennessee Health Science Center, Memphis, TN; [3]Department of Internal Medicine, University of Tennessee Health Science Center, Memphis, TN.*

Postoperative bacterial infections continue to be a significant cause of morbidity and mortality after solid organ transplantation. There have been few studies on surgical prophylaxis in liver transplantation and no clinical standards have been developed. Herein, we present short-term follow-up for 96 liver recipients transplanted between January 2000 and October 2002. The original disease for liver transplant recipients consisted of primarily hepatitis C virus 56 (58%) and primary sclerosing cholangitis 9 (9%). Immunosuppression consisted of steroid induction 96 (100%) with a calcineurin inhibitor 89 (93%) or sirolimus 7 (7%) based maintenance immunosuppression. Nineteen (20%) patients received daclizumab for immunosuppression during renal insufficiency. Eighty one (84%) had cadaveric donors and 15 (15%) had living donors. Seventy-four (77.1%) received at least one dose of cefoxitin and eight (8.3%) patients received vancomycin alone intraoperatively. Average duration of antibiotic prophylaxis was 4 ± 1.6 days with a median of 5 days.

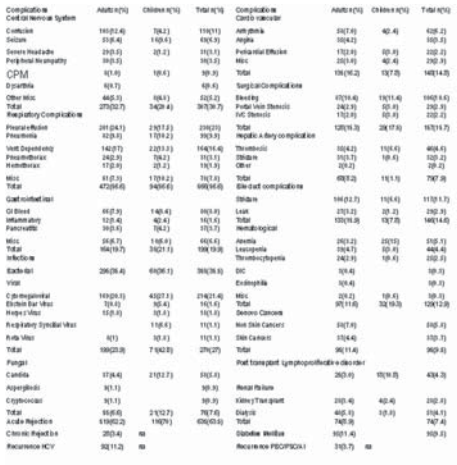
Results. The overall patient and graft survival were 88 (92%) and 90 (93%), respectively. Bacterial infection was identified in twenty-nine (30%) patients and six (20%) patients died as a result of infection. Bacterial infections developed in 14 (48%) patients less than 5 postoperative days, 19 (66%) patients after 5 postoperative days, and 5 (17.2%) patients in both time periods. The most common infections were pneumonia 19 (19.8%) and peritonitis 10 (10.4%). The most common isolated organisms were enterobacter (10.7%), pseudomonas (10.7%), and enterococcus (10.7%). Overall, eleven (11.5%) patients returned to the hospital with an infection within the first postoperative month. Factors associated with bacterial infection were hospitalization prior to transplant (31%), length of time on ventilator (225 ± 267 vs. 23 ± 15 hrs), MELD (21 ± 12 vs. 15 ± 8.3), and ICU stay (224 ± 267 vs. 85 ± 60 hrs). All patients that were retransplanted developed infection.

Bacterial infection post-transplant is still a major problem and early infections are related to prior hospitalization, duration of mechanical ventilation, and ICU length of stay, and living related transplantation. Overall, MELD and retransplantation are associated with postoperative infections. Our study suggests that patients with these factors need broader antimicrobial coverage for surgical prophylaxis.

Abstract# 739 Poster Board #-Session: P195-II

SURGICAL / NON SURGICAL, IMMUNOLOGICAL / NON IMMUNOLOGICAL, EARLY AND LATE COMPLICATIONS AFTER PRIMARY LIVER TRANSPLANTATION AND ITS IMPACT ON GRAFT AND PATIENT SURVIVAL IN ADULTS AND CHILDREN. AN ANALYSIS OF 1000 CONSECUTIVE PATIENTS WITH 9-12 YEARS FOLLOW-UP. Ashok Jain,[2] Reyes Jorge,[1] Randeep Kashyap,[2] Marcos Amadeo,[1] Mazariegos Gorge,[1] Eghtesad Bijon,[1] Fonte Paulo,[1] Cacciarelli Tom,[1] Marsh Wallis,[1] Devera Mike,[1] Fung John.[1] *[1]Surgery, T.E. Starzl Transplantation Institute, Pittsburgh, PA; [2]Surgery, University of Rochester, Rochester, NY.*

Introduction: Improvements in liver transplantation (LTx) survival outcome are widely acknowledged. However little is known about the types of longterm complications after LTx and their direct or indirect impact on patient and graft survival after liver transplantation. **Aim:** To examine the incidence of various types of complications in a large LTx population with a long-term follow-up. **Material:** One thousand consecutive patients (M=600,F=400) mean age 42.5 ±20.2, 834 adults (age>18years) and 166 children (age<18 years) received primary LTx between Aug, 1989 and Dec. 1992, were followed until Feb 2002 with respect to the nature and frequency of post LTx complications and the impact of the complications on patient and graft survival was determined, recognizing that many patients have more than one complication. **Results**: Overall patient / graft survival at 10 year 61.5%/ 56.9% (84.8%, 80.0% for children, 56.8%, 52.3% for adults. **Conclusion:** While multiple and often times, severe complications can occur following LTx, the overall survival of these patients is excellent. However, the impacts of various combinations of complications need further evaluation. Future studies should be directed towards reduction in rate of complications and prevention of multiple complications to improve patient and graft survival.Various complications, which occurred, in adults and children are grouped according to various systems and shown below.



Abstract# 740 Poster Board #-Session: P196-II

A SURVEY OF PROPHYLACTIC IMMUNICATION PRACTICES AT LIVER TRANSPLANT CENTERS IN NORTH AMERICA AND EUROPE - A WEB-BASED STUDY. Nadeem Anwar, Michael Ellis, Savant Mehta, Jennifer Daly. *Division of Gastroenterology and Infectious Diseases, UMass Memorial Healthcare, Worcester, MA.*

Background: Patients with end stage liver disease are at increased risk of infections. Immunization is an important strategy for preventing disease. There is no consensus on vaccination strategies in these patients.

Aim: To survey the current vaccination strategies employed at various liver transplant centers in North America and Europe using an internet based tool.

Methods: E-mail addresses of liver transplant personnel were identified using the ILTS membership database. A web-based 15-item questionnaire was sent via e-mail to all the addresses. The questionnaire was designed to be completed in under two minutes and only allowed one response to each question, namely, Always, Sometimes, or Never. The questions related to the administration of Hepatitis B, Hepatitis A, combined Hepatitis A&B (Twinrix), Tetanus toxoid, dT for adults, DPT for children, Influenza, Varicella, PPD/BCG, HiB, MMR vaccines and stool for ova and parasites. Number of liver transplants done at each center and the person answering the survey were noted. The responses were tabulated for the group as a whole and for centers in North America versus the rest.

Results: There were 58 responses out of the 288 programs surveyed (20.1%). Of the North American centers 24% responded (41/166) and of the other centers 14.75% (18/122) responded. The respondents included 27 hepatologists, 22 surgeons, 3 ID specialists, 5 transplant coordinators and 1 other. Of the centers surveyed 9 did 0-20, 20 did 20-50, 19 did 50-100 and 10 did more than 100 transplants/yr. Appropriate immunization was performed Always or Sometimes in appropriate patients in the following percentage of centers: 41% for Twinrix , 83% for Hepatitis A, 67% for Hepatitis B (single dose), 79% for Hepatitis B(double dose), 59% for tetanus, 45% for DT, 86% for influenza, 45% for varicella, 57% for BPD/BCG, 47% for HIB, 45% for stool for ova and parasites, 41% for MMR, and 57% for DPT in children. Comparing North America and Europe revealed that Hepatitis A vaccination was performed 92% of the centers in North America versus 61% of other centers [p value<0.003]. There were no other differences.There was only one center out of the 58 centers surveyed in which all of the 13 items were done Sometimes or Always in appropriate patients.

Conclusion: Appropriate use of different vaccines ranges between 42-92% of the transplant centers. There is a need for a consensus statement and quality assurance to ensure uniformity.

Abstract# 741 Poster Board #-Session: P197-II

REFRACTORY ASCITES AFTER LIVER TRANSPLANTATION. Seigo Nishida,[1] Noboru Nakamura,[1] Juan Madariaga,[1] David Levi,[1] Jang Moon,[1] Tomoaki Kato,[1] Gennaro Selvaggi,[1] Debbie Weppler,[1] Phillip Ruiz,[2] Guy Neff,[3] Andreas Tzakis.[1] *[1]Surgery, University of Miami, Miami, FL; [2]Pathology, University of Miami, Miami, FL; [3]Hepatology, University of Miami, Miami, FL.*

Purpose: To analyze the incidence, pathogenesis, clinical features, therapy and prognosis of refractory ascites (RA) after liver transplantation.

Methods: A retrospective review of orthotopic liver transplantation (OLT) between June 1994 and July 2002 was performed. RA was defined as ascites that cannot be mobilized or the early recurrence by medical therapy (the international ascites club definition).

Results: Overall 1191 liver transplant recipients were evaluated with a median follow up of 704 days. Sixty-three patients (5.3%) had RA requiring large volume paracenthesis as a result of recurrent hepatitis C(RHCV) 19, 7 with liver failure secondary to chronic rejection, 5 with bacterial peritonitis, 3 with tumor recurrence, 3 with caval anastomotic stenosis, 3 with acute cellular rejection with central venulitis, 1 with portal vein thrombosis (PVT), 1 with liver dysfunction after hepatic artery thrombosis (HAT), 1 with heart failure, 1 with small liver graft for recipient size, 1 with recurrent hepatitis B, 1 with hepatic vein outflow block and 16 with unknown causes. Doppler ultrasonography was done in all patients, while 30 had angiography. Of the 30 patients, 3 had caval anastomotic stenosis, 1 had tumor thrombus in the inferior cava and hepatic veins, 1 had PVT, 1 had HAT and 1 had hepatic artery stenosis. The patients with caval or hepatic artery stenosis were treated with radiological intervention. Of the 63 patients, 8 underwent retransplantation, 5 remain alive, 3 died. LeVeen shunts were placed in 7 patients, 4 remain alive and 3 are dead (1, recurrent HCV with heart and renal failure, 1 recurrent HCVwith infected shunt and 1 tumor recurrence). Transjugular intrahepatic portosystemic shunt (TIPS) was done in 2 patients and both are dead (1 RHCV and 1 sepsis due to infected TIPS). Of the 63 patients, 33 are alive (28 without ascites, 2 with less ascites and 3 with RA) and 29 patients are dead (15 RHCV, 5 sepsis due to bacterial peritonitis, 3 tumor recurrence, 3 liver failure due to chronic rejection, 1 heart failure, 1 alcoholic liver failure, 1 sepsis and 2 unknown). Twelve patients with renal insufficiency are dead. Overall 3-and 5-year patient survival were 60% and 50%.

Conclusion: RA after OLT is associated with a relatively high mortality rate. Common causes include RHCV, bacterial peritonitis, tumor recurrence, rejection, hepatic venous outflow block, PVT and HAT. Early detection and treatment are vital for patient survival.

Abstract# 742 Poster Board #-Session: P198-II

NO RECURRENCE IN PATIENTS TRANSPLANTED FOR YMDD GENOTYPIC MUTATIONS USING PRE-EMPTIVE LAMIVUDINE FOLLOWED BY COMBINATION PROPHYLAXIS. Lucio Caccamo,[1] Raffaella Romeo,[2] Luca S. Belli,[3] Marcello Vangeli,[3] Giorgio Rossi,[1] Alberto B. Alberti,[3] Giovambattista Pinzello,[3] Massimo Colombo,[2] Luigi R. Fassati.[1] *[1]UO Trapianto Fegato e Polmone, Ospedale Maggiore IRCCS Università Degli Studi, Milano, MI, Italy; [2]UO Epatologia Medica, Ospedale Maggiore IRCCS Università Degli Studi, Milano, MI, Italy; [3]Epatologia e Gastroenterologia, Ospedale Niguarda, Milano, MI, Italy.*

This study investigates the impact of lamivudine started before liver transplantation and followed by combined lamivudine and specific immunoglobulins post-transplantation on the risk of HBV recurrence.

Methods: 35 consecutive recipients with HBV-related chronic liver disease and with positive serum HBV-DNA were studied. Lamivudine (100 mg/day) was administered for a mean of 12 months (range 1-32) before transplantation. Patients were actively listed once serum HBV-DNA was negative by standard assay. At transplantation, serum HBV DNA was detected using a highly sensitive in house nested polymerase chain reaction (PCR). Lamivudine-resistant mutations at the YMDD motif of HBV P gene were determined by direct sequencing of PCR positive products. After transplantation, lamivudine was continued and anti-HBV immunoprophylaxis was added, aiming at anti-HBs trough levels of at least 200 U/l. The mean post-LT follow-up was 37 months (range 6-80).

Results: At time of transplantation, despite DNA negativity by standard assay 29 patients (82.8%) were found HBV-DNA positive by in house PCR, and 12 of them (46%) were harbouring YMDD mutations. Notably, none of these 12 patients developed a clinical HBV recurrence. Overall, three patients (two with YMDD mutations at transplantation) died of non-HBV related complications (8, 25 and 28 months after transplantation), and 2-years actuarial survival rate was 87%.

In conclusion, Combination prophylaxis is effective in preventing clinical recurrence in HBV viremic patients undergoing liver replacement in whom HBV-DNA could be reduced before transplantation by lamivudine, and even in presence of genotypic YMDD mutations pre-transplant.

Abstract# 743 Poster Board #-Session: P199-II

HEPATITIS C RECURRENCE FOLLOWING LIVER TRANSPLANTATION: DOES HISTOLOGIC SEVERITY OF INFLAMMATION IN THE EXPLANT COORELATE WITH RECURRENCE? Paul J. Gaglio,[1] Simona Jakab,[1] Alex Novogrudsky,[1] Jean C. Emond,[1] Robert S. Brown.[1] *[1]Center for Liver Disease and Transplantation, Columbia University P & S PH-14 622 W 168th St, NY, NY.*

Introduction: Hepatitis C (HCV) associated graft injury following liver transplantation involves a complex interaction between donor, recipient, and viral factors. However, the severity of histologic injury in the liver explant has not been adequately assessed as a factor associated with histologic recurrence of HCV following transplant. The innate immunologic response to HCV in the recipient manifested as pre-transplant histologic injury may influence post-transplantation histologic recurrence.

Methods: All 103 HCV infected patients (72 Deceased donor (DD) and 31 Living donor (LD) recipients) transplanted at our institution from 7/99-6/03 were included in this analysis. Histologic injury due to HCV following transplantation including the incidence and time to recurrence was assessed and correlated with the degree (grade) of histologic injury in the liver explanted at the time of transplant. Elevated serum transaminases, positive HCV RNA, and liver biopsy consistent with histologic evidence of HCV defined histologic recurrence. Grade of inflammation (grades 1-4) and Stage of Fibrosis (1-4) were assessed using a modification of Scheuer's system. An expert Hepatopathologist (JL) reviewed all liver biopsies.

Results: The overall rate of histologic recurrence of HCV was 78%; 70% in DD and 87% in LD (p = 0.008). The overall time to histologic recurrence of HCV was 18.4 weeks: 17.26 in DD and 19.17 in LD (p = NS). The incidence of histologic recurrence of HCV stratified by degree of inflammation (Grade) in the liver explanted at the time of transplantation was not different when comparing Grade 1 (G1) to G2 (66% vs 70%). However, 93% of G3 developed histologic recurrence of HCV (p = 0.027 G3 vs G1 and G2). When comparing DD to LD, there were no differences in time to recurrence as well as incidence of recurrence stratified by grade of inflammation on explant. Conclusions: 1) Histologic evidence of recurrent hepatitis C following transplantion was common in this patient population. 2) The severity of necroinflammatory activity (grade of inflammation in the explant) prior to transplantation was positively correlated with the incidence of post-transplantation histologic recurrence. 3) Factors which explain this phenomenon including innate immunologic response to HCV and virus specific factors (including quasispecies) require further study.

Histologic HCV Recurrence vs Explant Histology

	Overall (78%)	DD (70%)	LD (87%)
G1	66%	63%	83%
G2	70%	63%	82%
G3	93%	87%	100%

Abstract# 744 Poster Board #-Session: P200-II

IMPACT OF RENAL TRANSPLANTATION ON HEPATIC FIBROSIS PROGRESSION OF CHRONIC HEPATITIS C VIRUS (HCV) INFECTION IN PATIENTS WITH END-STAGE RENAL DISEASE (ESRD). Richard K. Sterling,[1] Kevin H. Peacock,[1] R. Todd Stravitz,[1] Anne L. King,[1] Velimir A. Luketic,[1] Arun J. Sanyal,[1] Melissa J. Contos,[1] A. Scott Mills,[1] Mitchell L. Shiffman.[1] *[1]Virginia Commonwealth University Medical Center, Richmond, VA.*

Approximately 20-40% of patients (pts) with ESLD have chronic HCV infection. Although liver histology in many pts with ESRD on chronic dialysis is generally mild (Am J Gastroenterol 1999; 94:3576), the impact of renal transplantation (RT) on the histologic severity of chronic HCV and fibrosis progression remains undefined. Aims: To evaluate the impact of RT on the rate of fibrosis progression and disease severity in pts with chronic HCV and ESRD. Methods: We performed a retrospective cohort analysis of 68 pts with ESRD (50 awaiting RT on dialysis and 26 pts following RT) and positive HCV RNA (Amplicor, Roche) who underwent liver biopsy (LBx) 1994-2002. Paired LBx, prior to and following RT, were available from 5 pts. Liver histology was assessed according to Knodell histologic activity index (HAI) and advanced fibrosis (adv fib) was defined as bridging fibrosis or cirrhosis. Duration of infection was calculated from time of first possible exposure and rate of fibrosis progression defined as fibrosis score/ duration of infection. Results: The two cohorts were similar with respect to age (mean: 47 yrs) , gender (79% male), racial distribution (20% Caucasian and 80% African Americans) and duration of HCV infection (15.8 yrs). Mean duration to LBx after RT was 8.7 yrs. Comparing pts who underwent LBx following RT to those on dialysis with ESRD, mean creatinine was significantly lower (3.4 vs 10.2; p<.001), ALT values were greater (78 U/L vs 35 U/L; p=.003) while total HAI (4.3 vs 5.1), inflammation (4.3 vs 3.5) and fibrosis scores (0.9 vs 0.9) and proportion with adv fib (15% vs 18%) were similar. The rate of fibrosis progression was higher in those following RT compared to those with ESRD pre-RT (0.057 vs 0.055 fibrosis units/yr) with an increased rate of 0.0056 fibrosis units/yr following RT suggesting that RT increases the rate of fibrosis progression. In support of this, in the 5 patients with paired biopsies (mean duration to LBx after RT was 36 months), total HAI (5 vs 9), liver inflammation (3.8 vs 7.0) and fibrosis scores (0.9 vs 1.2) all increased with a rate of fibrosis progression of 0.1 units/ yr. Conclusions: Worsening inflammation and progressive fibrosis is observed in pts with HCV and ESRD who undergo RT. The impact of this accelerated fibrosis on the natural history and long-term impact of HCV prior to and following RT requires further study.

LIVER TRANSPLANTATION: MANAGEMENT OF IMMUNOSUPPRESSION

Abstract# 745 Poster Board #-Session: P201-II

CLINICAL USEFULNESS OF THE MLR ASSAY USING THE CFSE-LABELING TECHNIQUE TO MONITOR THE IMMUNOSUPPRESSIVE STATE AFTER LIVING-DONOR LIVER TRANSPLANTATION. Yuka Tanaka, Hideki Ohdan, Toshimasa Asahara. *Department of Surgery, Division of Frontier Medical Science, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan.*

Background: Anti-donor alloreactivity, defined as the number and phenotype of alloreactive precursors in the recipient, can be used to monitor rejection or for withdrawal of immunosuppression. Mixed lymphocyte reaction using intracellular fluorescent dye 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling technique (CFSE-MLR) enables determination of the number of proliferating cells in response to allogeneic stimulation and simultaneous determination of the phenotype of proliferating cells by use of multiparameter FCM analysis. We investigated the clinical usefulness of the CFSE-MLR as reliable immunological monitoring after living-donor liver transplantation (LDLT).

Methods: Eighteen patients undergoing LDLT were enrolled in this study. CFSE-MLR assays were performed at regular intervals as follows; CFSE-labeled peripheral blood mononuclear cells (PBMC) from recipients were used as responders. Irradiated autologous, donor, and third-party PBMC were used as stimulators. After coculture, the responder cells were stained with either CD4 or CD8 mAbs-PE together with CD25 mAb-APC. To determine the stimulation index, the reactive precursor frequency and CD25-expression in each $CD4^+$ and $CD8^+$ T cell subsets, the cells were analyzed by FCM.

Results: One of the 18 patients had an episode of rejection reaction within three months after LDLT. Before making a clinical diagnosis of rejection, a more vigorous proliferation of $CD8^+$ T cells was observed in anti-donor MLR, when compared with those in anti-third-party MLR. The $CD8^+$ T cells responding to donor stimulators raised their expression of CD25 in parallel with proliferation. In two patients, a mild increase in transaminases, which hindered the reduction of immunosuppressants, sometimes occurred. Such episodes were always associated with a more marked proliferation of $CD4^+$ T cells in anti-donor MLR than those in anti- third-party MLR, regardless of the proliferation of $CD8^+$ T. One patient, in whom immunosuppressants had been satisfactorily withdrawn, showed a complete lack of proliferation of $CD4^+$ and $CD8^+$ T cells in anti-donor MLR and normal proliferation of $CD4^+$ T cells in anti-third-party MLR.

Conclusions. The proliferation of $CD8^+CD25^+$ T cells in response to anti-donor MLR would be a useful index of ongoing acute rejection. In contrast, the proliferation of $CD4^+$ T cells would represent an immunosuppressive state and be a useful marker to regulate immunetherapy.

Abstract# 746 Poster Board #-Session: P202-II

MONITORING OF CIRCULATING DC AND T CELL SUBSETS IN PATIENTS RECEIVING THYMOGLOBULIN INDUCTION IN LIVER TRANSPLANT. Mario Arpinati,[1] Michele Masetti,[2] Alessandra Zagnoli,[1] Damiano Rondelli,[3] Antonio D. Pinna.[2] *[1]Research Center on Transplant Immunology, University of Bologna, Bologna, Italy; [2]Liver and Multivisceral Transplant Center, University of Modena and Reggio Emilia, Modena, Italy; [3]Section of Hematology/Oncology, University of Illinois at Chicago, Chicago, IL.*

INTRODUCTION. Anti-lymphocyte antibodies can efficiently prevent alloimmune reactions, but induce profound immunosuppression in graft recipients.

MATERIALS and METHODS. In this study, 22 patients undergoing orthotopic liver transplantation from either living (n=17) or cadaveric donor (n=5) received high dose (5mg/kg) infusion of RATG induction just prior to surgery. Maintenance immunosuppression was TAC monotherapy at the dose of 0.15 mg/kg daily for 4 months, followed by gradual tapering. Numbers of various leukocyte subsets, including plasmacytoid and myeloid dendritic cells (DCs) [identified as BDCA-2+CD123+ and lineage$^-$, CD11c+, HLA-DR+, respectively], B cells (CD19+), NK cells (CD56+), CD4+ and CD8+ T cells, naive CD4+ (CD45RA+) and CD8+ T cells, and CD4+CD25+ T regulatory cells, were determined in recipients' peripheral blood before and at 1 and 6 months after transplant.

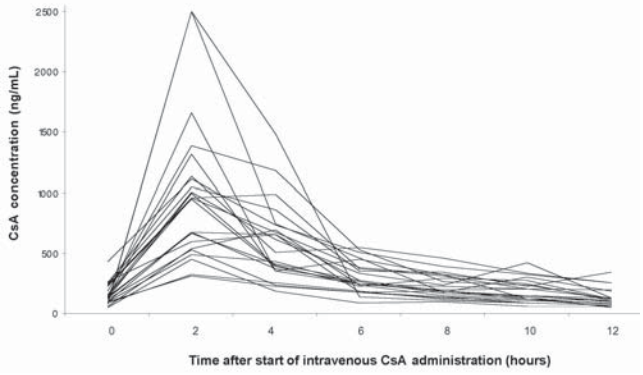
RESULTS. RATG administration was associated with reduced median numbers of CD4+ T cells (162/μL, range 7-768, vs 513/μL, range 74-1576; p=0.001), naive CD4+ T cells (41/μL, range 2-344 vs 194/μL, range 14-445; p=0.003) and CD4+CD25+ T regulatory cells (43/μL, range 9-310 vs 194/μL, range 22-1018; p=0.003) at 1 month after transplant as compared to pretransplant values, whereas DC numbers were comparable. In patients who did not experience graft rejection, we could still observe a significant reduction of CD4+ T cells (p=0.04), naive CD4+ T cells (p=0.05) and CD4+ CD25+ T regulatory cells (p=0.04) at 1 month after transplant. However no difference was observed at 6 months. Pretranplant circulating DC and T cells numbers were similar in patients who experienced rejection (8 patients, at a median of 66 days after transplant, range 6-144) as compared to the others. However, graft rejection was associated with significantly reduced levels of plasmacytoid DC (0.7/μL, range 0.4-1.5 vs 2.4/μL, range 0.5-14.4 in patients who did not reject; p=0.02) at 1 month after transplant, whereas mDC as well as T cell counts were comparable. Finally, in 3 patient samples obtained at the time of rejection (before the start of high dose corticosteroids) we observed a selective reduction in pDC numbers (0.4/μL, range 0.4-1.5) as compared to control samples (2.4/μL range 0.5-14.4) (p=0.04)

CONCLUSION. Single dose RATG administration can selectively deplete circulating CD4+ T cells subsets, whereas DC are not affected. However, loss of pDC may correlate with graft rejection.

Abstract# 747 Poster Board #-Session: P203-II

C_2 MONITORING USING 4-HOUR INTRAVENOUS INFUSIONS OF CYCLOSPORINE IN DE NOVO LIVER TRANSPLANT RECIPENTS. R. Lück,[1] J. Böger,[1] E. Kuse,[1] J. Klempnauer,[1] B. Nashan.[2] *[1]Klinik für Viszeral- und Transplantationschirurgie, Medizinische Hochschule Hannover, Hannover, Germany; [2]Multi Organ Transplant Program, QEII Health Sciences Centre, Halifax, NS, Canada.*

Intravenous administration of cyclosporine (CsA) avoids the problem of CsA absorption dysfunction that can occur in the first few days after liver transplantation. However, 24-hour infusions using C_0 monitoring have proven unfavourable in terms of efficacy and toxicity. To our knowledge, this is the first study to assess use of C_2 monitoring of CsA using 4-hour intravenous infusions, or to evaluate starting dose of intravenous CsA based on graft function. **Methods:** Four-hour intravenous infusions of CsA (2mg/kg/day) were given twice daily to 20 consecutive recipients of a liver allograft for a minimum of 4 days. Full CsA pharmacokinetic profiles were undertaken on day 3 post-transplant in all patients and also on day 7 in five patients. Patients received basiliximab and mycophenolate mofetil (n=11) or steroids (n=9). **Results.** Across all 20 patients, mean $AUC_{0\text{-}12}$ was 4,500ng.h/mL. The period of greatest exposure was during the period 2-4 hours after the start of infusion (32% of total $AUC_{0\text{-}12}$). The correlation between C_2 and $AUC_{0\text{-}12}$ was 0.91, while the correlation between C_0 and $AUC_{0\text{-}12}$ was 0.43. The CsA dose/kg body weight and total CsA dose correlated only poorly with $AUC_{0\text{-}12}$ (r=0.44 and r=0.61, respectively). When patients were stratified according to initial or delayed graft function, there was a good correlation between total CsA dose and $AUC_{0\text{-}12}$ (initial graft function, r=0.84; delayed graft function, r=0.93). In patients with initial graft function, $AUC_{0\text{-}12}$ could be predicted by the formula (24.5 x CsA dose) + 454. For patients with delayed graft function, the formula was (37 x CsA dose) + 1293. **Conclusions.** C_2 monitoring provides a more reliable indication of CsA exposure than C_0 monitoring with twice-daily 4-hour intravenous CsA. Calculating starting dose of intravenous CsA based on presence or absence of initial graft function is a more precise technique than use of body weight.



Abstract# 748 Poster Board #-Session: P204-II

CHRONIC REJECTION AND ITS RISK FACTORS FOLLOWING LIVER TRANSPLANTATION. Tom P. Theruvath,[1] Ulf P. Neumann,[1] Volker Schmitz,[1] Ruth Neuhaus,[1] Stefan G. Tullius,[1] Jan M. Langrehr,[1] Peter Neuhaus.[1] *[1]General and Transplantation Surgery, Charite Campus Virchow Clinic, Berlin, Germany.*

Chronic allograft rejection (CR) after liver transplantation (OLT) remains a common indication for retransplantation and cause of graft failure. This may be a rising problem since long-term complications of immunosuppressive drugs led to low immunosuppressive maintenance therapy in OLT recipients. Therefore we reviewed factors to identify patients at risk to develop CR after OLT.

Data from 1200 liver transplants was prospectively analyzed. Immunosuppression was commenced as either Cyclosporine A (CsA) or Tacrolimus (Tac) based therapy in different protocols. Follow-up period ranged from 24 to 168.8 months (median 78 months). Risk factors were identified by using multivariate analysis of graft, recipient, and posttransplant variables.

The actuarial graft survival was 88% after one year, 82% after three years, and 78.8% after five years. It was similar in Tac- and CsA treated patients. The overall incidence of histologically proven CR was 21/1200 (1.75%). In the multivariate analysis only CsA as primary immunosuppressant ($p<0.05$, CsA 16/496, 3.2% vs. Tac 3/704, 0.42%), history of an acute rejection episode ($p<0.01$), and transplants for primary sclerosing cholangitis (PSC) (5/43, 11%, $p<0.05$) contributed to an increased risk for the development of CR. In the analysis neither crossmatch nor HLA-compatibilities had a significant influence on the prevalence of CR. However, when analyzing only transplants for PSC more HLA-DR compatibilities were associated with an increased risk for CR.

The incidence of CR has decreased after the introduction of Tac in recent years. In contrast to this the morbidity and mortality caused by side effects of immunosuppressive therapy is increasing. This study identified patients with PSC and a history of acute rejection at risk for the development of CR. When minimizing long-term maintenance immunosuppressive regimens in these patients, early diagnostic is mandatory to prevent severe complications from underimmunosuppression and CR.

Abstract# 749 Poster Board #-Session: P205-II

INCREASING ADHERENCE TO THERAPY FOR RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION BY STARTING AT LOWER DRUG DOSES AND ADDING GROWTH FACTORS. Nazir A. Rahim,[1] Katherine Suggett,[1] Christoph Troppmann,[2] Colette Chambers,[1] John P. McVicar,[2] Rajen Ramsamooj,[3] Michael O'Brien,[4] Christopher L. Bowlus,[1] Lorenzo Rossaro.[1] *[1]Internal Medicine, University of California Davis Medical Center, Sacramento, CA; [2]Transplant Surgery, University of California Davis Medical Center, Sacramento, CA; [3]Pathology, University of California Davis Medical Center, Sacramento, CA; [4]Anatomic Pathology, Boston Medical Center, Boston, MA.*

BACKROUND: Adherence to antiviral therapy for recurrent hepatitis C (HCV) has been poor due to hematologic toxicity.

AIM: To increase adherence to interferon (IFN) and ribavirin (RBV) in patients with recurrent HCV after liver transplantation (LT) by starting at lower drug doses and adding growth factors.

METHODS: We enrolled 10 consecutive patients with recurrent HCV after LT. IFN was started at 1 MIU tiw and increased by 0.5 MIU biweekly to a maximum dose of 3 MIU tiw. RBV was started at 200 mg bid and increased by 200 mg biweekly to 1000-1200 mg daily for a total of 48 weeks. Those with end-of treatment viral response (ETVR) also received an additional 6 months of RBV monotherapy. Filgrastim was started at 300 ug three times weekly if absolute neutrophil count decreased to ≤1,500/cc, and erythropoietin was started at 40,000 U weekly if hemoglobin decreased to ≤ 10 g/dL. Liver biopsies were performed at baseline and at end of therapy.

RESULTS: Ten consecutive patients (1F/9M, mean age 47, 60% genotype 1, viral load >2 MIU, tacrolimus-based immunosuppression) with recurrent HCV on liver biopsy (grade 2-7, stage 0-6, Ishak score) were enrolled. Seven (70%) tolerated 12 months of combination therapy. Two discontinued for decompensation of liver cirrhosis at weeks 16 and 36, and one other discontinued for depression at week 32. No episodes of acute cellular rejection or discontinuation of therapy for hematological side effects were observed. All patients received >80% of the expected doses of RBV and IFN, but 70% required filgrastim and 80% required erythropoeitin. Four had negative HCV-RNA at the end of treatment (40% ETVR, ITT). Two relapsed while on RBV monotherapy. Two continued to be HCV-RNA negative during and 6 months following RBV monotherapy (sustained viral response or SVR: 20%, ITT). Nine paired biopsies showed stable or improved fibrosis scores in 6 patients (2.0 ± 1.5 vs 1.1 ± 0.5) and worsening fibrosis in 3 patients (1.6 ± 0.5 vs 3.5 ± 1.0). There was no correlation between viral response and fibrosis changes.

CONCLUSION: (1) SVR (20%) is lower compared with historical standard IFN and RBV in the non-transplant setting, (2) adherence can be achieved starting at lower doses of antivirals and adding growth factors, (3) most of the patients showed improved or stable fibrosis scores.

Abstract# 750 Poster Board #-Session: P206-II

DECREASED CELLULAR IMMUNE RESPONSE IN HEPATITIS C PATIENTS AWAITING LIVER TRANSPLANTATION. James Huang,[1] Mayra Lopez-Cepero,[2] James Mayes,[1] Angel Alsina,[1] David Bruce,[1] John Leone,[1] Victor Bowers.[1] *[1]LifeLink Transplant Institute, Tampa, FL; [2]Transplant Immunology Laboratory, LifeLink Foundation, Tampa, FL.*

Background: Hepatitis C viral infection has been shown to suppress host cellular immune response. A new assay (ImmuKnow™, Cylex Inc., Columbia, MD) assesses quantitatively the cell-mediated immune response by measuring the ATP release in $CD4^+$ T cells when stimulated by phytohemagglutinin. A pilot study with this assay was undertaken to assess baseline immune response of patients with end stage liver disease. The cell-mediated immune response in Hepatitis C versus non-Hepatitis C patients awaiting liver transplantation was determined.

Methods: Blood from Hepatitis C and non-Hepatitis C patients with end stage liver disease awaiting liver transplant were obtained and assayed for their cell-mediated immune response using the ImmuKnow™ assay. ATP release was classified as: low (<225ng/ml); moderate (226-524ng/ml); and strong (>525ng/ml). Healthy controls without liver disease were included in each assay run.

Results: In Hepatitis C patients (n=16), 15/16 (93.8%) had low ATP release, 1/16 (6.3%) had a moderate release, and 0/15 had a strong release. In non-Hepatitis C patients (n=5), 2/5 (40%) had a low ATP release, 3/5 (60%) had a moderate release, and 0/5 had a strong release. In healthy controls (n=16), 0/16 had a low ATP release, 10/16 (62.5%) had a moderate release, and 6/16 (37.5%) had a strong release. Average ATP release in Hepatitis C patients is 127.9ng/ml, in non-Hepatitis C patients is 264.6ng/ml, and in healthy controls is 468.9ng/ml.

Conclusions: The use of this assay demonstrates impaired and decreased ATP release in Hepatitis C infected patients with end stage liver disease. These findings corroborate previous studies demonstrating decreased cell-mediated immune response in Hepatitis C patients. This assay, when obtained in pre-liver transplant patients, establishes a baseline cell-mediated immune response that may be useful when compared to the cell-mediated immune response in the post-transplant setting to monitor levels of global immunosuppression. Further studies on the utility of the assay are warranted.

Abstract# 751 Poster Board #-Session: P207-II

ASPECTS OF SPONTANEOUS MICROCHIMERISM AFTER ORTHOTOPIC LIVER TRANSPLANTATION. G. Junge,[1] T. Härtl,[1] M. Nagy,[2] U. Neumann,[1] R. Neuhaus,[1] P. Neuhaus.[1] *[1]Department of Surgery, Virchow-Hospital Charité, Berlin, Berlin, Germany; [2]Forensic Medicine, Charité, Berlin, Berlin, Germany.*

Introduction: The phenomenon of persistent donor cells in peripheral blood, lymph nodes and bone marrow, even for years after transplantation is called microchimerism (MC). Based on this observations in long-term survivors it has been hypothesized that the development and persistence of donor-specific MC may play a role in the induction of allograft tolerance and hence result in a reduction of rejection episodes and immunosuppressive drug therapy. Other studies failed to support this assumption, so the role of MC is still on debate. Patients and method: We enrolled 50 patients, each 25 patients with/without MC and followed all recipients for 2 years after OLTx. Detection of MC was based on STR amplification technique of 9 STR regions plus the amelogenin gen in the CD3+ and CD34+ cell populations. Amplification-products were determined by calculating the proportion of peak areas of donor/recipients signals using an ABI PRISM 310 genetic analyser (5). Patients were 49.3/45.2 years old (16 to 70 years); 27 of 50 were men. We analyzed graft/patient-survival, laboratory data as ALT/AST, AP/GGT and Bilirubin at 0.25, 0.5, 1, 2, 3, 6, 12, 24 months after OLTx as well as drug-levels of immunosuppressive therapy and frequency of rejection episodes. Results: There was a lower incidence of acute rejection episodes in the MC group. Due to maximal donorspecific proportion we divided the MC groups into (1) MC < 10 % and (2) MC > 10 % and could show a significant ($p = 0.022$) difference in the incidence of acute rejection. We could as well demonstrate significant lower tacrolimus levels ($p = 0.0245$) in the MC group. Conclusion: Cells of donor origin can be found in recipient's peripheral blood. We could also demonstrate MC in the CD34+ population. The clinical relevance of MC is not defined yet. Some authors suppose that MC simply reflects a sufficient immunosuppression and is more the cause than the effect. Despite this our results suggest that recipients derives advantage from spontaneous and especially high proportion of MC: patients without MC had significant more episodes of acute rejection than patients with MC, they also need higher blood-levels of immunosuppressive drugs.

Abstract# 752 Poster Board #-Session: P208-II

CALCINEURIN (CNI) WITHDRAWAL IN LIVER TRANSPLANT (LTx) RECIPIENTS WITH LATE RENAL DYSFUNCTION—A VIABLE AND WORTHWHILE OPTION. Abhinav Humar,[1] Kristin Horn,[1] Ann Kalis,[1] Brooke Glessing,[1] Angelika Gruessner,[1] Rainer Gruessner,[1] Raja Kandaswamy,[1] William D. Payne,[1] John R. Lake.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Background: Late renal dysfunction and eventual renal failure is an increasing concern and problem in liver transplant recipients. Long-term CNI use is believed to play a contributing role in many of these cases. There is increasing interest in CNI withdrawal from maintenance regimens in an attempt to improve renal function.

Methods: We instituted a protocol of CNI withdrawal in liver transplant recipients with deteriorating renal function. Patients who were at least 6 months posttransplant with a rising serum Cr and no recent acute rejection (AR) episode were converted from a CNI maintenance protocol to a regimen consisting of full-dose MMF (1g bid) and low-dose prednisone (5 mg/day). Recipients that were off prednisone, were restarted on it at the time of the conversion.

Results: Since Jan '99, we have converted 13 recipients using this protocol. Mean recipient age was 57.3 yrs. Causes of liver failure were Hep C (3), PBC (2), Hep B (1), ETOH (4), and others (3). Mean time posttransplant at time of switch was 46.1 months (range=6-108 months). Six of the recipients were on CsA; 7 were on tacrolimus. Mean serum Cr at time of switch was 2.4 mg/dl (range=1.6-5.9); estimated Cr clearance=37.9 mls/min (range=18-63). With a mean follow up of 22.9 months (range 4-58) since CNI withdrawal, the following results have been seen

i) None of the recipients have had an episode of AR after the switch

ii) 12 recipients remain on MMF/Pred; 1 has restarted on FK with the MMF being stopped because of a very low WBC

iii) No recipient has progressed to ESRD and dialysis

iv) 11 of the 13 recipients have had an improvement in renal function, and 2 have had no change. No recipients have had a deterioration of renal function.

v) Mean serum Cr for all recipients at present is 1.7 (range=1.0-3.0); estimated Cr clearance= 51.8 (range= 19-86).

Conclusions: CNI withdrawal with conversion to MMF/Pred is a viable option in LTx recipients with deteriorating renal function. There does not seem to be an increased risk for acute rejection in this small group of patients. Renal function was noted to improve in the majority of recipients after stopping the CNI.

Abstract# 753 Poster Board #-Session: P209-II

FOURTY EIGHT HOUR DELAY IN IMMUNOSUPPRESSIVE THERAPY FOLLOWING LIVER TRANSPLANTATION: IMPLICATIONS FOR ACTIVATION-ASSOCIATED TOLERANCE. A. Agarwal,[1] J. A. Fridell,[1] D. A. Rouch,[1] W. C. Goggins,[1] P. Y. Kwo,[2] N. P. Chalasani,[2] L. Lumeng,[2] M. L. Milgrom,[1] M. D. Pescovitz,[1] A. J. Tector.[1] *[1]Department of Surgery, Indiana University, Indianapolis, IN; [2]Department of Medicine, Indiana University, Indianapolis, IN.*

Introduction: The induction of tolerance following liver transplantation in animal models is associated with the rapid and exhaustive activation of the recipient immune system. There is evidence that immunosuppression given immediately posttransplantation inhibits this form of tolerance. This single center, nonrandomized, retrospective study demonstrates the feasibility of delaying immunosuppression for 48 hours postoperatively to allow activation of the recipient immune system in adult cadaveric liver transplantation. **Methods:** Between July 2001 and October 2003, 226 patients underwent liver allografts. Exclusion criteria included: perioperative death, and previous liver allograft recipient, excluding 20 patients. Patients were divided into two groups: no delay (ND) with 106 patients and delayed immunosuppresion (DI) with 100 patients. The protocol with delayed immunosuppression consists of no immunosuppressive drugs during the perioperative period (48 hours), 3 doses (2 mg/kg/dose) of rabbit anti-thymocyte globulin (RATG, Sangstat) given on postoperative day (POD) 2, 4, 6, steroids with rapid taper started on POD day 2, and tacrolimus started on POD day 3 (trough levels 10-12). ND received the identical regimen except steroids were initiated immediately postoperatively, and RATG was started on POD1. The chi-square test and Student's t test were used for univariate analysis. **Results:** There was no difference in patient (ND: 96% vs. DI: 92%; p=ns) or graft survival (ND: 96% vs. DI: 91%; p=ns). The rejection rates were identical in both groups (10%). Two patients from DI were treated with anti-lymphocyte therapy for severe rejection, while the rest responded to steroids and reversal of weaning. There was no difference in serum transaminases, total bilirubin, and alkaline phosphatase between either group at any interval. No patient has developed post-transplant lymphoproliferative disorder. Recurrence of HCV hepatitis (biopsy proven) was 31% (ND) vs. 43% (DI) (p=ns). CMV infections (ND: 5% vs. DI: 2%; p=ns) were treated with oral valgancyclovir. **Conclusion:** Prospective trials evaluating delayed immunosuppressive therapy in liver transplantation for tolerance induction can be performed without increasing the incidence of rejection, graft loss, or patient death.

Abstract# 754 Poster Board #-Session: P210-II

COMPARATIVE ANALYSIS OF DELAYED TACROLIMUS IMMUNOSUPPRESSION, +/- ANTIBODY INDUCTION, IN ORTHOTOPIC LIVER TRANSPLANT PATIENTS WITH PRE-OPERATIVE RENAL DYSFUNCTION. Mark Johnson,[1] Jane Salm,[1] Paula McIver,[1] Robert Dupuis,[2] Ruthann Conoley,[2] Roshan Shrestha,[3] Steven Zacks,[3] David Gerber,[1] Jeffrey Fair,[1] Kenneth Andreoni.[1] *[1]Departments of Surgery; [2]Pharmacy; [3]Medicine, Division of Transplantation, University of North Carolina, Chapel Hill, NC.*

Introduction: Renal dysfunction is a major risk factor after liver transplantation (OLT), a predictor of waitlist mortality. Immunosuppressive regimens aimed at sparing renal function might improve patient outcomes post-transplantation.

Objective: To evaluate the efficacy of delayed tacrolimus, with or without induction therapy, in patients with renal insufficiency undergoing OLT.

Methods: Between January 1998 and December 2003, 60 patients had renal dysfunction (as defined as serum creatinine ≥ 1.6 mg/dl at the time of transplant) and received a renal sparing immunosuppression protocol of which 58 were eligible for this review. Group 1 (n=32) patients received MMF 1.5 gm, PO, BID, steroids starting on POD #0, and delayed Tacrolimus, 0.05 mg/kg PO BID starting on POD#4-7. Group 2 patients (n=26) received Simulect™ (N=8) induction 20mg IV POD #0 and POD #4 or Thymoglobulin™ (N=18) induction 2.0mg/kg IV intra-operatively, and 1.5mg.kg IV on PODs #1 and #3 along with steroids and delayed Tacrolimus. Goal trough levels were similar in each group. 30 day data were collected and analyzed using student's t-test.

Results: There was a higher incidence of post-transplant dialysis in the non-induction group (56% vs 27%, p=0.0265). There was also a higher incidence of opportunistic infections in Group 1, most common being CMV and fungal. Three patients in Grp 1 converted to Cya due to neurotoxicities, where as 1 patient in Grp 2 converted to FK secondary to neurotoxicity.

Conclusions: Antibody Induction therapy with delayed Tacrolimus and steroids, is a safer immunosuppressive protocol for patients with renal dysfunction undergoing liver transplantation than delayed Tacrolimus, MMF and steroids, and appears to provide clinically-apparent renal protection post-transplant.

	Group 1 (no induction)	Group 2 (induction)	p value
N	32	26	
Female (%)	31	38	
AGE	46.78 ± 2.03	47.85 ± 2.18	
UNOS STATUS			
1	8		
2A	17		
2B	6		
3	1		
MELD SCORE	N/A	33 ± 2.0	
LOS (days)	34.66 ± 8.03	24.58 ± 2.44	0.277
30 DAY REJECTION RATE (%)	31	27	
30 DAY GRAFT SURVIVAL(%)	81.20	88.50	0.4462
30 DAY PT SURVIVAL(%)	93.80	92.30	
PT W/PRE-TX DIALYSIS(%)	28.00	23.00	0.665
PT W/POST-TX DIALYSIS(%)	56.00	27.00	0.0265
PRE-TX CREATININE (mg/dl)	3.19 ± 0.28	3.02 ± 0.29	0.676
POD #14 CREATININE (mg/dl)	1.91 ± 0.29	2.44 ± 0.24	0.18
POD #30 CREATININE (mg/dl)	1.78 ± 0.20	2.09 ± 0.25	0.32
OPPORTUNISTIC INFECTIONS (%)	59	19	0.0021

Abstract# 755 Poster Board #-Session: P211-II

A PROSPECTIVE RANDOMIZED TRIAL COMPARING STEROID-FREE IMMUNOSUPPRESSION INDUCTION WITH TACROLIMUS AND MMF VERSUS TACROLIMUS AND STEROIDS IN PATIENTS WITH HCV. Martina T. Mogl,[1] Ulf P. Neumann,[1] Jan M. Langrehr,[1] Peter Neuhaus.[1] *[1]Department of Surgery, Charite Virchow-Klinikum, Humboldt-University, Berlin, Germany.*

Introduction: End-stage hepatitis-C-virus (HCV) infection is a frequent indication for orthotopic liver transplantation (OLT). In these patients recurrence of HCV infection after OLT is almost universal and while graft hepatitis is often mild in a considerable number of patients, severity of graft hepatitis increases during the long-term follow-up and approximately 10% of patients develop severe graft hepatitis and cirrhosis. Known risk factors for the development of severe HCV-related hepatitis after OLT are the HCV genotype and steroid administration after OLT. The aim of this study was to examine whether the immunosuppressive induction regimen would be safe without steroids and possibly be beneficial for the course of recurrent HCV-hepatitis after OLT.

Patients and methods: In a prospective analysis 53 consecutive patients with end-stage HCV hepatitis were randomized to receive either Tacrolimus (Tac)/ Prednisolone (Pred) or Tacrolimus/ Mycophenolate Mofetil (MMF). The groups consisted of 27 and 26 patients respectively. Follow-up ranged from 6 to 52 months (median = 28 months).

Results: The overall patient survival was 81% with 4 of 26 dying in the Tac/MMF-group compared to 6 of 27 in the Tac/Pred-group. The incidence of acute rejection in the Tac/MMF-group was 31% (8/26) whereas 30% (8/27) of patients in the Tac/Pred-group developed an acute rejection episode. Only one of these patients developed a steroid resistant rejection with the need of OKT3 because of severe non-compliance.

Discussion: Our data demonstrate that a steroid-free immunosuppressive induction protocol with Tac/MMF after OLT for HCV is safe and effective reaching a similar risk for rejection compared to the Tac/Pred-group. However due to the short follow-up no final conclusion on the severity of recurrence of Hepatitis-C can be made up to date.

Abstract# 756 Poster Board #-Session: P212-II

36 MONTH RESULTS WITH EVEROLIMUS (E) IN LIVER TRANSPLANTATION. G. Levy,[1] V. M. Moeller,[2] J. Jaffe,[2] J. Punch,[3] P. Neuhaus,[4] D. Mayer.[5] *[1]Director, Multi-Organ Transplantation, Toronto General Hospital, Toronto, ON, Canada; [2]Transplant and Immunology, Novartis Pharmaceuticals Corp., East Hanover, NJ; [3]Department of Hepatic Transplantation, University of Michigan, Ann Arbor, MI; [4]Chirurgische Klinik, Universitaetsklinikum Rudolf Virchow, Berlin, Germany; [5]The Liver Unit, The Queen Elisabeth Hospital, Edgbaston, Birmingham, United Kingdom.*

Patients randomized to Neoral, E 0.5 mg, bid, Group I; E 1.0 mg, bid, Group II; E 2.0 mg, bid, Group III; and placebo (PLA) Group IV in this investigational study were followed for 36 months. Ninety-one percent of patients who received E at 1.0 mg per day completed the M36 visit; 72.7% of patients who received E at 2.0 mg per day completed the M36 treatment, whereas only 33.3% of patients who received E at 4.0 mg per day were able to complete the full course due to adverse events.

There were no dose-related or statistically significant differences between treatment groups and the incidence rate of efficacy failure or its single components. A trend towards lower incidence of death was observed in the two highest dose levels of E. All graft losses and most deaths were secondary to typical post-transplant complications. None were attributed to the study medication. The incidence of hypercholesterolemia was proportional to the dose of E given and was 3%, 7%, 10% and 10% (PLA, E 1.0, 2.0 and 4.0 mg per group). Renal dysfunction was higher in patients receiving E (7%, 21%, 10% and 16% in the PLA, E 1.0, 2.0 and 4.0 mg per group). Neoplasms were rare in all groups. Viral infections appeared to be associated with E dose and were highest in the two highest E dose levels (13%, 14%, 23% and 23% in the PLA, E 1.0, 2.0 and 4.0 mg groups respectively). From M3 onward, mean values of alkaline phosphatase appeared inversely associated with E dose and were 298, 223, 174 and 146 IU/L in the PLA, 1.0, 2.0 and 4.0 mg groups at M12. After an initial increase, mean creatinine values remained relatively stable from M1 in all treatment groups. Creatinine clearance decreased in all groups with relatively stable values from M1 onward and no treatment-related trend. Mean total cholesterol increased from baseline in all treatment groups and maximum levels were generally attained at M6. Changes from baseline were generally dose-related, but were not significant between treatment groups. Mean triglycerides revealed dose-related increases and maximum values were obtained also at M6.

This study demonstrates that the use of everolimus in liver transplant patients is safe and effective with a dose-associated effect on creatinine, increases of cholesterol and triglyceride. These studies provide data for additional studies in this indication.

Abstract# 757 Poster Board #-Session: P213-II

SWITCH FROM CALCINEURIN INHIBITORS TO MYCOPHENOLATE MOFETIL IN LIVER TRANSPLANT PATIENTS WITH LONG TERM SIDE EFFECTS OF IMMUNOSUPPRESSION. Giuseppe Tisone,[1] Andrea Cardillo,[1] Alessandro Anselmo,[1] Tommaso M. Manzia,[1] Claudia Ciceroni,[1] Linda De Luca,[1] Nicola De Liguori Carino,[1] Carlo U. Casciani.[1] *[1]Department of Surgery, Universita' Degli Studi Tor Vergata, Rome, Italy.*

BACKGROUND: Long-term side effects of immunosuppressive therapy with calcineurin inhibitors (CNI) in liver transplant patients are major causes of morbidity.

PATIENTS AND METHODS: We undertook a prospective study to assess the safety and efficacy of CNI withdrawal and replacement by mycophenolate mofetil.

33 patients with a minimum follow-up of 2 years after liver transplantation were included in the study. They were all on monotherapy of one of the two CNI. Of these 30 had renal dysfuction attributable to suspected CNI toxicity and 3 had hyperlipidaemia. 10 of these patients had both renal dysfunction and hyperlipidaemia. 20 of these patients had also arterial hypertension.

Renal function, blood pressure and lipid profile were measured before and 12 months after study entry. A sequential renal scintigraphy was also performed on every patients before and 12 months after study entry, to appraise renal damage and possible improvement. Side effects of medication and graft function were recorded during the study.

RESULTS: At the end of the study there was a significant decrease in serum creatinine (by 28%) and urea levels (by 36%). Blood pressure improved significantly with a systolic decrease of 20% and diastolic decrease of 12%. There was also an improvement of cholesterol (decrease of 21%) and triglyceride (decrease of 56%). None of the patients had to stop the study because of side effects of the new therapy. No episodes of active graft rejection occurred during the convertion period and after remaining on mycophenolate mofetil monotherapy.

CONCLUSIONS: Substitution of CNI by mycophenolate mofetil can improve renal function, blood pressure and cholesterol and triglyceride concentration of liver transplant patients without an increased rejection risk with mycophenolate mofetil monotherapy.

Abstract# 758 Poster Board #-Session: P214-II

IMPROVEMENT IN RENAL FUNCTION FOLLOWING INTRODUCTION OF SIROLIMUS IN LIVER TRANSPLANT RECIPIENTS. Moreno Luna Laura, Gregory J. Gores, Walter K. Kremers, Russell H. Wiesner, David J. Brandhagen, K. V. Narayanan Menon. *Transplant Center, Mayo Clinic, Rochester, MN.*

Renal failure related to the use of calcineurin inhibitors (CIs) is an increasing problem following orthotopic liver transplantation (OLT). Sirolimus, a potent immunosuppressive agent, has less nephrotoxicity than CIs and is a potential alternative immunosuppressive agent in patients who develop renal insufficiency. **Aim:** 1) To determine if switching from CIs to sirolimus results in improvemed renal function following OLT and, 2) To determine the side-effect profile of sirolimus. **Patients and Methods:** Between May 1998 and August 2003, 41 liver transplant recipients received sirolimus as part of their immunosuppressive regimen. Sixteen patients received sirolimus immediately after liver transplantation (either alone or in combination with tacrolimus) and 25 patients went on sirolimus either discontinuing or reducing CI doses at least one month or more post-OLT. Trough sirolimus levels of 5-10 ng/mL were maintained. Renal function was assessed by serum creatinine levels measured before starting sirolimus and at one month or more after starting sirolimus. **Results:** The median age at OLT was 57 years (range 13-71, 64% males). The most common etiologies of liver disease were alcohol (24%) and alcohol and hepatitis C (20%). The median follow up after starting sirolimus was 102 days (range 38-307). Common side effects were hyperlipidemia 25% (9), infections 22% (8), anemia 16%(6), leucopenia 11% (4), mouth ulcers 8% (3), obesity 5% (2), thrombocytopenia 5% (2). Hepatic artery thrombosis occurred in 3 patients all within <30 days of OLT. Wound dehiscence occurred in 4 patients who were on SRL < 30 days after OLT. Among the 25 patients who switched to sirolimus mean creatinine declined from 1.91 to 1.58 mg/dL (p=0.02). For patients in whom CIs were discontinued, mean creatinine declined from 2.03 to 1.48 mg/dL (p=0.015) and for patients who had reduced CI dose, creatinine decreased from 1.78 to 1.68 mg/dL (p=NS). **Conclusion:** Following switching to sirolimus and reduction or discontinuation of CIs there were significant reductions in serum creatinine levels. The most common side effects were hyperlipidemia and infections.

Abstract# 759 Poster Board #-Session: P215-II

MYCOPHENOLATE MOFETIL IN LIVER TRANSPLANT PATIENTS WITH CALCINEURIN INHIBITOR WITHDRAWL. Maria Lucia Zanotelli,[1] Ana Luiza Gleisner,[1] Alfeu Fleck, Jr.,[1] Eduardo Schlindwein,[1] Alvaro Cassal,[1] Ian Leipnitz,[1] Tomaz De Jesus Grezzana,[1] Mario Henrique Meine,[1] Lisia Hope,[1] Ajacio de Mello Brandão,[1] Claudio Augusto Marroni,[1] Guido Pio Cracco Cantisani.[1] *[1]Liver Transplantation, Santa Casa de Porto Alegre - Hospital Dom Vicente Scherer, Porto Alegre, RS, Brazil.*

Purpose: Analyse the effect of Mycophenolate Mofetil (MMF) as routine immunosuppression after liver transplantation (LTx) and the safety as calcineurin-inhibitor (CI) replacement.

Methods: This prospective randomized trial included 71 adult patients transplanted in our center from March 2002 to June 2003. Patients were allocated immediately after LTx in two groups according immunosuppression protocol: Tacrolimus (Tac) + Steroids (St) (controls) and Tac + St + MMF (cases) in a 1:3 proportion. Both groups had similar clinicals characteristics and HCV cirrhosis was the main LTx indication. At the sixth month post-operative all patients were submitted to liver biopsy. If no rejection was found, patients in the MMF group were randomized in three branches: Tac + St + MMF; Tac + MMF and St + MMF. One year follow-up is reported.

Results: Two patients died and other was lost follow-up before second randomization at six months. Two additional patients were excluded due abnormal liver tests and MMF intolerance. Renal dysfunction was less frequent and glomerular filtration rate were higher at six months among patients that received MMF although the differences were not significant. The incidence of rejection was similar.There was no statistical significance increase in infection rate either. CI withdrawl was attempted in 13 patients. In three of them with persistent increase in liver enzymes Tac was partially wean (50% the initial dose) however liver biopsy was rejection free but showed steato-hepatitis in one and HCV recurrence in other. In 7 patients CI were discontinued and all of them had alteration in liver function tests (5 with biopsy proven acute rejection). All but one respond to CI re-introduction. The patient that did not improve had moderate rejection with progressive cholestasis associated with infection and graft failure causing death. Due to this case we ended the study before including 4 cases in the second randomization and did not wean Tac in 3 others patients previously random.

Conclusions: MMF in addition to Tac and St as early routine immunosuppression was well tolerated after LTx without increasing side-effects although the acute rejection rate was similar.The MMF efficacy in renal function and HCV recurrence need further consideration. In this study CI withdrawl couldn't be safely attempted in the first year after LTx.

Abstract# 760 Poster Board #-Session: P216-II

POPULATION PHARMACOKINETIC STUDY OF TACROLIMUS (FK506) IN ADULT LIVER TRANSPLANT RECIPIENTS. Hamim Zahir,[1] Ameeta Nelson,[2] Margaret Gleeson,[3] Geoffry McCaughan,[3] Andrew J. McLachlan,[2] Fatemeh Akhlaghi.[1] *[1]Applied Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI; [2]Faculty of Pharmacy, University of Sydney, Sydney, NSW, Australia; [3]AW Morrow Liver and Gastroenterology Centre, Royal Prince Alfred Hospital, Sydney, NSW, Australia.*

Purpose: We have investigated post transplant changes in the apparent clearance (CL/F) of tacrolimus in 67 consecutive adult liver transplant patients using a population pharmacokinetic approach.

Methods: A total of 694 tacrolimus trough blood concentrations (C_{min}) and corresponding hematocrit, ALT, AST, GGT and bilirubin levels were retrospectively collected. Blood samples were analyzed for tacrolimus using MEIA II (Abbott IMx Analyzer). In this cohort, 45 patients were male with an age range of 17 to 68 yr and mean (SD) weight of 77.8 (17.4) kg. Tacrolimus dose and concentration data were modeled using a nonlinear mixed effect modelling technique implemented in the NONMEM population pharmacokinetics program. As only tacrolimus trough concentrations were available, a one-compartmental oral pharmacokinetic model with first order absorption and elimination and an absorption rate constant of 4.5 hr^{-1} was used to describe the drug disposition.

Results: Average tacrolimus C_{min} was 11.1 ng/mL (range: 1.5 to 29.5 ng/mL) and tacrolimus dose was 7.6 mg/day (range: 1 to 24 mg/day). No correlation was observed between the dose of tacrolimus and C_{min} values (R^2=0.014). Using the base model, average tacrolimus CL/F was estimated to be 19.0 L/h (95% CI: 14.6 - 23.4 L/h) with an inter-subject variability of 50.8%. Apparent volume of distribution was found to be 302 L (95% CI: 169 - 435 L). Patient age, weight and gender did not have any influence on tacrolimus CL/F. Tacrolimus CL/F was 21% higher during the first post transplant month. Patients with moderate anemia (hematocrit less than 35%) also had a 24.2% higher tacrolimus CL/F. Coadministration of fluconazole decreased tacrolimus CL/F by 13% and explained 10% of the inter-patient variability in CL/F. There was no significant impact of ALT, AST, GGT and bilirubin on tacrolimus CL/F, when these indices were modeled as continuous variables. However, 7 – 10% decrease in CL/F was observed in patients with elevated (>55 U/L) ALT and AST levels.

Conclusion: Tacrolimus CL/F was found to be dependent on time post transplant, hematocrit, concurrent administration of fluconazole, ALT and AST levels. The influence of hematocrit on CL/F has not been described before and warrant further investigations.

Abstract# 761 Poster Board #-Session: P217-II

COMBINATION OF CYTOCHROME P-450 3A4, 3A5, AND P-GLYCOPROTEIN AS PHARMACOGENOMIC PREDICTORS OF TACROLIMUS PHARMACOKINETICS AND CLINICAL OUTCOMES IN LIVER TRANSPLANT RECIPIENTS. Christine M. Formea,[1] Tuan Luu,[1] Hossein Yarandi,[1] Taimour Langaee,[1] Valerie Greene,[2] Shiro Fujita,[2] Willem van der Werf,[2] Alan Hemming,[2] Richard Howard,[2] Alan Reed,[2] Janet L. Karlix.[1] *[1]College of Pharmacy, University of Florida, Gainesville, FL; [2]College of Medicine, University of Florida, Gainesville, FL.*

Tacrolimus is a macrolide immunosuppressant used to prevent allograft rejection in organ transplantation. CYP3A4, CYP3A5, and P-glycoprotein (P-gp) may play significant roles in drug disposition of xenobiotics including tacrolimus. Genetic variants in CYP3A4 (A-290G), CYP3A5 (A22893G), and P-gp (C3435T) may result in altered *in vivo* catalytic activity. This study explored the pharmacogenetic impact of CYP3A4, CYP3A5, and P-gp variability on tacrolimus dosing and clinical outcome in liver transplant recipients.

In 48 patients, clinical and biochemical data were collected for the first 10 days post-transplant and at Month 4. Genomic DNA was isolated from human liver tissue with genotypic determination done via PCR-based detection methods. T-tests, MANOVA, chi square tests, and mixed models with various covariant structures were used to compare genotype groups and categorical or continuous data. P<0.05 was considered significant.

Significantly, CYP3A4 AA genotype had higher mean tacrolimus dose requirements during the initial 10 days post transplant than AG/GG (0.052±0.02 vs 0.036±0.03 mg/kg/day, p=0.027). Individuals with CYP3A5*3/*3 required higher tacrolimus doses than CYP3A5*1 (0.053±0.02 vs 0.035±0.02 mg/kg/d, p=0.0165). Renal clearance significantly declined from transplant to Month 4 in all groups (p<0.0001). Rejection rates significantly decreased from transplant to Month 4 in all groups (p<0.0001).

Thus, genetic variants of CYP3A4 and CYP3A5 may be useful predictors of tacrolimus requirements in liver transplant patients and may contribute to the construction of clinically relevant pharmacogenetic models in transplantation.

Abstract# 762 Poster Board #-Session: P218-II

PHARMACOKINETICS OF MYCOPHENOLIC ACID AND CLINICAL OUTCOMES IN LIVER TRANSPLANT RECIPIENTS WITH HEPATITIS C (HCV). Theodore M. Sievers,[1] Curtis D. Holt,[1] R. Mark Ghobrial,[1] Lucy Artinian,[1] Sue V. McDiarmid,[1] Ronald W. Busuttil.[1] *[1]Surgery, UCLA Med CTR, Los Angeles, CA.*

BACKGROUND: Pharmacokinetic (PK) and pharmacodynamic data on mycophenolic acid (MPA) are lacking in adult orthotopic liver transplant (OLT) recipients.

OBJECTIVE: To evaluate the pharmacokinetics of MPA in OLT HCV recipients receiving mycophenolate mofetil (MMF) as part of an IRB approved randomized, open-label study and determine if these parameters correlate with their clinical outcomes.

METHODS: Upon administration of a steady-state morning MMF dose (500 or 1000mg orally twice daily), blood samples were collected at 0, 0.5,1, 2, 4, 6, and 12 hours after dosing on post-operative days (POD) 7 and 90. Total MPA concentrations were measured by a validated HPLC method with ultraviolet detection. PK parameters (dose-normalized C_{max}, C_{min}, and AUC) were calculated by traditional noncompartmental methods. Acute rejection (ACR) and HCV recurrence were biopsy proven.

RESULTS: PK data on 5 male and 3 female OLT recipients, age 46.8±11.9 years receiving concurrent prednisone and tacrolimus (FK) were included in this interim analysis. Comparison of PK parameters obtained at POD 7 and 90 demonstrated no significant differences. POD 7 and 90 MPA AUCs were 20.42±9.96 (range 8.88-34.79) and 21.16±8.14mg*h/L (range 7.91-31.65), respectively, (p=NS) and did not correlate with patient gender or C_{min} values. One subject experienced ACR on POD 40 (MPA AUCs 8.88 and 7.91mg*h/L) with concurrent FK levels ≥8ng/mL and an ongoing biliary leak. Five subjects were noted to have mild HCV recurrence based on POD 90 biopsies (HAI ≥ 3); MPA AUC values did not differ significantly in subjects with or without recurrence (18.81±7.95 vs. 19.24±6.13mg*h/L, respectively). Of note, in the patient with the highest AUC values, the HCV viral load on POD 90 was reduced 56% compared to baseline.

CONCLUSIONS: Preliminary data show wide inter- and intrapatient variability in MPA PK parameters. Mean, dose normalized AUC values were found to be lower in OLT HCV recipients than the recommended range of 30-60mg*h/L in other transplant populations. Low MPA AUCs were associated with ACR, perhaps suggesting the need to individualize dosing of MMF in OLT recipients, especially in those with externally diverted biliary drainage. MPA AUCs did not correlate with HCV recurrence, but high MPA AUCs could impact HCV viral load following transplantation. Further studies with larger sample sizes are needed to help identify optimal MMF dosing strategies in this patient population.

Abstract# 763 Poster Board #-Session: P219-II

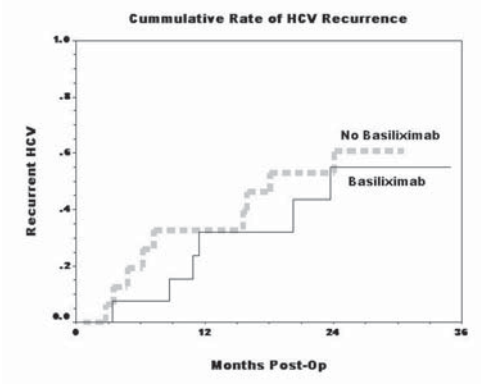
PHARMACOKINETICS OF NEORAL® IN PATIENTS RECEIVING MARGINAL LIVER GRAFT. Francesco Lupo,[1] Chiara Stratta,[1] Guido Liddo,[1] Gian Paolo Zara,[2] Roberto Passera,[1] Federico Casale,[2] Donatella Moscato,[1] Roberto Mosso,[1] Alessandro Ricchiuti,[1] Andrea Brunati,[1] Stefano Skurzak,[1] Stefano Mirabella,[1] Elisabetta Cerutti,[1] Mario Eandi,[2] Mauro Salizzoni.[1] *[1]Centro Trapianto Fegato, Molinette Hospital, Turin, Italy; [2]Department of Farmacology, University of Turin, Turin, Italy.*

Introduction. Cyclosporine (CyA) C_2 monitoring improved the management of post-transplant immunosuppression. Nevertheless, there is a lack of pharmacokinetics (PK) in patients receiving marginal livers (age>65 yrs and/or histological macrosteatosis>15%). **Methods**. We prospectively compared 24 liver transplanted patients: 10 pts received marginal graft (M group) while 14 received standard graft (control, C group). All patients received Neoral® as primary immunosuppression, with tapered steroids and azathyoprine. On post-operative day 3 (d3) and 10 (d10), 12 blood samples were collected during 12 h post drug administration (respectively at 0, 15,30,45,60,75,90,120,240,360,480 and 720 minutes). Rejection episodes (follow-up 3 months), graft and renal function data were registered. CyA concentrations were measured by fluorescence polarisation immunoassay and non-compartmental PK analysis was applied. **Results**. Considering samples taken between 60 and 180 min, we found increased CyA concentrations from d3 to d10 (99-204% in C group and 32-106% in M group). In such interval, C_{max} raised in C group from 537 to 1016 ng/ml ($p<0.001$) and AUC_{0-12} from 3779 to 5307 ng*h/ml ($p<0.05$), while in M group C_{max} raised from 524 to 749 ng/ml ($p<0.05$) and AUC_{0-12} from 3836 to 4049 ng*h/ml (p=ns). T_{max} progressively shortened from d3 to d10 in both groups (from 3.5 to 2 h in M group and from 3 to 2 h in C group). According to literature data, on d3 C2 provided best AUC_{0-4} estimate in C group ($r^2=0.95$), while C3 was best for M group ($r^2=0.88$); at d10 C2 is a good surrogate marker of AUC_{0-4} ($r^2=0.89$ and 0.88, for C and M groups respectively). No correlation was found between clinical data and PK parameters. No difference was noted between groups about graft and renal function, probably due to small subjects number. 43% of C group patients had graft rejection, compared to 20% of M group patients; 11 episodes occurred, 9 in C and 2 in M group. **Conclusions**. Patients receiving marginal graft showed different PK behaviour compared to control patients. As previously reported, largest PK variations occured during absorption phase: compared to C group, absorption in M group is poor and delayed at d10. All these informations could have a clinical impact for immunosuppressive management in the early post-transplant period.

Abstract# 764 Poster Board #-Session: P220-II

BASILIXIMAB INDUCTION ALLOWS THE DELAY OF TACROLIMUS FOLLOWING LIVER TRANSPLANTATION IN PATIENTS WITH RENAL INSUFFICIENCY. Benjamin Philosophe,[1] Sharon L. Wilson.[2] *[1]Surgery, University of Maryland, Baltimore, MD; [2]Pharmacy Services, University of Maryland, Baltimore, MD.*

Introduction. In renal transplantation basiliximab is a safe and effective induction agent allowing the delay of tacrolimus in the presence of delayed graft function. Delaying a calcineurin inhibitor in this context following liver transplantation has not been reported. We describe the use of basiliximab induction and tacrolimus delay in liver transplant recipients with renal insufficiency. **Methods**. Basiliximab was administered to 42 patients with renal insufficiency prior to their transplants. These were compared to 82 that received no induction and immediate tacrolimus. In the basiliximab group, tacrolimus was delayed for up to 14 days. Maintenance immunosuppression was based primarily on tacrolimus and prednisone. **Resuslts.** The mean serum creatinine on POD 0 was higher in the basiliximab group, 1.7 vs. 1.1 mg/dl (p=0.02). The mean number of days to achieve therapeutic tacrolimus levels was 6.2 in the basiliximab group, compared to 1.8 in the control group (p=0.002). Overall 3 year patient and graft survival was 75% and 71% respectively, similar for both groups. 56% of the patients had hepatitis C (HCV), all genotype 1a or 1b, similar for both groups. The cummulative recurrence of HCV was similar for both groups (figure 1). 30 month biopsy-proven HCV recurrence was 55% in the basiliximab group compared to 61% in the control group (p=0.613). the mean grade and stage of recurrent HCV was 2.0 and 0.83 respectively for the basiliximab group compared to 2.14 and 1.00 for the control group (p=0.858 and 0.719 respectively). There was no difference in viral load between the groups. 30 month cummulative rejection was lower in the basiliximab group, 5% vs. 11% in the control group (p=0.467). **Conclusion.** Basiliximab induction in liver transplantation allows the safe delay of tacrolimus in patients with renal insufficiency. This is associated with a low rejection rate and there is no increase in HCV recurrence at 3 years.



Abstract# 765 Poster Board #-Session: P221-II

IMPROVED RENAL FUNCTION IN ADULT LIVING-DONOR LIVER TRANSPLANTATION (LDLT) RECIPIENTS USING POLYCLONAL ANTILYMPHOCYTE INDUCTION THERAPY. M. S. Cattral,[1] I. McGilvray,[1] L. Adcock,[1] L. Lilly,[1] N. Girgrah,[1] G. A. Levy,[1] M. Walsh,[1] R. Kim,[1] S. Sakamoto,[1] C. Moulton,[1] P. D. Greig,[1] D. R. Grant.[1] *[1]Mutliorgan Transplant Program, University of Health Network, Toronto, ON, Canada.*

Chronic renal failure has emerged as a significant cause of morbidity and mortality after liver transplantation. Although this has been attributed mostly to long-term exposure to nephrotoxic immunosuppressive therapy, recent evidence indicates that the development of acute renal failure in the immediate post-operative period is a major risk factor. Thymoglobulin (Thymo) induction with delayed introduction of maintenance calcinerin inhibitor (CNI) therapy may reduce the risk of early renal failure and improve long-term renal function. To further define this strategy in adult right lobe LDLT we compared renal function of 32 patients (group 1) that received Thymo with 29 patients (group 2) that received no polyclonal antibody induction therapy. **Methods.** Thymo was initiated within the first 6 hrs after LDLT and continued for 7-10 days; after the initial dose of 1.25 mg/ kg body weight, daily dosage was adjusted to maintain absolute lymphocyte counts of <0.2. All patients received a tapering dosage of methylprednisolone and tacrolimus (Tac; group 1, 92%; group 2, 80%) or Neoral cyclosporine (Cya; group 1, 8%; group 2, 20%; p=NS). Five patients in group 2 received 2 doses of basiliximab. In group 1, initiation of Cya/Tac was routinely delayed to the 3rd -5th post-op day. Target levels for Tac/Cya after the first week were identical in both groups. Pre- and post-operative serum creatinine was measured, and the % increase over preoperative values was calculated. **Results.** Demographic characteristics of the two groups were similar with respect to age, sex, etiology of liver disease, medical status at transplantation, and preoperative serum creatinine values. Temporary dialysis was required in 1 patient in group 1 and two patients in group 2. Table 1 summarizes the results.

Conclusion. Following LDLT, induction therapy with polyclonal antilymphocyte induction and delayed introduction of CNI appears to reduce the severity of postoperative renal dysfunction. This may be particularly valuable in patients with evidence of preoperative renal dysnfunction.

Table 1.

Characteristic	14 days after LDLT Group 1 (Thymo)	14 days after LDLT Group 2 (No Thymo)	p	3 mo after LDLT Group 1 (Thymo)	3 mo after LDLT Group 2 (No Thymo)	p
Proportion of patients with elevated Cr above pre-LDLT value	45%	59%	ns	53%	77%	ns
Median delta increase of Cr above pre-LDLT value	14%	40%	0.04	26%	46%	0.02
Tacrolimus level (mean± S.D; ng/ml)	14±5	11±6	ns	11±3	12±2	ns

Cr, creatinine.

Abstract# 766 Poster Board #-Session: P222-II

THYMOGLOBULIN INDUCTION THERAPY ALLOWS DELAY OF CALCINEURIN INHIBITORS IN LIVER TRANSPLANT RECIPIENTS WITH RENAL INSUFFICIENCY. Iman E. Bajjoka,[1] Rozelle H. Dingle,[1] Atsi Yoshida,[2] Kim Dean,[2] John Jerius,[2] Kate O'Dell,[3] Marwan S. Abouljoud.[2] *[1]Transplant Surgery and Pharmacy, Henry Ford Hospital, Detroit, MI; [2]Transplant Surgery, Henry Ford Hospital, Detroit, MI; [3]Pharmacy, Hery Ford Hospital, Detroit, MI.*

Calcineurin inhibitors (CIs) are known to cause nephrotoxicity. Immediately after transplant, delaying their use may be beneficial in liver transplant patients with renal insufficiency. Data is lacking on the use of thymoglobulin (thymo) as induction therapy in this selected population. The purpose of this study is to evaluate the use of thymo induction regimen in liver transplant patients with renal insufficency.

Methods: A retrospective analysis was conducted of all our liver transplant recipients transplanted after July 2001. Renal insufficiency was defined as baseline serum creatinine (SCr) ≥ 1.5 mg/dL. Induction treatment included thymo, mycophenolate mofetil (MMF) and steroids (ST). CIs were initiated one day prior to thymo discontinuation and once SCr stabilized. Biopsy-proven acute rejection in the first 3 months following transplantation was evaluated using the Banff criteria.

Results: One hundred forty-four patients were evaluated. Thirty-seven patients (25.6%) were identified as renal insufficient and received thymo. Patient demographics include a mean age of 53.2±9.4 yrs with 27 Caucasians, 8 African Americans, and 2 others, pretransplant MELD score 22±7.8, baseline SCr 2.4±1.6 mg/dL, and baseline creatinine clearance 33.5±18.4 mL/min. Patients received a mean thymo cumulative dose of 2.7±1.4 mg/kg over 4.9±2.8 days period. Baseline (pre thymo) and end of thymo treatment parameters are summarized below:

	Results		
	Baseline (mean±SD)	End of Treatment (mean±SD)	P-value
SCr(mg/dL)	2.4±1.6	1.4±1	<0.001
White blood cells (K/uL)	7.8±3.4	6.9±3.8	0.675
Platelets(K/uL)	94.4±43.9	64.7±50.1	<0.001
AbsoluteCD3(Cell/uL)	436.6±234.1	14.2±15.1	<0.001

Seven patients (19%) required a decrease in thymo dosage due to decrease in platelets and/or white blood cells. At 3 months, 9/37 patients (24.3%) developed biopsy-proven acute rejection (1 severe, 1 moderate and 7 mild); 12 patients developed infections from various sites (4 blood, 2 catheter, 3 sputum and 3 urine). Only 1 patient died of sepsis 35 days post transplantation.

Conclusion: We conclude that in liver transplant recipients with marginal renal function, induction therapy with thymo/MMF/ST allows the delay use of CIs and significant improvement in renal function without higher risk of developing acute rejection or other adverse effects

Abstract# 767 Poster Board #-Session: P223-II

INTERLEUKIN-2 RECEPTOR ALPHA SUB-UNIT ANTIBODY (IL-2Rα-Ab) THERAPY ALLOWS DELAYED INTRODUCTION OF CALCINEURIN-INHIBITOR (CI) BASED IMMUNOSUPPRESSION IN LIVER TRANSPLANT PATIENTS WITH PERIOPERATIVE RENAL IMPAIRMENT (RD). Barry Rosser,[1] Martin Mai,[1] David Kramer,[1] Hani Grewal,[1] Raj Satyanarayana,[1] Andrew Keaveny,[1] Rolland Dickson,[1] Denise Harnois,[1] Winston Hewitt,[1] Justin Nguyen,[1] Christopher Hughes,[1] Thomas Gonwa,[1] Jeffrey Steers.[1] *[1]Department of Transplantation, Mayo Clinic, Jacksonville, FL.*

Utilization of IL-2Rα-Ab therapy in renal transplantation as a CI sparing strategy is well established but data in liver transplant (LT) patients with renal dysfunction (RD) is limited. We evaluated our experience using IL-2Rα-Ab therapy in patients undergoing LT with perioperative RD. **Results:** From Sept 1, 2002 to Oct 30, 2003, 32 patients (21M;11F) undergoing 36 liver or liver/other organ transplants with perioperative RD defined as need for renal replacement therapy (RRT) or Cr >1.5 mg/dl with oliguria were treated with prednisone, MMF, and basiliximab (Simulect® Novartis), at an initial dose of 20 to 40 mg followed by repeat dose 4 and 14 days later if required. CI was introduced if RRT was discontinued, creatinine decreased to < 2 mg/dl without oliguria or acute cellular rejection (ACR) requiring therapy occurred. Mean patient age was 58 yrs (range 43 to 71). Mean pre-transplant MELD score was 23 (8 to 40). 3 grafts lost within 72 hours due to primary non-function (2) and early patient death (1) from multiorgan system failure (MOSF) were excluded from the analysis. Patients received a mean of 1.9 (1-3) infusions with mean basiliximab dose of 40 mg (20-60). Duration of RRT, initial ICU and hospital stays were 5.5 (0-114), 4.6 (0-23) and 25.6 (4-86) days respectively. Time to therapeutic immunosuppression averaged 12.5 days (4-27). Mean day 21 and 120 creatinines were 1.6 (0.6-2.5) and 1.5 (1-1.9) mg/dl. 5 patients developed ACR responsive to a single solumedrol infusion. 11 patients required sirolimus but only 5 patients remained on sirolimus as primary immunospuppression at follow up. Major infections due to CMV (19%), bacteria (42%) or fungus (6%) infections were common. 2 graft losses due to arterial complications required retransplant. 2 patients died of MOSF beyond 21 days post LT and 1 patient died after being lost to follow up. 3 patients had residual RD defined as need for RRT or creatinine >2 mg/dl at mean F/U of 211days (range 42 to 431). **Summary:** IL-2Rα-Ab therapy allows delayed introduction of CI with a low incidence of ACR allowing for renal function recovery in LT patients with perioperative RD. Frequent infectious complications need to be further analyzed but may be due to the critically ill status of these patients.

Abstract# 768 Poster Board #-Session: P224-II

THYMOGLOBULIN INDUCTION IN LIVER TRANSPLANTATION AND RECURRENCE OF HEPATITIS C. Antonio D. Pinna,[1] Nicola De Ruvo,[1] Alessandro Cucchetti,[1] Augusto Lauro.[1] *[1]Department of Surgery, Center for Liver and Multiorgan transplantation, Bologna, Italy.*

Introduction: Induction immunosuppression with Thymoglobulin, a potent anti-thymocyte policlonal antibody, might allow a tolerogenic regimen of recipient pretreatment and low-dose immunosuppression. The effect of this novel approach on HCV recurrence after liver transplantation has never been investigated. This analysis aimed to discover a relationship between Thymoglobulin induction and pattern of HCV recurrence after liver transplantation. **Methods:** we used Thymoglobulin induction + Tacrolimus monotherapy in a group of 22 HCV+ patients receiving liver transplantation (Thymo group); 32 historical HCV+ patients with different Tacrolimus based immunosuppression represented the comparison group (non-Thymo group). **Results:** Patients survival is equal in both groups, with 1-year survival rate of 82% (Thymo group) versus 86% (non-Thymo group) (p=ns). Five patients (22,7%) in Thymo group experienced mild acute rejection versus 10 patients (31,3%) in non-Thymo group; among all Thymo patients, rejection grade was generally mild, requiring steroid recycle in 3 patients (13,6%), whereas 60% of patients in the non-Thymo group experienced moderate or severe acute rejection, requiring steroid recycle in all cases and OKT3 administration in one. Clinically HCV recurrence rate was equal in both groups (57% vs 55% of patients), although pattern of recurrence was distinct. With respect of mean ALT and AST elevation, patients in Thymo group reached higher levels than in the non-Thymo group (respectively mean ALT level: Thymo 326± 65 UI/mL, non-Thymo 94± 78 UI/mL, p=0,001; mean AST level: Thymo 202± 71 UI/mL, non-Thymo 152± 81 UI/mL, p=0,03). Patients in Thymo group expressed earlier increase of HCV RNA load in a median 96 days (range 21-284 days) when compared with non-Thymo group (187 days; range 13-838; p=0,044); interestingly the mean peak RNA load was lower in Thymo patients than in the non-Thymo patients (1494 U/L vs 3516 U/L; p=0,017). Histological recurrence of HCV was also earlier in Thymo patients with a median disease-free survival of 113 days (range 25-204) when compared with the other group (327 days; range 31-752, p=0,001). However no significant difference was observed in mean Ishak's histological grading (4 in both groups) and staging (Thymo S=1, non-Thymo S=2) of HCV recurrence. **Conclusion:** Induction immunosuppression with Thymoglobulin in liver transplant recipients is effective in protecting against rejection whereas demonstrated a peculiar relationship with HCV recurrence that deserves further investigations.

Abstract# 769 Poster Board #-Session: P225-II

POOR PREDICTION OF THE GLOMERULAR FILTRATION RATE USING CURRENT FORMULAS IN THE NOVO LIVER TRANSPLANT PATIENTS. M. Cantarovich,[1] E. Yoshida,[2] P. Marotta,[3] P. Greig,[4] N. Kneteman,[5] D. Marleau,[6] K. Peltekian,[7] G. Facciponte,[8] B. Simpson,[8] R. Balshaw,[9] J. Barkun.[1] *[1]Royal Victoria Hospital, McGill University Health Centre, Montréal, QC; [2]British Columbia Transplant Society, Vancouver, BC; [3]London Health Sciences Centre, London, ON; [4]University Health Network, Toronto General Hospital, Toronto, ON; [5]University of Alberta Hospital, Edmonton, AB; [6]CHUM – Hôpital St-Luc, Montréal, QC; [7]Queen Elizabeth II Health Sciences Centre, Halifax, NS; [8]Hoffmann La-Roche Limited, Mississauga, ON; [9]Syreon Corporation, Vancouver, BC, Canada.*

Renal dysfunction is a well-known complication in liver transplant patients receiving calcineurin inhibitors. Serum creatinine (Scr) overestimates the glomerular filtration rate (GFR). Several formulas have been developed to estimate the GFR. However, their application in liver transplant patients is not well described. Purpose: To determine the correlation between the radionuclide GFR and serum creatinine (Scr), and different formulas accepted to estimate the GFR in other settings. Methods: This is a sub-study of a Canadian multicenter randomized study that evaluates the safety and efficacy of daclizumab induction plus mycophenolate mofetil, tapered corticosteroids and delayed and low-dose tacrolimus vs. a standard-dose tacrolimus-based regimen in adult liver transplant patients (n=148). Baseline (pre-transplant) and 3-months GFR was performed in 32 and 35 patients, respectively. The formulas used to estimate the GFR were the following: Cockroft-Gault, Levey (MDRD, modification of diet in renal disease), and 1/Scr. Results: Results (means±SD) are shown in the tables.

	GFR (ml/min)	Scr (µmol/L)	1/Scr	Cockroft-Gault (ml/min)	Levey (ml/min)
Baseline	100±38	79±28	0.01±0.004	103±51	82±33
3-months post-Tx	76±27	86±32	0.01±0.004	88±32	78±23

Correlation (r^2) between the GFR, Scr and accepted formulas to estimate the GFR

	Scr	1/Scr	Cockroft-Gault	Levey
Baseline	0.18	0.15	0.33	0.27
3-months post-Tx	0.17	0.25	0.24	0.36

Conclusion: Although Scr was the least appropriate measure to estimate GFR, the use of any "accepted formula" provided a poor correlation with the radionuclide GFR during the first 3-months post-liver transplant. A modification of these formulas is therefore required to better assess the GFR after liver transplantation.

Abstract# 770 Poster Board #-Session: P226-II

EFFECT OF CYCLOSPORINE ON HEPATOCELLULAR CARCINOMA RECURRENCE AFTER LIVER TRANSPLANTATION. Marco Vivarelli,[1] Alessandro Cucchetti,[1] Giuliano La Barba,[1] Antonino Cavallari,[1] Antonio D. Pinna.[1] *[1]Department of Transplantation, University of Bologna, Bologna, Italy.*

Introduction: It is demonstrated that pharmacological immunosuppression can accelerate tumor growth; however, the role of immunosuppression as risk factor of tumor recurrence after liver transplantation for hepatocellular carcinoma (HCC) has never been investigated. **Methods:** 70 patients with HCC transplanted between January 1991 and November 2002, who received cyclosporine (CsA) as main immunosuppressant, were retrospectively reviewed to ascertain whether tumor recurrence was influenced by tumor pathology or by the blood levels of the immunosuppressant drug administred. **Results:** Tumor recurrence occurred in 7 of 70 patients (10%): age, sex, etiology of underlying liver disease (viral versus non-viral), presentation of tumor (incidental vs non-incidental), Milan criteria (fulfilled vs unfulfilled) and histological grading (G1-G2 vs G3-G4) did not influence recurrence-free survival. Recurrence-free survival was significantly worse in patients with tumor microvascular invasion (89%, 80% and 75% at 1,3 and 5 years) than in patients without evidence of tumor microvascular invasion (100%, 100% and 96% at 1,3 and 5 years; p=0.009) and in those with pT2-T3 stage (93%, 88% and 21% at 1,3 and 5 years) than pT1 stage (100%, 100% and 100% at 1,3 and 5 years; p=0.05). The analysis of the means throught blood levels of CsA at 14 days, 1, 3, 6 months and 1 year after transplantation showed significantly higher blood levels in patients in which tumor recurrence occurred (p-value ranged from 0.013 at 14 days to 0.034 at 1 year at the Student t-test). Using the ROC-curve analysis on CsA blood level in relationship with tumor recurrence we obtained an optimal cut-off that correlates with significantly different recurrence-free survival (AUC ranged from 0.71 at 6 months to 0,82 at 14 days). Recurrence-free survival of those patients with CsA blood level equal or above to 380, 460, 265, 250 and 220 ng/mL at 14 days, 1 ,3 , 6 months and 1 year was significantly lower (p-value ranged from 0.001 at 1 month to 0.006 at 14 days). CsA blood levels at 1-month and 1-year were the only independent predictors of recurrence-free survival at Cox regression analysis among all the parameters considered. **Conclusion**: Immunosuppression can play an important role on HCC recurrence after liver transplantation.

Abstract# 771 Poster Board #-Session: P227-II

POSITIVE T-CELL FLOW CYTOMETRY CROSSMATCH IN THE LIVER TRANSPLANT POPULATION: IS IT CLINICALLY SIGNIFICANT? T. D. Merchen,[1] B. Susskind,[1] M. Gupta,[1] J. F. Buell,[1] E. S. Woodle,[1] S. M. Rudich.[1] *[1]Division of Transplantation, University of Cincinnati, Cincinnati, OH.*

Positive (pos) T-cell flow crossmatch (X-match) results in the liver transplant population have been associated with early acute rejection, biliary complications, and a lower allograft and patient survival. Although there is little evidence to support waiting for X-match results before liver transplantation, there is still the question of whether pos results have a clinical significance. **Purpose:** To evaluate if T-cell flow X-match pos OLT recipients demonstrate any greater degree of rejection, biliary or vascular complications, or lower allograft survival compared to X-match negative (neg) recipients. **Methods:** Demographic, morbidity and mortality data were reviewed for OLTs performed at our center over a one year period to assess the influence that pos flow X-match has on clinical outcomes. Biliary (stricture) and vascular (hepatic artery thrombosis and stenosis) complications were tabulated. **Results:** 80 patients were identified who underwent 81 liver transplants over the study period. Mean follow-up was 6.2 months. Fifteen (18.5%) pos flow X-matches and 66 (81.5%) neg flow X-matches were noted. The pos X-match group consisted of 4 men (26.7%) and 11 women (73.3%); the neg X-match group comprised 48 men (73.9%) and 17 women (26.1%). (p<0.005) There were no significant differences between the pos and neg X-match groups with respect to age, match and laboratory MELD, CTP, ABO blood type, or etiology of liver disease.

	Positive X-match	Negative X-match	p value
Acute rejection (%)	26.7	23.1	0.77
Biliary complications (%)	13.3	12.3	0.91
Vascular complications (%)	0	7.7	0.27
Allograft survival (%)	100	93.9	0.39

Conclusion: A higher number of women have positive X-matches compared to men. There was a similar rate of early rejection in both groups. We did not find any difference in biliary or vascular complications or in allograft survival between the positive and negative X-match groups. Therefore, it would appear that the clinical implications of a positive T-cell flow X-match in the early post-OLT period is minimal.

Abstract# 772 Poster Board #-Session: P228-II

LIVER TRANSPLANTATION RECIPIENTS WITHOUT HEPATITIS C RECEIVING SIROLIMUS AS PRIMARY IMMUNOSUPPRESSION HAVE MILD ELEVATIONS IN ALT. Steven R. Kaptik,[1] Julie C. Osborne,[1] James F. Trotter.[1] *[1]Division of Gastroenterology/Hepatology, University of Colorado Health Sciences Center, Denver, CO.*

Background: Sirolimus (SIR) is a new immunosuppressant agent in liver transplantation. We have previously demonstrated favorable outcomes in over 200 patients who received SIR as part of a primary immunosuppressive regimen for liver transplantation. We have noted that many of the patients in our cohort have mild persistant elevations in alanine aminotransferase (ALT). We report the frequency and severity of this abnormality and speculate on its clinical significance. **Methods:** All patients without hepatitis C who received SIR as part of their primary immunosuppressive regimen were included in this study. Patients were censored from analysis following discontinuation of SIR. The control group included all non-HCV liver transplantation recipients from our institution between 1997 and 1999 who did not receive SIR as primary immunosuppression. These patients are designated "noSIR." **Results:** While the mean AST levels were not significantly different between SIR and noSIR patients at any of the intervals, the mean ALT levels were significantly higher in SIR patients at months 12 and 15, as shown in Table 1. The percentage of SIR patients with abnormal ALT (> 47 IU/L) was significantly greater at month 12 and approached statistical significance at months 15 and 18, as shown in Table 2. Fewer than 5 % of SIR and noSIR patients had ALT > 94 IU/l (greater than two-fold normal) at any of the monthly intervals (data not shown), p = ns for each interval. SIR levels were (mos 3, 6, 9, 12, 15, 18, 21): 6.4, 6.0, 6.1, 4.7, 5.6, 5.7 and 5.8 ng/ml. Progressively elevated ALT values were not seen in any of the patients on SIR. **Conclusions:** 1) Liver transplantation recipients without hepatitis C who receive SIR as primary immunosuppression have mild elevations in ALT. 2) We believe that this observation represents a drug effect of SIR and not hepatotoxicity, since progressive ALT elevations were not seen. 3) Since ALT is one of the laboratories used to monitor for acute cellular rejection, physicians administering SIR to liver transplantation recipients should be aware of these findings.

Table 1 Mean AST and ALT in SIR and noSIR patients

month	3	6	9	12	15	18	21
SIR ALT	36	36	44	36	46	36	43
noSIR ALT	33	29	29	32	32	32	28
p	ns	.10	.11	.02	.04	ns	ns
SIR AST	30	26	27	29	34	30	30
noSIR AST	27	25	26	26	26	32	29
p	ns	ns	ns	ns	ns	ns	ns

Table 2 % patients with ALT > 47: SIR vs. noSIR

month	3	6	9	12	15	18	21
SIR	19 %	21 %	24 %	33 %	28 %	27 %	15 %
noSIR	22 %	14 %	14 %	12 %	10 %	12 %	12 %
p	ns	ns	ns	,02	.06	.06	ns

Abstract# 773 Poster Board #-Session: P229-II

PRELIMINARY RESULTS OF ANTI-IL-2 RECEPTOR ANTIBODY (BASILIXIMAB) IN ADULT LIVING DONOR LIVER TRANSPLANTATION. Chih-Che Lin, Feng-Rong Chuang, Chih-Chi Wang, Yaw-Sen Chen, Chih Hsiung Lee, Yueh-Wei Liu, Yu-Fan Cheng, Chao-Long Chen. *Liver Transplantation Program, Department of Surgey, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Kaohsiung, Taiwan.*

PURPOSE: Basiliximab, a high affinity chimeric monoclonal antibody, is effective in reducing acute rejection episode in liver transplantation. The study was to evaluate the efficacy and postoperative renal function in adult living donor liver transplantation with basiliximab induction to delay and decrease the dosage of tacrolimus.

METHODS: Between February 2001 and June 2003, 36 recipients of adult living donor liver transplantation under the immunosupressants of tacrolimus, steroid, with/without mycophenolate were retrospectively reviewed. The induction group (n=18) was administrated basiliximab(20mg) on day 0 and 4; then tacrolimus was delayed until urine amount recovered. The target trough level of tacolimus was 5-10 ng/ml. The control group (n=18) didn't receive basiliximab; whereas tacrolimus was given on first postoperative day.The target trough level of tacolimus was 10-15 ng/ml.

RESULTS: The preoperative conditions were poorer (Child C: 67% vs 33%, p=0.04; UNOS 2a: 22% vs 0%, p=0.02) in the induction group with more intraoperative blood loss (3365 vs 811 ml, p<0.01). The one -year actuarial patient and graft survival rates were 100%. The median delay of tacrolimus administration was 36(24-108) hours. The incidences of acute celluar rejection, bacteremia, and CMV infection were similar between two groups. The serum creatinine levels at postoperative first and forth weeks were significantly lower in the induction group (0.73 vs 0.95, 0.95 vs1.21 mg/dl, p<0.05).

CONCLUSIONS: Basiliximab contributed to the delay and dose reduction of tacrolimus without increasing rejection and infection. The renal function could improve within one month after transplantation, especially for the patients with critical pretransplant status.

LIVING DONOR KIDNEY TRANSPLANTATION

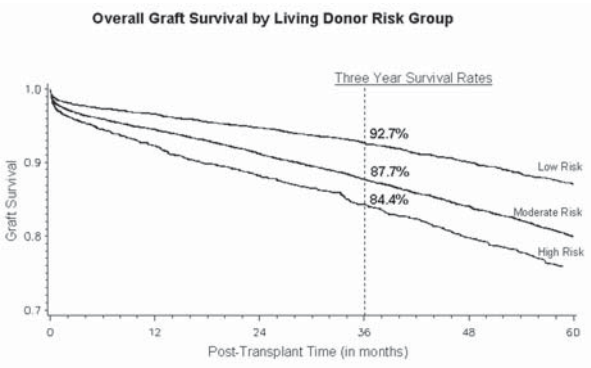
Abstract# 774 Poster Board #-Session: P230-II

ASSESSMENT OF THE QUALITY OF LIVING DONOR KIDNEYS. Jesse D. Schold, Shiro Fujita, Michael Bucci, Pamela R. Patton, Bruce Kaplan, Herwig-Ulf Meier-Kriesche. *University of Florida, Gainesville, FL.*

Living kidney donation (LD), as a therapeutic intervention for patients with end stage renal disease has markedly increased over the past decade. Despite the relative benefit of this donation type, well documented risk factors remain associated with characteristics of these organs. To assess the cumulative impact of these factors we performed an analysis to measure the relative risks associated with identified levels of quality of the living donated kidney.

We examined all first solitary LD transplants from 1995-2001 from the SRTR database in order to create a model for measuring the impact of the donated organ. We randomly assigned two-thirds of the records to a subset in order to construct a model, and tested the group risk designations on the remaining third representing the naive cohort. Parameter estimates of significant donor risk factors deriving from a Cox model for overall and death censored graft survival (including age, CMV status, relation to recipient, gender match, race, and HLA matching) were combined to generate risk scores. These scores were then assigned to three levels of risk via cluster analysis.

Univariate and multivariate models confirmed a significant distinction between the low, mid, and high level risk groups. Kaplan-Meier plots displayed a significant association (p<.0001) for overall graft survival between risk groups in the test data set.



The multivariate model, adjusted for recipient factors, found that with the low risk group level as reference, the mid group level incurred a 1.52 hazard, 95%CI (1.39, 1.66), and the high risk group a 1.80 hazard (1.57, 2.05).

The utility of such a rating system may be applicable in situations in which recipients and clinicians have the luxury of choosing among several living donor candidates, to help assess levels of therapeutic intervention, and for identifying the quality of organs in the context of living exchange programs.

Abstract# 775 Poster Board #-Session: P231-II

CONVALESCENCE AFTER LAPAROSCOPIC LIVE DONOR NEPHRECTOMY. S. Bergman,[1] L. S. Feldman,[1] F. Carli,[2] M. Anidjar,[1] P. P. Metrakos,[1] J. I. Tchervenkov,[1] S. Paraskevas,[1] D. Klassen,[1] M. C. Vassiliou,[1] C. G. Andrew,[1] D. D. Stanbridge,[1] G. M. Fried.[1] *[1]Surgery, McGill University, Montreal, QC, Canada; [2]Anesthesia, McGill University, Montreal, QC, Canada.*

Introduction: Laparoscopic live donor nephrectomy (LLDN) has become a favored technique in kidney transplantation. While recipient outcomes have been closely scrutinized, little is known about donor recovery. Our aim is to describe convalescence in LLDN using both objective and subjective measures.

Methods: This is a prospective study of consecutive patients undergoing LLDN at a single institution between September 2001 and September 2003. At baseline and four weeks postop, functional exercise capacity was measured using the six-minute walk test (6MWT). Health-related quality-of-life was assessed with the SF-36, using the physical component summary (PCS) and mental component summary (MCS) scores. Pain and fatigue were assessed on a 10-point verbal response scale. In addition, a structured interview focusing on recovery of usual activities was conducted. Data were analyzed using Student's t-test.

Results: 37 patients underwent LLDN and 33 participated in the study. Their median age was 41 years (IQR 35-51), 18 (55%) were female and 27 (82%) were employed. Postop assessment was done at 29 days (IQR 22-29). The number of days after which patients left the house, drove a car, and resumed work were 4.5 (IQR 3-7), 7 (IQR 5-10), and 34 days (IQR 13-43), respectively. At follow-up, median patient-stated recovery was 90% (IQR 75-94). The 6MWT distance and PCS scores were lower at follow-up, while pain scores were higher; MCS and fatigue scores were unchanged from baseline (Table). Postop patient-stated recovery correlated both with PCS (R=0.7, p<0.01) and the decline in 6MWT distance (R=0.6, p<0.01). When stratified into those who felt ?90% recovered (n=18) or <90% recovered (n=15), the former group had closer to baseline PCS scores (92 ±12% vs 66 ±18% of baseline, p<0.001), walked further (102 ±16% vs 86 ±14% of baseline distance, p<0.01), and had less pain (p=0.02).

Conclusion: Four weeks following LLDN, patients have not yet returned to baseline exercise capacity or general physical health. Patients' self-assessment of the extent of their recovery correlates with results obtained from other standardized measures of convalescence.

	Pre-op	Post-op	p
6MWT (meters)	549 (88)	513 (89)	<0.05
PCS	55.5 (5.3)	44.1 (9.8)	<0.01
MCS	53.3 (9.5)	54.7 (9.2)	0.33
Pain	0.2 (0.7)	1.5 (1.4)	<0.01
Fatigue	1.8 (1.9)	2.7 (2.3)	0.07

Data expressed as mean(SD)

Abstract# 776 Poster Board #-Session: P232-II

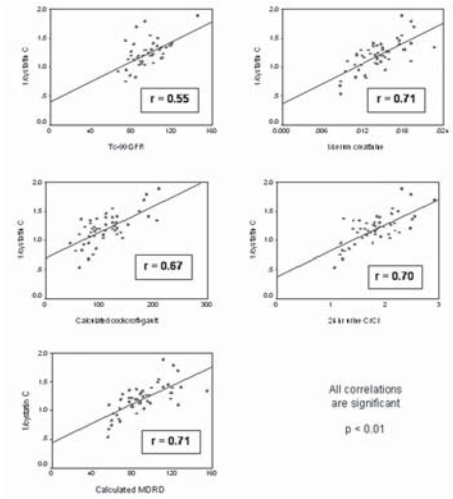
IMPLANT BIOPSIES AND DONOR OUTCOME FOLLOWING LIVING DONOR NEPHRECTOMY. Yook M. Woo,[1] William Gourlay,[2] Gerald Da Roza,[1] Alexander Magil,[3] Janet Holden,[3] Gary Nussbaumer,[4] John S. Gill,[1,5] David Landsberg.[1] *[1]Dept of Nephrology, University of British Columbia, Vancouver, BC, Canada; [2]Dept of Urology, University of BC, Vancouver, BC, Canada; [3]Dept of Pathology, University of BC, Vancouver, BC, Canada; [4]Renal Transplant, BC Transplant Society, Vancouver, BC, Canada; [5]Dept of Nephrology, Tufts New England Medical Center.*

Implant biopsies are routinely performed in most transplant centres at time of live donor renal transplantation. There is currently little information regarding the role of this investigation in assessing subsequent clinical outcomes of donors. The purpose of our study is to determine the utility of implant biopsy in predicting donor clinical outcome. Charts of all living donors between September 1997 to April 2003 were reviewed to obtain pre- and post-nephrectomy clinical information and laboratory data. GFR was estimated using the MDRD formula. All implant biopsies were reported by two pathologists. Reports were scored as normal or abnormal according to the presence of glomerulosclerosis beyond normal expectation for age, tubular atrophy or fibrosis and by severity of vascular changes. Post-donation BP and laboratory data were excluded from analysis if taken less than 30 days and 60 days following nephrectomy respectively. Over 68 months, 205 live donor nephrectomies were performed at this centre. Mean donor age was 42 (19-70) with sex ratio 41:59 (male:female). 33.7% donors were lost to direct follow-up. 77.1% had follow-up laboratory work. Data was available in 106 cases following time-restriction exclusion. No follow up beyond 1 year post nephrectomy was available. Median Cr pre-and post-nephrectomy were 74 µmol/L (46-112) and 102 µmol/L (65-159) respectively. Median delta GFR was -0.47 ml/s (-1.21-0.02). New onset proteinuria (>0.2 g/day) was present in 8 donors and new onset hypertension (>140 systolic or >90 diastolic) identified in 7 donors. Of the 205 transplants, 12 implant biopsies were insufficient for diagnosis. The remaining biopsies were classified as normal in 127 (65.8%) and abnormal in 66 cases (34.2%). There was no difference in delta GFR, change in BP or post-nephrectomy proteinuria between these groups. Conclusion: Implant biopsies at the time of donor nephrectomy revealed pathological abnormalities in 34% of cases where there was adequate tissue for analysis. Although there were no differences in short term outcomes between patients with and without abnormal implant biopsies in this study, long term follow information is needed to fully assess the clinical utility of the implant biopsy in living donor follow-up. More rigorous pathological analysis of implant biopsies (eg quantification of interstitial fibrosis) may yield additional useful information and is currently being pursued.

Abstract# 777 Poster Board #-Session: P233-II

SERUM CYSTATIN C DOES NOT PROVIDE AN ADVANTAGE OVER CONVENTIONAL TESTING IN EVALUATION OF POTENTIAL LIVING KIDNEY DONORS. Sita Gourishankar,[1] Gian S. Jhangri,[1] Philip F. Halloran.[1] *[1]Medicine, University of Alberta, Edmonton, AB, Canada; [2]Public Health Sciences, University of Alberta, Edmonton, AB, Canada.*

It is essential that candidates for living kidney donation are carefully screened to ensure safety of donation. It is particularly important to rule out any evidence of impairment in renal function and extensive and often expensive testing (ie. nuclear medicine GFR determination) occurs to accomplish this task. In the general population, serum cystatin C has been shown to be more sensitive than serum creatinine (SCr) in detecting subtle impairment in GFR earlier (88 ml/min versus 75 ml/min; $p < 0.001$) (AJKD 2000; 36: 29). The latter property could potentially be effective in evaluating potential living kidney donors for subtle changes in renal function but has not been previously evaluated. We examined 43 potential donors and 7 recent donors (within the past year) to determine the correlation between serum cystatin C and the following tests: (1) Tc-99 radioisotope nuclear medicine GFR (Tc-99); (2) SCr; (3) 24 hour urine creatinine clearance (CrCl); (4) calculated Cockcroft-Gault CrCl (CG); and (5) calculated MDRD CrCl (MDRD). **Results:** The mean age of the subjects was 44. 2 years (range 24-76) and 76% were female. The average values for the specific tests were: SCr 73.7 umol/L; Tc-99 96.8 ml/min; 24 hour urine CrCl 109.2 ml/min; CG 111.3 ml/min; MDRD 91 ml/min. Figure 1 describes the correlation of serum cystatin C with the other renal function tests. **Conclusions:** Serum cystatin C was highly correlated with SCr, 24 hour urine CrCl, calculated Cockcroft-Gault CrCl and MDRD CrCl (r=0.67-0.71;p<0.01). However, correlation with the predefined gold standard, Tc-99 radioisotope nuclear medicine GFR was lower (r=0.55; p<0.01). Thus serum cystatin C does not afford an advantage over the conventional tests presently used in living kidney donor evaluation and consideration of a new gold standard may be warranted.



Abstract# 778 Poster Board #-Session: P234-II

FATAL AND NON-FATAL HEMORRHAGIC COMPLICATIONS OF LIVING KIDNEY DONATION. Amy L. Friedman,[1] Lloyd E. Ratner,[2] Thomas G. Peters.[3] *[1]Surgery, Yale University School of Medicine, New Haven, CT; [2]Surgery, Thomas Jefferson University, Philadelphia, PA; [3]Surgery, Jacksonville Transplant Center at Shands Jacksonville, Jacksonville, FL.*

To define both fatal and non-fatal hemorrhagic complications of living kidney donation, a survey was sent to all ASTS members in October of 2003. Of 945 surveys, 203 (22%) were returned. Issues examined included open and laparoscopic surgical technique as applied to arterial and venous closure, failures of specific techniques to control hemorrhage, and the time of failure (at primary application or at various subsequent times). We also sought the outcome of any reported hemorrhagic complications and solicited comments deemed important by respondents.

HEMORRHAGIC FAILURES OF VASCULAR CONTROL IN LIVING DONOR NEPHRECTOMY

	CLOSURE TECHNIQUE			
FAILURE TYPE	**CLIPS**	**STAPLER**	**TIE alone**	**TRANSFIXION**
ARTERIAL-primary application	2	4	7	2
ARTERIAL-early intraop	8	9	5	5
ARTERIAL-delayed	11	2	3	2
ARTERIAL-unknown	3	0	1	1
VENOUS	1	21	7	7

Sixty-eight arterial control problems resulted in: 1 donor death, 2 donors with near fatal hemorrhagic shock and contralateral renal failure, at least 13 donors receiving transfusion (data not available for all) and 16 urgent reoperations. Vascular clips were involved in 11/18 (61%) of delayed failures. The most common failures were intra-operative (at application + immediate) associated with staple dysfunction. Importantly, delayed arterial control problems occurred on 18 occasions, 11 of which involved clips (locking 5 and standard 6). There were an additional 36 reports of failed renal vein control; none proved fatal, but 4 required re-exploration; 21/36 (58%) involved staplers. Significant vascular complications, some resulting in death or renal failure, occur with living kidney donation. Venous stump problems seldom result in life threatening hemorrhage, but arterial control problems may jeopardize a donor's life, especially if occurring in delayed (i.e. post-recover period) fashion. Both locking and standard clips, used to control the renal artery, appear to be associated with the highest risk of delayed arterial complication (although ligature and transfixion of the renal artery was associated with at least one post-operative donor death). Living donor nephrectomy, whether open or laparoscopic, requires careful vascular dissection, preservation of an adequate vessel stump for transfixion control, and clear definition of vessel anatomy (particularly arterial) identifying underlying disease or anomaly that might complicate suitable control. Arterial transfixion should be accomplished with multi-row arterial stapling devices, or with ligature plus suture ligature transfixion to assure a safe recovery from living donor nephrectomy.

Abstract# 779 Poster Board #-Session: P235-II

CONVENTIONAL AND UNCONVENTIONAL PAIRED KIDNEY EXCHANGES. Matthew Cooper,[1] Janet M. Hiller,[1] Jennifer Rickard,[1] Hamid Rabb,[2] Andrea A. Zachary,[2] Julie Graziani,[2] Robert A. Montgomery.[1] *[1]Department of Surgery; [2]Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: One third of kidney donors are eliminated on the basis of ABO incompatibility (ABOi) with an intended recipient. The paired kidney exchange (PKE) is one approach to allowing ABOi patients to receive a blood type compatible kidney. However, based on blood group distributions in the US it would be predicted that only 3% of donor/recipient pairs would benefit from conventional PKE (pairs with blood types A/B and B/A). This is because if either recipient is a blood type O then the exchange only benefits 1 pair. Many patients have a live donor who has been excluded due to a positive crossmatch (+XM). If +XM and ABOi patients are matched in exchanges both pairs can benefit without blood type restrictions and this could greatly expand PKE.

Methods: During the study period (6/00-11/03) 7 PKEs were performed at our institution resulting in 15 transplants.

Results: 4 conventional PKEs (donor/recipient A/B and B/A) were performed. 3 unconventional PKEs (table) were accomplished by pairing +XM, ABOi, and high immunologic risk patients (including blood type O). All PKE recipients received a – XM, ABO compatible kidney. 1 of the unconventional PKEs involved 3 donor/ recipient pairs. Patient survival was 100% and graft survival was 93%.

Unconventional	Donor ABO	Recipient ABO	Incompatibility	Benefit of Exchange
Exchange #1				
Pair #1	B	O	ABOi	ABO compatible
Pair #2	O	B	historic +XM, repeat MM	no repeat MM
Exchange #2				
Pair #1	O	A	repeat MM	no repeat MM
Pair #2	B	B	XM+ titer>1024 and PRA>80%	XM- and 1 Ag MM
Pair #3	A	B	ABOi	ABO compatible
Exchange #3				
Pair #1	B	B	5 Ag MM	3 Ag MM
Pair #2	O	B	XM+ titer>1024 and PRA>80%	XM-

Conclusions: PKEs represent an innovative approach to getting patients transplanted who have willing live donors that are incompatible. Unconventional PKEs are a less expensive and immunosuppressive alternative to desensitization of donor/recipient pairs with a +XM or ABOi.

Abstract# 780 Poster Board #-Session: P236-II

WHEN DOES THE GENDER DISPARITY DEVELOP IN THE KIDNEY DONOR EVALUATION PROCESS? Kathryn A. Tuohy, Scott Johnson, Khalid Khwaja, Martha Pavlakis. *Department of Medicine, Nephrology Division, Beth Israel Deaconess Medical Center, Boston, MA.*

Background. Gender differences in live donor kidney transplantation are well established. Females are more often donors than males. Our goal was to examine the potential donor pool and to determine at what point in the donor evaluation process this gender disparity becomes apparent so as to better understand the reasons for the disparity. **Methods**. We obtained records from our HLA tissue typing lab on all patients who came forward for blood typing to be a potential kidney donor for recipients being evaluated for live donor kidney transplant in our center between January 2000 and December 2002. We then reviewed the patients records to determine at which of the following points along the evaluation process that drop-out occurred: ABO incompatible, positive cross-match, no medical work-up, medical workup not completed, medical contraindication, social contraindication, approved but recipient sick, dead

or already transplanted, approved but unwilling to donate, approved and donated. **Results**. A total of 363 potential donors (54% females, 46% males, NS) were evaluated. Eighteen patients were lost to follow-up and were excluded. Fifty-two patients were ruled out due to ABO incompatibility (42% female, 58% male, NS). Nineteen patients were excluded due to a positive cross-match, which occurred more often in females (63% vs. 37%, NS). Ninety-eight patients never began a medical work-up (56% female, 44% male, NS). Of this group, the potential recipient was already transplanted (65%), was unable to undergo transplant because he or she had become too sick or had died (19%), or was still pre-dialysis (4%). Twelve patients began, but did not complete a medical workup (50% female, 50% male, NS). Fifty-eight patients were ruled out for medical reasons (57% female, 43% male, NS), and 6 patients were ruled out for social reasons (67% female, 33% male, NS). This left 100 potential donors who were approved for donation (55% female, 45% male, NS). Of these, 5 patients had a potential recipient who was already transplanted, dead, or too sick to undergo transplant. Of the remaining 95 potential donors, 73 went on to donate (64% female, 36% male, p=0.017) and 22 were unwilling to donate (27% female, 73% male, p=0.03). **Conclusion.** While males and females initially come forward as a potential living kidney donor equally as often, and are equally as likely to get through each step of the evaluation process, females approved for donation are significantly more likely to donate than approved males. The reasons for this remain to be determined.

Abstract# 781 Poster Board #-Session: P237-II

HEMODYNAMIC ADAPTATION AFTER LIVING DONOR NEPHRECTOMY. Sandra J. Taler,[1] Nancy Driscoll,[1] Mary Tibor,[1] Genie Sprau,[1] Jo Ellen Augustine,[1] Timothy S. Larson,[1] Mark D. Stegall,[2] Stephen C. Textor.[1] *[1]Hypertension and Nephrology; [2]Transplant Center, Mayo Clinic, Rochester, MN.*

Blood flow to the kidneys represents 20-25% of resting cardiac output. Increased use of living kidney donors warrants close evaluation of changes produced in the systemic circulation. The **aims** of this study were to examine changes in cardiac output and arterial resistance 6-12 months after donor nephrectomy.

Methods: We studied 98 living kidney donors (mean age 42± 1 years, 40 male, 58 female) using serial systemic hemodynamic measurements obtained before and within the first year (6-12 months) after donor nephrectomy. We measured clinic oscillometric blood pressure (BP) and heart rate (HR), and stroke volume (SV) by thoracic bioimpedance (TBI). We excluded donors with previously identified or newly diagnosed hypertension or taking antihypertensive medications. Cardiac output and systemic vascular resistance were determined from stroke volume, heart rate and BP measures and indexed to body surface area. Absolute thoracic impedance and impedance change with posture (supine to standing position) were used as indicators of cardiopulmonary volume.

Results:

	Baseline	Post nephrectomy
BP, mm Hg	121±1/74±1	118±1 */73±1
HR, bpm	70±1	68±1 **
Stroke volume index , ml/b	50±1	49±1
Cardiac index, L/min/m^2	3.42±0.06	3.24±0.06 **
Systemic vascular resistance index, d-sec-cm^{-5}-m^{-2}	2183±52	2258±53 *
Supine impedance, ohms	30.6±0.8	29.8±0.7
Standing impedance, ohms	34.2±0.8	34.4±0.8
Impedance change with standing, ohms	3.6±0.2	4.6±0.2 **

Mean±SEM: *p<0.05, **p<0.01 vs baseline

Blood pressure fell slightly after nephrectomy accompanied by a reduction in heart rate. Cardiac index fell while systemic vascular resistance index increased. Body weight did not change (85±2 to 84±2 kg). Absolute thoracic impedance measures were stable after nephrectomy, while impedance change with posture increased substantially.

Conclusion: These results before and after donor nephrectomy establish the magnitude of hemodynamic changes induced by the removal of this large vascular bed. The fall in heart rate and cardiac output with increased postural fluid loss from the cardiopulmonary bed suggest both reduced intravascular volume and sympathetic adrenergic tone, possibly related to partial loss of afferent sympathetic stimuli from the removed kidney. These changes offset a minor rise in systemic resistance and may explain stable or reduced arterial pressure observed in the first year after donor nephrectomy.

Abstract# 782 Poster Board #-Session: P238-II

ATTITUDINAL AND PSYCHOLOGICAL DIFFERENCES BETWEEN ALTRUISTIC STRANGERS AND THE GENERAL PUBLIC. L. Ebony Boulware,[1] Misty U. Troll,[1] Lloyd E. Ratner,[2] Andrew S. Klein,[1] Neil R. Powe.[1] *[1]Departments of Medicine and Surgery, Johns Hopkins School of Medicine, Baltimore, MD; [2]Department of Surgery, Thomas Jefferson School of Medicine, Philadelphia, PA.*

Background: Little is known about attitudinal and psychological differences between altruistic strangers and the general public. Identifying differences could lend insight to altruistic strangers' motivations for donation.

Methods: We performed a case control study interviewing 34 altruistic strangers (cases identifying themselves to our Live Kidney Transplant Program) and 68 zip code-matched persons from the general public (controls) to assess their attitudes regarding tolerable thresholds for risks (medical complications and kidney failure, out of pocket expenses, and failure of transplant) incurred with donation, other potential motivators for donation (prior donation and religiosity) and psychological factors (depression, and anxiety, assessed using a validated diagnostic questions). We used descriptive and comparative (Kruskal-Wallis and χ^2) statistics to compare altruistic strangers and controls.

Results: The mean (SD) age of participants was 45 (12) years, 64% were female, 84% were White, 52% were college graduates, 6% were employed full time, and 37% had household incomes ≥ $60,000. There were no demographic differences between groups. Altruistic strangers vs. controls were willing to donate given greater levels of potential risk for: medical complications (>50% vs. 35% risk, p<0.001), their own kidney failure (100% vs. 50% risk, p<0.001), time without monetary compensation (>3 vs. 2.5 months, p<0.001), out of pocket expenses (>$4500 vs. $4500, p<0.001), and failed transplant in the recipient (90% vs.70% risk, p<0.001). More altruistic strangers than controls donated blood in the past, (91% vs. 54%, p<0.001), but they were no more likely than controls to consider themselves "moderately or very" religious (70% vs. 82%, p=0.2) or spiritual and (82% vs. 82%, p=0.9). There were no differences in rates of depression (12% vs. 18%, p=0.4), panic disorder (3% vs. 0%, p=0.15), or suicidal ideation (2% vs. 2%, p=0.9) between altruistic strangers and controls.

Conclusions: While altruistic strangers have similar attitudes regarding religion/spirituality and similar rates of psychological disorders when compared to the general public, they are more likely to have had past donation experiences and are willing to accept greater personal risks for donation (medical and financial). When presenting potential risks of donation to altruistic strangers, transplant programs should seek to confirm altruistic strangers' full understanding of such risks prior to proceeding with donation.

Abstract# 783 Poster Board #-Session: P239-II

COMPLICATION RATES OF OPEN VS. LAPROSCOPIC DONATION NEPHRECTOMY AS REPORTED BY DONORS TO THE LIVING ORGAN DONOR NETWORK (LODN) REGISTRY. Thomas R. McCune,[1] Thomas A. Armata,[2] John W. Blanton,[2] Leroy R. Thacker.[2] *[1]Renal Transplant Program, Sentara Norfolk General Hospital, Norfolk, VA; [2]South-Eastern Organ Procurement Foundation, Richmond, VA.*

Background: In October 2000 the South-Eastern Organ Procurement Foundation (SEOPF) formed a living donor registry called the Living Organ Donor Network (LODN). This registry prospectively follows living kidney donors through self-reporting of general health to assess the impact of donation on donor health. This registry captures information on short and long-term donation related complications. As of October 2003, LODN has registered 294 donors; 270 (91.8%) have completed at least one follow-up survey.

Methods: Donors were classified based on whether they underwent laproscopic (LAP) or open/full incision (OPEN) for their donation surgery. Donor complications are reported on follow-up surveys at six months post-operatively and at annual intervals thereafter. The follow-up surveys are mailed directly to the donors and returned to the SEOPF offices. Complications are broadly categorized as Operative/Wound (OP/WOUND), Genito-Urinary/Renal (G-U/RENAL), Gastro-Intestinal (G-I) or Miscellaneous (MISC). Complication rates were examined by procedure type using Fisher's exact test.

Results: While participation is voluntary, response rates are excellent; 814 of 948 (85.9%) outstanding follow-up forms have been returned to the SEOPF. Overall, 19.6% of donors report some kind of complication post-donation; 11.9% report OP/ WOUND complications, 4.1% report G-U/ RENAL complications, 3.0% report G-I complications and 2.2% report MISC complications. Donors undergoing OPEN donation procedures reported higher rates of ALL , OP/WOUND and MISC complications. Donors undergoing LAP procedures reported higher rates of G-U/RENAL and G-I complications. These reported differences were not statistically significant. LAP donors required less time to recuperate before returning to work (42.5 vs. 49.5 days, p=.0493). LAP donors also reported fewer unpaid work days missed after donation than OPEN donors (11.6 vs. 18.8, p=.0349). Regardless of procedure type, the same number of paid sick/vacation days were used.

Conclusion: Using self-reporting of donation related complications, there are no differences in complications reported by donors undergoing laproscopic and open/full incision donation procedures. Donors who underwent laproscopic nephrectomy recuperated faster and lost less income due to unpaid sick leave.

DONOR REPORTED COMPLICATIONS

	OPEN	LAP	p value
ALL	22.2%	18.7%	.6033
OP / WOUND	15.3%	10.6%	.2936
G-U / RENAL	2.8%	4.6%	.7327
G-I	1.4%	3.5%	.6859
MISC	2.8%	2.0%	.6590

Abstract# 784 Poster Board #-Session: P240-II

LESSONS LEARNED IN 100 ROBOTIC-ASSISTED DONOR NEPHRECTOMIES FOR KIDNEY TRANSPLANTATION. Santiago Horgan,[1] Garth Jacobsen,[1] Antonio Manzelli,[2] Piero Fisichella,[1] Jason Harris,[1] Diego Bogetti,[2] Robert Berger,[1] Howard N. Sankary,[2] Giuliano Testa,[2] Enrico Benedetti.[2] *[1]General Surgery, University of Illinois at Chicago, Chicago, IL; [2]Transplant Surgery, University of Illinois at Chicago, Chicago, IL.*

We previously presented our initial experience with donor nephrectomy for kidney transplantation (KTx) using the Da Vinci Surgical System (Intuitive Surgical, Mountain View, CA). We report outcomes and lessons learned in a larger series of 100 consecutive patients.

Methods: From 8/00 to 11/03, 100 consecutive robotic-assisted donor nephrectomies for KTx were performed. All cases were completed using the hand-assisted technique. All charts were retrospectively reviewed noting intraoperative and postoperative complications, length of stay, estimated blood loss, warm ischemia time, operative time, and outcome in both donor and recipient.

Results: Our pt. population consisted of 49 males and 51 females with a mean age of 35 years (range 18-58); 38 were African-American, 23 Caucasian, 35 Hispanic, and 3 of other race. No deaths or life-threatening complications occurred. Postoperative morbidity included 1 pneumonia, 1 mild pancreatitis, and 3 superficial wound infections treated with conservative management. In all cases, the surgical dissection was successfully completed with the robotic system. However, 4 pts. required conversion to open surgery due to stapler line failure of the renal artery stump (3 pts.) and bleeding from the renal vein (1 pt.) just before kidney removal. To avoid this complication we modified the renal artery stapling technique by placing a locking clip on the renal artery takeoff followed by GIA stapling; there were no complications of this type in the last 40 cases. Median length of stay was 2 days (range 1-8). Average blood loss was 50 cc (range 10-1500). Mean warm ischemia time was 87 sec (range 60-120). Average operative time was 184 min (range 85-320).

Average operative time dropped from to 206 min (range 120-320) in the first 50 cases to 156 min (range 85-240) in the last 50 cases ($p<0.0001$) thus reflecting our increasing experience. In all recipients, graft function was immediate. 1-year patient survival was 100% with 98% graft survival. Two grafts were lost secondary to rejection and renal artery thrombosis. Average creatinine at 6 mo. was 1.3 mg/dl.

Conclusions: Our extended series confirms that robotic-assisted donor nephrectomy for KTx can be performed in a safe, fast, and accurate fashion. The length of the operation is now less than standard laparoscopic donor nephrectomy. Technical modification of the renal artery stapling virtually eliminates the chance of conversion.

Abstract# 785 Poster Board #-Session: P241-II

LONG-TERM OUTCOME OF RENAL TRANSPLANTION USING HLA MISMATCHED A2 DONOR KIDNEYS. Patrick P. W. Luke,[1] Vaishali Karnik,[1] Mahms Mohammed,[1] Andrew A. House,[2] Norman Muirhead,[2] David Hollomby,[2] Vivian C. McAlister,[1] Anthony M. Jevnikar.[2] *[1]Surgery, The University of Western Ontario, London, ON, Canada; [2]Medicine, The University of Western Ontario, London, ON, Central African Republic.*

It had been previously demonstrated that transplantation across the ABO barrier is feasible using A2 donor kidneys. However, the practice of A2→O/B transplantation has not been universally accepted due to fears of inferior long-term results. We report the long-term results of A2→O/B renal transplantation at our transplant centre. Between November 1990 and June 2001, 8 patients with blood group B (3) or O (5) received A2 renal transplants. Five of these kidneys were from living-related donors. The mean %PRA was 1% (0-6%), and the mean number of HLA matches was 3/6 (2-4/6). All patients received cyclosporine/prednisone immunosuppressive therapy. Four patients received mycophenolate mofetil and the remaining patients were treated with azathioprine as a third agent. One patient received thymoglobulin induction therapy for delayed graft function. In 4 patients identified to have > 1:16 pre-operative anti-A1 or anti-A2 antibody titres, plasmapheresis was carried out. One patient was pre-treated with cyclophosphamide due to the inability to lower anti-A antibody titres using plasmapheresis alone. Prior to our current established pre-transplant target of anti-A levels of <1:8, 2 patients were not given pre-operative plasmapheresis and went on to lose their grafts from acute humoral rejection within the first week post-transplantation. After a mean follow-up of 7.6 years (2.5-13) in remaining patients, none of the other patients had a documented acute rejection episode and all have remained off dialysis. Furthermore, renal reserve remained excellent with a mean serum creatinine at last follow-up of 1.6 mg/dl (1.0-2.1 mg/dl). One patient died from metastatic squamous cell carcinoma 6 years post-transplant with a functioning graft (creatinine 1.3 mg/dl). In summary long-term outcomes of A2→O/B renal transplantation are excellent and should be encouraged in all centres if pre-operative anti-A1 antibody titres can be reduced to < 1:8.

Abstract# 786 Poster Board #-Session: P242-II

COST-EFFECTIVENESS OF THE USE OF HTK (HISTIDINE-TRYPTOPHAN-KETOGLUTARATE) AS AN ALTERNATIVE PRESERVATION SOLUTION IN LIVE DONOR RENAL TRANSPLANTATION. Meelie A. DebRoy, Mark Gravel, Richard Chenault, Robert M. Merion, Jeffrey D. Punch, John C. Magee, Juan D. Arenas. *Surgery, Division of Transplantation, University of Michigan, Ann Arbor, MI.*

The development of safe organ preservation solutions revolutionized the field of transplantation. Currently, the solution most commonly used for organ preservation in the United States is University of Wisconsin (UW) solution. The use of UW solution is not without its price – high cost, high viscosity and the risk of hyperkalemic arrest in the reperfusion period. An alternative solution, HTK, has lower viscosity and is an adequate buffer. We studied the efficacy (cost-effectiveness and early graft function) of using HTK as preservation solution in adult patients undergoing live donor renal transplants.

Methods: Adult patients undergoing live donor renal transplantation at our institution between 9/01 and 8/03 were included in the study. The patients were divided into 2 groups – those whose organs were preserved using UW solution (n=106) and those using HTK solution (n=102). Total cost of solution used (irrespective of volume, as unused solution was discarded) was calculated for each group, as well as clinical evidence of early graft function. Mean age of patients in the UW group was 42±12 yrs, compared to 47±11yrs in the HTK group (p=NS). No differences in warm or cold ischemic times were found between groups. Immunosuppression regimens were followed according to institutional protocol.

Results – At the time of organ recovery, ~ 600 cc of preservation solution was used for each patient. This necessitated the use of 1L of solution/patient. The total cost of preservation solution alone was $31,800 for the UW group, compared to a cost of $15,300 for solution for the HTK group. The pre-operative serum creatinine levels were 7.4±3.1mg/dl for the UW group and 6.7±2.6 mg/dl for the HTK group (p=NS). Both groups had a significant drop in creatinine within the first week after transplant, sustained at 3-month follow-up. Two patients (1.8%) in the UW group and one patient (0.9%) in the HTK group had delayed graft function, necessitating dialysis within the first week after transplant. There was one death with a functioning graft in the HTK group one week post-transplant.

Conclusion: Organs preserved with HTK solution for live donor renal transplants have equivalent early graft function compared to those preserved with UW solution. This is achieved with an appreciable cost benefit to using HTK solution and should spur the use of this solution for recovery of other organs (liver, pancreas, heart) as well.

Abstract# 787 Poster Board #-Session: P243-II

PROSPECTIVE PSYCHOSOCIAL EVALUATION AND OBJECTIVE ASSESSMENT OF MOTIVATION IN LIVE RENAL DONATION. Mukut Minz,[1] Navalkishor Udgiri,[1] Munish K. Heer,[1] Randeep Kashyap,[1] Ritu Nehra,[2] Vinay Sakhuja.[3] *[1]Transplant Surgery Unit, Deptt. of Surgery, Post-Graduate Institute of Medical Education and Research, Chandigarh, U.T., India; [2]Deptt. of Psychiatry, Post-Graduate Institute of Medical Education and Research, Chandigarh, U.T., India; [3]Deptt. of Nephrology, Post-Graduate Institute of Medical Education and Research, Chandigarh, U.T., India.*

INTRODUCTION: Motivation may play a role in low psychosomatic morbidity seen in live renal donors. We objectively evaluated the role of motivation and psychosocial factors affecting the psychosomatic outcome in living renal donation.

METHODS: Psychosocial evaluation of the donors was performed based on a structured questionnaire preoperatively and at three months postoperatively. Motivation was objectively assessed (Score 0-66). Donors were interviewed for depression, anxiety and social support according to "Beck's depression inventory", "Spielbergs State and trait anxiety" and "social support" questionnaire. Postoperative pain was quantified based on Visual Analog Scale (0-100).

RESULTS: 57 donors were evaluated. Mean age 41.6±12. M:F :18:39. 84.2% donors volunteered for donation and only 9% found it hard to make the choice. The median Motivation Score was 61[interquartile range 59-63]. 15.8% donors had negative impact on their health at the end of three months. None of the donors had regrets about their decision. Median pain score during follow up period was 8 [interquartile range 2-15]. Donors returned to their presurgical activity in an average of 41.4 ± 25.3 days. Preoperative and post operative state anxiety, trait anxiety, social support and depression scores are given in the table1. All the donors would definitely encourage others to donate and if given a chance would donate again.

Conclusion: Motivation scores of living donors is high. Though negative impact on health was observed none of them regretted. Depression scores in postoperative period were found to be high, majority of them due to worry about their recipient or social taboo related to donation. We recommend psychosocial evaluation and objective assessment of motivation to be incorporated as part of donor's routine evaluation.

Assessment scores (range)	Pre-operative scores median [interquartiles]	Post-operative scores median [interquartiles]	Comparison by wilcoxon signed ranked test
State anxiety (24.9-42.4)	31 [27-34]	26 [22-31]	P=0.001
Trait anxiety (30.6-47.8)	32 [29-37]	28 [25-33]	P=0.002
Social support (59.3-70.1)	57 [54 -60]	59 [56- 63]	P=0.002
Depression Range: 0-39 psychosocial factor scores	0[0-1]	0[0-2]	P=0.050

Abstract# 788 Poster Board #-Session: P244-II

WHATEVER IT TAKES: RULED OUT KIDNEY DONORS' WILLINGNESS TO PARTICIPATE IN DONOR SWAPPING. Amy D. Waterman,[1] Daniel C. Brennan,[2] Barry Hong,[3] Emily Schenk,[1] Tonie Covelli,[1] Tracye Davis,[4] Anne Barrett,[5] Sarah Stanley,[5] Mark A. Schnitzler.[4] *[1]General Medical Sciences, Washington University School of Medicine, Saint Louis, MO; [2]Transplant Nephrology, Washington University School of Medicine, Saint Louis, MO; [3]Psychiatry, Washington University School of Medicine, Saint Louis, MO; [4]Health Administration, Washington University School of Medicine, Saint Louis, MO; [5]School of Public Health, Saint Louis University, Saint Louis, MO.*

Purpose: Living kidney donors who are ABO- or HLA-incompatible with their recipients could still help their recipients receive kidneys through donor-swapping. We surveyed ruled-out living donors to determine how willing they were to participate in altruistic donation and other donor-swapping alternatives.

Methods: We surveyed 54 living donors who were ABO- or HLA-incompatible with their recipients and whose recipients still needed transplants to assess their donation situation, attitudes about and willingness to participate in donor-swapping. We asked donors to rate how willing they would be to donate their kidney through four types of donor swapping programs on a scale from '1' (very unwilling) to '10'(very willing).

Results: Ruled-out donors were predominately Caucasian (82%), family members of the recipients (70%), and female (54%). Most reported being somewhat or extremely upset after being ruled-out (80%), with 9 donors (17%) being upset for over 2 months. Ninety-six percent trusted that their recipients would receive the transplant benefits promised if they participated, and 78% thought that many people would participate in donor-swapping. Donors were most willing to participate in a living donor swap (Table 1), with immediate family members and donors of recipients without other donors being more willing than others.

Conclusions: Donors were upset after being ruled out and very willing to participate in donor-swapping if it could help their recipients obtain kidneys. Living donor swapping was most appealing because their recipients were guaranteed to receive kidneys. Further study is required to determine if reported willingness translates into actual transplants.

Willingness to Participate in Donor-Swapping

Donor-Swapping Benefit*	Mean Willingness (SD)	No. of Donors with High Willingness (N=54)**
Recipient Receives Living Donor Kidney	7.54 (SD=2.60)	42 (78%)
Recipient Receives Next Cadaveric Kidney	6.19 (SD=2.90)	30 (56%)
Recipient Moves into Top 20% of the Cadaveric Waiting List	4.94 (SD=2.72)	21 (39%)
Recipient Receives No Benefit-Altruistic Donation	4.09 (SD=2.64)	13 (24%)

* Benefits to the ruled-out donors' recipients; **On a scale of 1-10, number of individuals who reported willingness greater than 5

Abstract# 789 Poster Board #-Session: P245-II

IMAGE ANALYSIS OF STRUCTURAL INTERSTITIAL REMODELING, TOTAL MATRIX AREA AND GLOMERULOSCLEROSIS ARE CLOSELY CORRELATED IN POTENTIAL DONOR BIOPSIES. Paul C. Grimm,[1] David C. Rayner,[2] Adam L. Merry,[1] Philip F. Halloran,[3] Anette Melk.[3,4] *[1]Pediatrics, University of California at San Diego, La Jolla, CA; [2]Pathology, University of Alberta, Edmonton, AB, Canada; [3]Nephrology, University of University of Alberta, Calgary, AB, Canada; [4]Pediatrics, University of Heidelberg, Heidelberg, Germany.*

Background. Quantitating the interstitial fibrosis area of a renal allograft biopsy may be a surrogate marker of graft outcome. Some groups measure interstitial fibrosis (VIntFib) using the total matrix area (Trichrome or Sirius Red imaged by White Light (SRWL) whereas others measure the area of fibrotic change represented by Collagen Type 1 & 3 as determined by Sirius Red stain imaged by Polarized Light (SRPL). In allografts, the total matrix area is increased by acute inflammatory processes with transient, reversible expression of matrix components such as hyaluronan. In protocol allograft biopsies SRPL is more predictive of long term outcome than SRWL, especially in allografts with acute rejection. We hypothesize that in organs without injury nor inflammation such as donor biopsies, these measures may be very close.

Methods. We studied 46 autopsy and nephrectomy (healthy tissue removed at tumor nephrectomy) specimens (age range 1 month to 88 years). After staining with Sirius Red, cortical portions were outlined with a black pen, 400X images were obtained and archived using polarized and white illumination. The interstitial fibrosis fraction VIntFib obtained with white light (SRWL) represents the entire matrix, while VIntFib obtained with polarized light (SRPL) represents Collagen 1& 3. These measurements were compared with age, Banff score and glomerulosclerosis %. The operator was blinded to the clinical data.

Results. VIntFib from both SRPL and SRWL were tightly correlated ($p<0.0001$, $r=0.85$). Donor age was more highly correlated with SRWL ($p<0.0001$, $r=0.59$) than SRPL ($p=0.0003$, $r=0.51$). This is in contrast to the findings in allograft biopsies were only SRPL is highly correlated with outcome.The difference between the SRWL and SRPL (total vs fibrotic matrix) increased with increasing age ($p=0.004$, $r=0.42$). Glomerulosclerosis was highly correlated with age ($p<0.0001$, $r=0.61$),SRWL ($p=0.0002$, $r=0.53$) and SRPL($p<0.0001$, $r=0.57$).

Summary. Glomerulosclerosis, interstitial fibrosis and interstitial matrix area increase with advanced age and are tightly correlated. This is contrary to findings in transplant biopsies where inflammation leads to marked discrepancies between interstitial fibrosis and interstitial matrix area. Sirius red staining of potential donor biopsies may be useful in assessing organ quality, especially in biopsies with a limited sample of glomeruli.

Abstract# 790 Poster Board #-Session: P246-II

A PROSPECTIVE SURVEY OF CONCERNS IN KIDNEY TRANSPLANTATION: DONOR AND RECIPIENT PERSPECTIVES. Prabhakar Baliga,[1] Gilbert Smalls,[1] Greg Gilbert,[2] Lilless Shilling,[3] Margaret Martin,[3] Michele Norman,[4] Jennifer Milton,[2] Laura Hildebrand,[2] Kenneth Chavin.[1] *[1]Department of Surgery/Tranplant Division, Medical University of South Carolina, Charleston, SC; [2]Transplant Services, Medical Universtiy of South Carolina, Charleston, SC; [3]Department of Health Administration and Policy, Medical University of South Carolina, Charleston, SC; [4]Department of Rehabilitation, Medical Universtiy of South Carolina, Charleston, SC.*

Existing information about barriers to living donation in African-Americans (AA) is sparse. Utilizing focus groups of health care workers and interviews from previous living donors, we developed a questionnaire to assess the concerns of potential donors and recipients. The purpose of this study was to prospectively use our living donor organ survey (LDOS) with all potential donors and recipients referred for a transplantation evaluation to identify barriers to living organ donation in AA.

Methods:

LDOS was pilot tested and revised. Sixty (60) potential donors and recipients referred to the transplant center were asked to complete the LDOS and FACES II. The Brief COPE was administered only to recipients. A factor analysis of the content-specific items was completed. Coefficient alpha was calculated for each instrument. Test-retest reliability coefficients, (Kuder-Richardson 20), were also calculated for each instrument. Using a two-sample Student's t-test demographics (race, age, occupation, education, income, marital status) were tested against each of the factors and total score. Because of the multiple tests performed we adjusted for the number of comparisons made (Bonferroni method).

Results:

The factor analysis identified six factors from the potential recipient instrument accounting for 83.44% of the variance and identified five factors from the potential donor instrument accounting for 81.71% of the variance. There were no significant differences for any of the factors on any of the demographic variables for potential recipients. However, potential AA donors differed significantly in relations to personal concerns. The survey captured concerns about making a big sacrifice, time away from family, feeling obligated to donate, living with one kidney, sexual activity and having children, surgical complications, scarring, and care from unfamiliar doctors. General concerns included, religious beliefs about donating, improving the health of the recipient and time away from work, ($p<0.0001$ and $p=0.0031$, respectively). The mean scores for AA were significantly higher for both groups.

Conclusion:

Racial differences exist in the concerns of potential donors and recipients. Addressing these concerns by transplant professionals may increase the number of AA living donors.

Abstract# 791 Poster Board #-Session: P247-II

THE SPECTRUM OF CHRONIC HISTOLOGIC ABNORMALITIES IN LIVING DONOR KIDNEY TRANSPLANTS AT THE TIME OF IMPLANTATION: CORRELATION WITH DONOR AND RECIPIENT CLINICAL DATA. Montserrat M. Diaz Encarnacion,[1,2] Matthew D. Griffin,[1] Stephen C. Textor,[1] Fernando C. Cosio,[1] James R. Gregoire,[1] Thomas R. Schwab,[1] James M. Gloor,[1] Sandra J. Taler,[1] Timothy S. Larson,[1] Mark D. Stegall,[3] Joseph P. Grande.[1,2] *[1]Dept Medicine, Divisions of Nephrology and Hypertension, Mayo Clinic and Foundation, Rochester, MN; [2]Dept of Laboratory and Pathology, Mayo Clinic and Foundation, Rochester, MN; [3]Dept. Surgery, Division of Transplantation Surgery, Mayo Clinic and Foundation, Rochester, MN.*

Background and Aims: Living kidney donors (LD) now outnumber deceased donors in the USA and acceptance of a wider spectrum of LD is becoming more prevalent. We sought to examine the presence of pre-existing structural abnormalities in a recent cohort of LD kidneys and correlate the findings with relevant clinical indices. **Methods:** The following histologic analyses were carried out on biopsies taken intraoperatively from 44 LD kidneys: (a) Assignment of Banff 97 "chronicity" indices (cg, ci, ct, cv, ah). (b) Estimation of % tubulointerstitial fibrosis (%TIF) by a renal pathologist as well as by computerized digital analysis of Sirius Red-stained sections. The biopsies were divided into two groups: those for which cg+ci+ct+cv+ah was 0 or 1 and those for which cg+ci+ct+cv+ah was ≥2. Clinical data was derived from donor and recipient records. GFR was measured by iothalamate clearance. BP readings during initial clinic visit and by Ambulatory BP Monitoring (ABPM) were separately recorded. **Results:** See table:

Conclusions: (a) A subgroup of LD kidneys have mild chronic histologic abnormalities at the time of transplantation which are primarily associated with older donor age. (c) We did not observe significant functional differences between LD kidneys with and without mild chronic histologic abnormalities. (d) %TIF was low in both subgroups of LD kidneys. (e) Correlation of the baseline histology of LD kidneys with subsequent graft biopsies, long-term graft function, and donor clinical follow-up will be important for optimization of the practice of LD kidney transplantation.

	Sum of Chronicity Scores 0-1 (n = 24)	**Sum of Chronicity Scores ≥2 (n = 20)**	**p**
Donor Age (years)	36.8±7.6	47.0±12.4	0.001
Donor GFR (ml/min/SA)	105.5±15.4	106.9±23.6	0.8
Donor Body Mass Index	27.3±4.6	27.6±3.9	0.6
Donor systolic BP (Clinic BP/ABPM, mmHg)	132±13 / 123±8	134±18 / 124±14	0.7 / 0.7
Donor diastolic BP (Clinic BP/ABPM, mmHg)	75±9 / 75±8	78±11 / 77±7	0.2 / 0.4
%TIF (Pathologist estimated)	2.3±0.6 %	3.4±1.8 %	0.01
%TIF (Digital image analysis - Sirius Red non-polarized)	3.1±1.8 %	3.7±2.4 %	0.3
Recipient GFR at 1 month post=transplant	57.4±14.1	57.2±14.7	1.0

All results expressed as mean ±SD

Abstract# 792 Poster Board #-Session: P248-II

NONDIRECTED DONATION (NDD) - CONTINUED OBSERVATIONS AND PROGRAM EVOLUTION. Catherine Garvey,[1] Cheryl Jacobs,[1] Deborah Roman,[1] Arthur Matas.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Since 1998, we have had 360 enquires about NDD, done detailed medical and psychosocial evaluations of 42 potential NDDs, and done 22 NDD transplants. As our program has evolved, we have noted differences for NDDs and have made changes in our practice: 1) We have insisted that potential NDDs travel to our center for evaluation (unlike directed donors who may be evaluated at a local center). NDDs, like all donors, must pay their travel costs. After an early experience with positive viral serology (which was a contraindication) we began to require the potential NDD obtain an H&P, blood work (including electrolytes, CBC, and hepatitis B, C, and HIV), prior to coming for evaluation. 2) We require that the NDD and recipient remain anonymous (to each other), so we assigned each donor an alias at the time of admission for surgery. This practice led to logistic problems and was abandoned. 3) We had a minimum age of 18 for NDDs (similar to directed donors). The few inquirers who have been under 21 had voiced their parents' concerns about donating, or avoided telling them altogether, for fear of disapproval, or their angered response. This has raised concerns about family stress or lack of support post donation. We have now raised the minimum age to 21: any volunteers under 21 are informed about our reasoning, provided with our donor educational materials, and are welcomed to re contact us when 21. 4) One advantage of NDD (in contrast to directed donation) is that there is no family pressure to donate. In fact, we have noticed in a few cases that family and friends have tried to dissuade the potential donor. In the majority, these issues were discussed and resolved, and at the time of surgery, family and friends were at the hospital and were supportive. However, in 3 cases, we learned that spouses were upset with the donation at some point in the process (1 during surgery because of their own limited support, 1 because the donor was out of work longer than expected, 1 who we later learned was opposed all along. As a consequence, we have intensified our efforts to have family involvement. We encourage family to come to the initial screening (and information providing) interview but we recognize that this is not always feasible. We have made a video that is mailed to prospective donors (NDD and directed) that details the process and risks. And we counsel prospective donors on the importance of discussing donation with the family. These issues will be discussed in detail.

Abstract# 793 Poster Board #-Session: P249-II

PERCEPTIONS OF FINANCIAL INCENTIVES, PRESUMED CONSENT, AND FAMILY VETO AMONG NEXT-OF-KIN WHO REFUSED VS. CONSENTED TO ORGAN DONATION. James R. Rodrigue,[1] Danielle L. Cornell,[2] Richard J. Howard.[3] *[1]Clinical and Health Psychology, University of Florida, Gainesville, FL; [2]LifeQuest Organ Recovery Services, Gainesville, FL; [3]Surgery, University of Florida, Gainesville, FL.*

Background: Strategies for bridging the gap between the supply of organs and the demand for transplantation are the topic of considerable debate. These include financial incentives, presumed consent legislation, and not permitting family members to exercise veto power over the decision when the deceased has documentation of intention to donate. This study examined whether family members who were approached about organ donation differ in their perceptions of these strategies for optimizing donation. Methods: Data were collected via telephone interviews with 184 next-of-kin who were previously approached about the donation of their deceased relative's organs. Participants were recruited as part of a larger NIH-funded study on factors influencing organ donation decisions. The majority of participants (78%) were from UNOS Region 3. Interviews lasted about 45 minutes and included questions about the donation request process, attitudes toward donation, and other contextual factors surrounding the donation decision. Data reported here are for several questions related to financial incentives, presumed consent, and the role of family in donation decision-making. Results: Univariate analyses (Chi square test when the variable had 3 or more categories or Fisher exact test when the variable had 2 categories) were conducted to examine the relationship between the survey responses and the next-of-kin's donation decision. All tests were 2-tailed and significance was set at $P \leq .05$. When compared to those who consented to donation, next-of-kin who refused donation were more likely to report that financial incentives would have made a difference in their donation decision, that they personally would be more likely to donate organs if financial incentives were available, and that families should retain veto authority over donation decisions. Those who consented to donation (vs. those who did not) reported more favorable attitudes toward presumed consent and proceeding with procurement with or without family permission if the deceased's donation intentions are known. Conclusions: Next-of-kin who have been faced with a donation decision have an important voice in the debate on strategies to increase organ donation. Findings from this study suggest that there may be important differences between next-of-kin donors and non-donors in their attitudes toward financial incentives, presumed consent, and whether families should be permitted to override the donation wishes of the deceased.

Abstract# 794 Poster Board #-Session: P250-II

THE LIVING ORGAN DONOR NETWORK: THREE YEARS EXPERIENCE WITH A MODEL REGISTRY TO PROSPECTIVLY FOLLOW THE HEALTH AND WELL-BEING OF KIDNEY DONORS. Thomas R. McCune,[1] Leroy R. Thacker,[2] Thomas A. Armata,[2] John W. Blanton.[2] *[1]Renal Transplant Program, Sentara Norfolk General Hospital, Norfolk, VA; [2]South-Eastern Organ Procurement Foundation, Richmond, VA.*

Since 2001 the largest source of kidneys for transplantation has been living donors. Despite calls for a national registry to track the health of donors post-donation and provide comprehensive data on medical complications, there is currently no registry. In October 2000 the South-Eastern Organ Procurement Foundation (SEOPF) announced the formation of the Living Organ Donor Network (LODN), a model registry to prospectively follow the health and psychological well being of living kidney donors through patient self-reporting to SEOPF. Donors at participating centers become eligible for insurance to cover accidental death related to the donation, income during recuperation from complications and medical expenses related to complications. The total potential benefit is $550,000. A charge of $550 per donor funds the insurance policy. Participating transplant programs enroll all living donors and provide limited medical data. Additional information is provided by the donor directly to SEOPF at the time of donation, 3 and 6 months post-donation and yearly thereafter. As of October, 2003 the thirteen participating centers have enrolled 290 donors. In addition, 4 individual donors from non-participating centers have enrolled for a grand total of 294 living donors. Currently, 85.9% of all donor follow-up questionnaires have been returned. Data provided reveal that even though donor income levels are similar to the national average they still experience costs related to travel, lodging and child-care during the donation process. On average donors had 14 days of unpaid leave during recuperation. Donor reports of medications perscribed found that 5.1% of donors have been treated for hypertension and 9.9% for depression after donation. Complications were reported in 19.7% of donors, the majority of which were self-limited and related to the operative

wound. Serious complications which required hospitalization or re-operations occurred in 3.1% of donors. LODN proves that donors are willing to participate in prospective follow-up after donation. The insurance policy also encourages participation. LODN data reveals that donors continue to accept and experience financial costs and that donors report more complications than transplant centers do. This registry of donors can provide information on short-term donation related complications and can also prospectively track donors for long-term complications.

Pancreas and Islet Transplantation

Abstract# 795 Poster Board #-Session: P251-II

TYPE 1 DIABETES FOLLOWING PANCREAS TRANSPLANTATION: DO AUTO-ANTIBODIES MARK RECURRENCE OF AUTO-IMMUNITY? George W. Burke,[1] Gaetano Ciancio,[1] Joshua Miller,[1] Gloria Allende,[2] Alberto Pugliese.[2] *[1]Department of Surgery, Division of Transplantation, University of Miami School of Medicine, Miami, FL, 33136; [2]University of Miami, Diabetes Research Institute, Miami, FL, 33136.*

INTRODUCTION. Hyperglycemia following pancreas transplantation (PT) is typically due to 1) chronic rejection (CR) of the pancreas, 2) insulin resistance associated with obesity or immunosuppressive medication (calcineurin inhibitors/steroids), or rarely 3) recurrence of type 1 diabetes (T1D). While recurrence of T1D is unusual and occasionally associated with islet cell auto-antibodies, the prevalence of islet cell auto-antibodies following PT is not known.

MATERIALS AND METHODS. Since 1990, over 250 SPK transplants have been performed at this center. Over the past 1 ½ years, five SPK recipients (5-9 years following transplantation) have become hyperglycemic, associated with prior development of islet cell auto-antibodies, and loss of insulin secreting cells on PT biopsy (4/5), while maintaining pancreatic exocrine function (urine amylase) and kidney transplant function. In addition, we have identified 28 other SPK recipients who have become hyperglycemic, as well as 14 patients with normal glucose tolerance, and evaluated them for islet cell auto-antibodies.

RESULTS. Anti-GAD65 and anti-IA2 antibodies were assessed retrospectively in these 42 patients:

		GAD	1A-2	Either Ab	
Hyperglycemic	Pancreas CR (n=9)	5	6	7	78%
	T1D Recurrence (n=7)	5	4	5	71%
	Kidney-Pancreas CR (n=4)	0	1	1	25%
	T2D/IGT (n=13)	2	1	2	15%
Normoglycemic	NGT (n=14)	3	0	3	21%

CONCLUSION. Of 33 patients with hyperglycemia, 15 (45.4%) were positive for one of the islet cell auto-antibodies. The data show a higher percentage of islet cell auto-antibodies in recurrence of T1D and CR of the pancreas transplant, than in the other 3 groups, CR-SPK, T2D/impaired glucose tolerance (IGT), or normal glucose tolerance (NGT). Therefore in recipients of pancreas transplants, islet cell auto-antibodies may mark the presence of an auto-immune response in those patients with clinical evidence of T1D recurrence and, surprisingly, CR.

Abstract# 796 Poster Board #-Session: P252-II

A MULTICENTER ANALYSIS ON THE SIGNIFICANCE OF HLA MATCHING ON OUTCOMES FOLLOWING KIDNEY-PANCREAS TRANSPLANTATION. Robert J. Stratta,[1] Rita R. Alloway,[2] Agnes Lo,[3] Ernest Hodge.[4] *[1]Surgery, Wake Forest University, Winston-Salem, NC; [2]Nephrology, University of Cincinnati, Cincinnati, OH; [3]Pharmacy, University of Tennessee, Memphis, TN; [4]Roche Research Laboratories, Nutley, NJ.*

Introduction: The significance of HLA matching on transplant outcomes has been widely debated and remains controversial. The purpose of this study was to determine the influence of HLA matching on outcomes in simultaneous kidney-pancreas transplant (SKPT) recipients enrolled in a multicenter trial.

Methods: From March 1999 to May 2001, a total of 297 SKPT patients were enrolled into a prospective, multicenter, randomized, open-label, comparative trial of two daclizumab dosing strategies versus no antibody induction in combination with tacrolimus, mycophenolate mofetil, and steroids in SKPT recipients. The main outcomes of this study have previously been reported. Subanalyses using both univariate and multivariate models were performed to identify factors associated with acute rejection, graft loss or death at 1 year. Potential risk factors evaluated were treatment group, African-American ethnicity, HLA-A mismatches (mm), HLA-B mm, HLA-DR mm, total HLA mm, surgical technique (portal-enteric, systemic-enteric, systemic-bladder drainage), CMV donor and recipient status, and delayed graft function (DGF).

Results: Univariate analyses revealed that treatment group, HLA-A mm, HLA-B mm, >3 total HLA mm, and DGF were significantly associated with acute rejection.

HLA mismatch (mm)	Acute Rejection	p
HLA-A 0 mm	17.8%	0.038
HLA-A 1 mm	24.2%	
HLA-A 2 mm	31.9%	
HLA-B 0 mm	7.7%	0.066
HLA-B 1 mm	26.6%	
HLA-B 2 mm	28.5%	
HLA-DR 0 mm	15.0%	0.514
HLA-DR 1 mm	30.6%	
HLA-DR 2 mm	25.2%	
Total <3 mm	18.4%	0.015
Total >3 mm	29.3%	

These variables were then entered into logistic and Cox regression analyses. HLA-A mm and DGF were the only variables that remained significantly associated with acute rejection in the multivariate model. The relative risk of acute rejection in recipients with HLA-A mm was 1.56 (95%CI 1.07-2.28, p=0.02) and with DGF was 2.88 (95%CI 1.25-6.61, p=0.01). None of the variables tested were significantly associated with graft loss or death at 1 year posttransplant in both univariate and multivariate analyses.

Conclusions: Despite contemporary immunosuppression, the degree of HLA mm, particular HLA-A, and DGF are associated with an increased risk of acute rejection in SKPT recipients at one year. Less rejection was noted in 0 mm patients at all 3 HLA loci, as well as in patients with a total HLA-mm <3. However, none of these factors affected patient or graft survival.

Abstract# 797 Poster Board #-Session: P253-II

IMPROVED HUMAN ISLET ISOLATION OUTCOME BY ADDITION OF VITAMIN E AND NICOTINAMIDE TO THE ISLET PROCESSING MEDIUM. Hirohito Ichii,[1,2] Ismail Al-Abdullah,[1] Joel Szust,[1] Jorge Montelongo,[1] Itzia Iglesias,[1] Day Longsomboom,[1] Aisha Khan,[1] Yoshikazu Kuroda,[2] Rodolfo Alejandro,[1] Camillo Ricordi.[1] *[1]Diabetes Research Institute, University of Miami School of Medicine, Miami, FL; [2]Gastroenterology Surgery, Kobe University School of Medicine, Kobe, Japan.*

Background. Despite recent improvements in clinical islet transplantation outcome, the current isolation methods have not produced consistent islet yields and several islet preparations could not be used for clinical transplantation. Many factors could limit the yield of the currently employed islet isolation procedures. A significant number of islets could be lost during pancreas digestion, islet purification and culture. There is a need to develop strategies that can reduce islet damage and improve islet yields. Vitamin E (VitE) and Nicotinamide (NA) have cytoprotective and antioxidant properties that can be beneficial to islets. In this study, we investigated the effect of addition of VitE and NA to the islet processing medium used during human islet isolation.

Methods. 81 pancreata were processed using a modification of the automated method and continuous density gradient purification. Pancreata were divided into 4 groups. Group I (n=19): islets were processed using standard isolation medium, without VitE and NA, following pancreas preservation with UW alone. Group II (n=14) VitE and NA were added to the medium, following preservation with UW alone. Group III (n=10): islets were processed using the same medium as Group I and preservation with the two-layer method (TLM). Group IV (n=38) Islets were processed using isolation medium with both Vitamins, following pancreas preservation with TLM.

Results. There were no significant differences in donor related factors (i.e. age, body mass index, pancreatic weight, etc) between Groups. In Group I and II, where the pancreas was preserved in UW alone; the addition of VitE and NA in Group II resulted in a significant increase in islet yields (Group I: 262,003±99,315; Group II: 326,507±77,201 IEQ, P<0.05). Similarly in Group III and IV, where the pancreas was preserved using TLM, a significant increase in islet yields was observed in Group IV (Group III: 320,829±69,535; Group IV 383,896±127,909 IEQ, P<0.05). The rate of successful transplantation was 15.7% in Group I, 42.3% in Group II, 50% in Group III and 60.5% in Group IV.

Conclusions. Independently on the pancreas preservation method, the addition of VitE and NA to the islet isolation medium could increase islet yields and improve islet transplantation outcome.

Abstract# 798 Poster Board #-Session: P254-II

FACTORS CORRELATED WITH SUCCESSFUL PANCREATIC ISLET ISOLATION. Adam M. Frank,[1] Shaoping Deng,[1] Ergun Veledeoglu,[1] Niraj M. Desai,[1] Xialun Huang,[1] Mohammed M. Mohiuddin,[1] Moh Moh Lian,[1] Thavachenthan Thambi-Pillai,[1] Yong Suk Bae,[1] Chengyang Liu,[1] Clyde F. Barker,[1] Ali Naji,[1] James F. Markmann.[1] *[1]Surgery, Division of Transplantation, Hospital of the University of Pennsylvania, Philadelphia, PA.*

Background: Recovery of large numbers of isolated islets per donor organ is critically important to successful islet transplantation (IT). The digestion process, although considerably more standardized in recent years, continues to suffer form significant variability. In addition, it remains unanswered which human pancreases (HPs) are optimal for islet production.

Methods: 193 HPs were processed for islets between 2/00 and 11/03. Beginning in 9/01, HPs began to be processed for IT. Since then, of 97 isolations 25 HPs have been used in 12 IT recipients via 22 infusions. 31 additional isolations were begun with the intent to transplant but did not meet clinical criteria for yield or quality.

Results: Islet yields were markedly impacted by liberase lot. 6 different lots were used for the 56 HPs that comprised transplanted preparations (TP) and failed clinical preps (FCP). 2 Liberase lots were preferentially used in the majority of the TP (21/25) and the FCP (16/31). These superior liberase lots (SLLs) yeilded a higher mean IEQ# pre cobe than poor liberase lots (PLLs) (653,947 versus 457,083). In addition, SLL were more likely to result in pre cobe yields of >700,000 IEQ than with PLLs (17/37 vs 1/16 $p \leq 0.05$).

The TP had significantly higher mean islet yields pre and post cobe than the FCP (750,887 IEQ and 484,963 IEQ vs 439,917 IEQ and 270,743 IEQ). TP donors ranged in age from 27 to 65. The age range for FCP was broader (14-66), but both had identical mean ages (49). Women donors were more common in the TP than in the FCP (68% vs 50%) but not to a statistical significant degree. Mean BMI was higher in the TP (34.3 vs 26.3 kg/m²), but mean pancreas weight was higher in the FCP (122.4 vs 108.8g) and more of these HPs were excessively fatty (7 vs. 4).

Combining TP and FCP, the 30-39 year old HP donors had the highest percentage of TPs (4/6), highest IEQ# pre cobe (892506), and the highest mean IEQ#postcobe/gram pancreas (6965) of any decade age group. Donors from this group, however, had an elevated mean BMI (37.1kg/m²) and had 5 of 6 isolations preformed with the best liberase lot.

Conclusions: Vast differences in the performance of liberase lots significantly impacts islet yields. Female donors and heavier donors appears to generate higher islet yields. HPs from young donors age 30-39 are most likely to yield transplantable preparations.

Abstract# 799 Poster Board #-Session: P255-II

EFFICACY OF HUMAN ISLET ISOLATION FROM TAIL PART OF PANCREAS FOR THE POSSIBLE LIVING DONOR ISLET TRANSPLANTAION. Shinichi Matsumoto,[1] Teru Okitsu,[1] Yasuhiro Iwanaga,[1] Hirofumi Noguchi,[1] Yukihide Yonekawa,[1] D. Michael Strong,[2] Jo Anna Reems,[2] Koichi Tanaka.[1] *[1]Department of Transplantation and Immunology, Kyoto University, Kyoto, Kyoto, Japan; [2]Islet and Cell Processing Laboratory, Puget Sound Blood Center, Seattle, WA.*

Background/Purpose: Islet transplantation became more popular for the treatment of type 1 diabetes and shortage of donor pancreata became more apparent. If we were able to use living donor pancreata, the shortage would be alleviated. The critical issue for using the living donor pancreas is islet yields from a part of pancreas. Currently, whole pancreas is used for islet isolation, however, it is known that pancreas head is not suitable for islet isolation due to complex anatomy. The purpose of this study is to evaluate whether pancreatic tail could provide high enough islet yield for transplantation.

Methods: After obtaining human pancreata, islets were isolated from the head part (N=20, head group) or tail part (N=23, tail group) or whole pancreata (N=24, whole group). Islets were isolated by enzymatic digestion followed by purification. We compared islet yield, purity, viability using AO/PI staining and stimulation index of glucose challenge test.

Results: Fifteen out of 20 cases (75%) with head group, all cases (100%) with tail group and 23 out of 24 cases (96%) with whole group were successfully completed for islet isolation and head group was significantly difficult to complete compared to tail ($P<0.02$) and whole groups ($P<0.05$). Further analyses were performed with completed cases. The islet yield per gram pancreas was significantly higher in the tail group compared to both the head ($P<0.001$) and whole ($P<0.01$) group (head; 1,472 ± 326IE/g, tail; 4,256 ± 574 IE/g, whole; 2,424 ± 506 IE/g). Total islet yield with head group was significantly low compared to both tail ($P<0.003$) and whole ($P<0.002$) groups (head; 75,016 ± 18,933 IE, tail; 197,469 ± 28,236 IE and whole; 208,207 ± 43,414 IE) and interestingly, tail group showed similar islet yield to the whole group ($P=0.79$). Whole group showed significantly lower purity when compared to both head ($P<0.03$) and tail ($P<0.02$) groups (head; 76.4 ± 6.7%, tail; 76.7 ± 5.6%, whole; 55.5 ± 12.1%). Islet viability and stimulation index were 89.6 ± 2.7%, 1.5 ± 0.2 in the head group, 94.4± 1.4%, 2.5 ± 0.6 in the tail group and 92.6 ± 23.1%, 2.4± 0.7 in the whole group and there were no significant differences among the groups.

Conclusions: Tail part of human pancreas is suitable for islet isolation and total islet yield from tail part was similar from whole pancreata. Living donor islet transplantation could be possible with tail part of donor pancreas.

Abstract# 800 Poster Board #-Session: P256-II

THE EFFECT OF DONOR AND ISOLATION FACTORS ON HUMAN ISLET IN-VIVO FUNCTION. Omaima Sabek,[1] Patricia Cowan,[1] Daniel Fraga,[1] Malak Kotb,[1] Osama Gaber.[1] *[1]Surgery, University of TN, Memphis, TN.*

Intoduction: Islet isolation has steadily improved over the past few years aided by technical modifications and the introduction of new and defined enzyme mixes. Although, islet yield is an important consideration, it is crucial that isolated islets be shown to demonstrate in vivo function. We examined factors influencing islets recovery and in vivo function with emphasis on donor related factors.

Methods and Results: Islets were isolated either by mechanical shaking (n=95) or hand shaking (n=123) from human donor pancreata, the majority of which were unsuitable for whole organ transplant. Islet yield was reported as islet equivalent per gram pancreatic tissue. Donor factors were collected for each isolation and correlation statistics performed between donor variables and islet yield. Data analysed in 95 human isolations indicated a differential effect of enzyme mixes on yield with Collagenase P digestion most suitable for increased ischemic time ($R^2 = 0.1$; $P< 0.08$), Liberase with small donor pancreas size and elevated pre-procurement glucose ($P<0.05$) and Serva with female donor gender ($R^2 = 0.17$; $P< 0.06$). Results from the 123 hand shaking isolations regression analysis with donor factors only showed age correlates with islet equivalent per gram ($R^2 = 0.05$; $P<0.03$), while regression analysis with isolation as well as donor factors showed that hypertension and length of digestion are the main factors that correlate with islet equivalent per gram ($R^2 = 0.1$; $P<0.01$). Islets from mechanical isolations (n=20) and hand shaking isolations (n=64) were further tested by transplantation under the kidney capsule of immune-deficient NOD-SCID mice following short-term culture (≤7 days). In-vivo function was assessed by measuring the production of human insulin and C-peptide. Age was the only donor factor to correlate with in-vivo function. Young donor age (33.3±18) was associated with better function than older age (56.4±6.6; $p<0.001$), in the mechanical isolation group. In the hand shaking islet isolation using wilcoxon two-sample test cold ischemia (CIT) was the most significant factor were mean CIT 11.4±5.6 (function) vs. 14.9 ±5.1 (non function), $p \leq 0.03$, age 42.1+1±12.8 function) vs. 46.4 ±13.2 (non function), $p \leq 0.07$ and lipase 88.3±91 (function) vs. 252.9 ±113 (non function), $p \leq 0.08$ were also significant.

Conclusion: Results show that while older donors may produce higher yield, younger donors with short CIT and non-inflamed pancreas will result in better function islet.

Abstract# 801 Poster Board #-Session: P257-II

NUMBER OF ISLET β CELLS BETTER PREDICTS SUSTAINED INSULIN-INDEPENDENCE THAN NUMBER OF ISLET EQUIVALENTS (IE) TRANSPLANTED IN TYPE 1 DIABETIC PATIENTS. Hui-Jian Zhang,[1] Jeffrey Ansite,[1] Sung-Hee Ihm,[1] Jeremy Oberbroeckling,[1] Andrew Friberg,[1] Bernhard J. Hering.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Background Pancreatic islet cell identity, purity, and mass are central components of islet product characterization that may predict insulin-independence in islet recipients. To analyze the cellular composition of islet products we modified current immunocytostaining techniques by using microporous polyethelene terephthalate membranes instead of glass slides for cell adhesion. All cells were captured on the membrane without loss; cell detachment during staining process was avoided and cellular morphology was preserved. **Method** Islets were prepared from human cadaver donor pancreases using controlled perfusion, Ricordi digestion and COBE purification. We examined cell composition of islet products by dissociating islets into single cells, fixing cells on membranes, and applying antibodies to identify β, α, δ, PP, acinar and ductal cells. After staining, photomicrographs were taken using a digital camera and images analyzed by computer. The ratio of positive cell # to nuclei # was calculated. Total DNA of products was measured to determine # of β cells (=DNA(pg)/7 (pg/cell) x % of β cells). Islets cultured for 2 days prior to transplant (tx) were infused into the portal vein of Type 1 diabetic patients. We assessed 16 consecutive tx's. **Results** Ratio of β, α, δ, pp, acinar, ductal, and other cells was 29±6, 17±9, 12±5, 5±2, 23±6, 9±9, and 6±7% respectively (mean±SD); islet purity was 62±12%; DNA content/IE ranged from 4.7 to 31 ng; # of β cells/IE ranged from 150 to 1250. Eleven single-donor islet recipients achieved sustained insulin-independence (I-ID). We retrospectively analyzed correlations of recipient outcome with # of β cells or # of IE transplanted. Insulin-dependent (I-D) and I-ID patients received 3.1±1.1 and 6.6±2.6 x10^6/kg BW of β cells with range of 1.2-4.2 and 3.7-11.0 x10^6/kg BW respectively, showing minimal overlap (n=2, p=0.002) between the 2 groups. However, I-D and I-ID patients received 6.6±1.5 and 7.6±1.7 x10^3/kg BW of IE, with range of 4.5-8.0 and 6.0-11.5x10^3/kg BW, showing more overlap (n=7, p= NS) between the 2 groups. The rate of insulin-independence increases linearly with increasing β cell number: <3 (0%), 3-5 (50%), and >5 x10^6/kg (100%) but not linearly with IE number: <5 (0%), 5-6 (50%), 6-7 (80%), 7-8 (50%), and >8 x10^3/kg (100%). **Conclusion** The # of transplanted islet β cells seems to be more predictive than # of conventional IE for sustained insulin-independence in human recipients.

Abstract# 802 Poster Board #-Session: P258-II

POTENTIAL ROLE OF TELOMERASE AND TELOMERES IN HUMAN ISLETS. Annie T. L. Young,[1] Douglas Wu,[1] Ronald B. Moore,[1] Jonathan R. T. Lakey.[1] *[1]Surgery, Surgical Medical Research Institute, University of Alberta, Edmonton, AB, Canada.*

Rationale: Undividing islet tissue poses a huge limitation on clinical islet transplantation given the scarcity of donor tissues and the variable islet mass acquired per isolation. The genetic engineering of human islets to express telomerase (hTERT) may trigger islet cell division. This application may have potential for islet tissue expansion *in-vitro* and the attenuation of replicative senescence via telomere maintenance.

Methods: Telomerase expression and telomere length were determined in fresh and frozen human islets by Western blot and a modified flow-FISH procedure. Western blotting was achieved with mAb (mouse) detection based on known amount of load protein. Flow-FISH was performed using a FITC-labelled telomere-specific PNA probe that hybridized to only the telomeric DNA region of dissociated human islet cells. The resulting signal intensity of fluorescence was standardized into molecules of equivalent soluble fluorochrome (MESF) unit (M) that directly correlates to telomere length. Telomerase activity was determined using a telomeric repeat amplification protocol (TRAP). A green fluorescent protein reporter gene (EGFP) was delivered into islet tissues using lipofection reagents, DOTAP and FuGene 6. Both transfection efficiency and glucose stimulation index were evaluated in hTERT-transfected islets for clinical potential and feasibility.

Results: Telomerase gene expression was not detected in both fresh and frozen human islets, but detected in samples containing exocrine tissue using monoclonal hTERT antibody. Telomerase activity in dithizone-stained, handpicked islets from young (8 yrs) and old (64 yrs) human donors was also undetectable. *In-vivo* telomere shortening was found in human islets of advancing age. Young pancreas donors (7-18 yrs) demonstrated average telomere length correlating to mean MESF value of 35±7.5 kM (n=5) whereas, donors of intermediate (29-47 yrs) and old (55-65 yrs) age had mean MESF values of 18±3.8 kM (n=5) and 17±7.7 kM (n=3) respectively. Transfection efficiency reached 40±4.6% (n=13) and 22±4.0% (n=9) using DOTAP and FuGene 6 respectively. EGFP-transfected islets remained responsive to a glucose challenge similar to untreated controls.

Conclusions: Islet cells senesce with aging and injury. Lipofection is a feasible strategy for the delivery of the hTERT gene into human islets, with no apparent toxicity on islet viability. It is hoped that telomerase-expressing islets could be stimulated to proliferate and create more robust islets for clinical transplantation.

Abstract# 803 Poster Board #-Session: P259-II

ISLET TRANSPLANTATION ALONE IN TYPE 1 DIABETES: SINGLE CENTER EXPERIENCE. Paola Maffi,[1] Federico Bertuzzi,[1] Francesca De Taddeo,[1] Rita Nano,[1] Massimo Venturini,[2] Alessandro Del Maschio,[2] Antonio Secchi.[1] *[1]Medicine - Transplant Unit, San Raffaele Scientific Institute, Milan, Italy; [2]Radiology, San Raffaele Scientific Institute, Milan, Italy.*

After Edmonton experience islet transplantation is a real alternative therapy to insulin in the cure of type 1 diabetes.The aim of our study was to analyze advantages and risks of this procedure.14 intraportal islet transplants were performed: 10 according to Edmonton protocol (daclizumab, sirolimus and tacrolimus), 4 after a period of pre-transplant treatment, 3-6 months, with statin and sirolimus, followed by the Edmonton protocol. Infusions were: 3 in 2 cases, 2 in 6, 1 in 6. Patients received 8,210.4 ± 1,076.7 islets equivalent number /kg body weight.Rate of insulin independence was 50% (duration 5.7±1.6 months), 3 patients were insulin free for more than 6 months (maximum duration: 14 months). 50% of patients reduced the exogenous insulin requirement < 50% of the pre transplant dose. Metabolic parameters were considered at the following time: pre-transplantation, after 2 weeks and after1,3,6,12 months. C-peptide (ng/mL): 0.1+0, 1+0.1, 0.7+0.1, 0.9+0.1, 0.7+0.1, 1.3+0.4; glycaeted haemoglobin (%): 8.6+0.4, 7.9+0.2, 6.7±0.2,6.8+0.2, 6.7+0.2, 6.3+1.4. Kidney function got worse in 2 patients(serum creatinine > 3 mg/dL), who were treated with ACE inhibitors because of mild proteinuria; in both cases immunosuppression was stopped, without regression of renal damage. Transient increase of liver enzymes and of fibrin degradation products (FDP) were observed immediately after the infusion as the expression of local inflammatory reaction. AST (U/L) ALT (U/L) and FDP were: pre transplant: 22±1, 24±2, 0.5±0.1; 1st week: 123±25, 145±28, 1.0±0.2; 2nd week: 61±10, 116±26, 2.3±0.8; 4th week: 35±4, 65±11,0.9±0.5. Other complications were: peritoneal hemorrhage (2 cases); peripheral vein thrombosis (2 cases); artero-venous fistula (1 case); mouth ulcers (10 cases); acne lesions (4 cases); arthritis (2 cases); leucopenia (2 cases); hyperlipemia (1 case). They resolved spontaneously or after arrangement of therapy.Conclusion. Islet transplantation alone is an alternative to insulin therapy and it is capable to improve the metabolic control in all cases leading 50% of the patients to insulin independence. Strict inclusion criteria are required, due to potentially severe complications, mainly related to immunosuppressive therapy.

Abstract# 804 Poster Board #-Session: P260-II

COLLABORATIVE ISLET TRANSPLANT REGISTRY (CITR). Bernhard J. Hering,[1] Nicole C. Close,[2] Ravinder Anand,[2] Thomas L. Eggerman.[3] *[1]Diabetes Institute for Immunology and Transplantation, The University of Minnesota, Minneapolis, MN; [2]The EMMES Corporation, Rockville, MD; [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.*

Currently there are over 30 transplant centers in the world focusing their efforts on the challenges and methods of islet cell transplantation. As the field of islet transplantation matures and the number of islet transplants performed increases, detailed analyses on factors that predict patient and graft survival are needed. In response to the need for more complete information in the field, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is sponsoring the Collaborative Islet Transplant Registry (CITR). The mission of CITR is to expedite progress and promote safety in islet/beta cell transplantation through the collection, analysis and communication of comprehensive and current data on all islet/beta cell transplants performed in North America. Compiling and analyzing data from all transplant centers in North America will accelerate the identification of both critical risk factors and key determinants of success, and thereby guide transplant centers in developing and refining islet/beta cell transplant protocols, leading to an advancement in the field of islet transplantation. Participating in CITR is voluntary and over 22 transplant centers have been invited to join, with 12 activated centers currently contributing to the efforts. All islet transplants performed in North America since January 1, 1996 are planned to be captured by the CITR database. Through an electronic, internet based, data capture system, quality control procedures, and the minimization of duplicate efforts at the transplant center, the most relevant and succinct information are entered. From these data a comprehensive report will be published annually. In addition, special analyses will be performed and published periodically. To date, over 83 islet transplant recipients have been entered in the CITR database and information on over 133 processed pancreata have been reported to the Registry. Data from the first Annual Report will be presented.

Abstract# 805 Poster Board #-Session: P261-II

SIROLIMUS AND TACROLIMUS IN PREPARATION FOR ISLET CELL TRANSPLANTATION IN TYPE 1 DIABETIC PATIENTS RECIPIENT OF RENAL ALLOGRAFTS. Enrico Cagliero,[1] Anil K. Chandraker,[2] Arthur Dea,[1] Susan Fritz,[1] Kadir Omer,[3] Martha Pavlakis,[4] Gordon C. Weir,[3] Hugh Auchincloss, Jr.,[1,2] David M. Nathan.[1] *[1]Diabetes Center, Massachusetts General Hospital, Boston, MA; [2]Medicine, Brigham and Women's Hospital, Boston, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Medicine, Beth Israel Deaconess Medical Center, Boston, MA.*

Steroid-free immunosuppression with sirolimus and tacrolimus is the hallmark of the successful "Edmonton protocol" for islet cell transplantation. However, sirolimus has been associated with hyperlipidemia and hypertension, and tacrolimus can induce hypertension; two major cardiovascular risk factors that are especially important in diabetic patients. The goal of this study was to assess the impact of switching immunosuppression to sirolimus and tacrolimus in type 1 diabetic patients, recipient of renal allografts, prior to islet cell transplantation. Twenty-three type 1 diabetic patients (mean age 45±7 years, duration of diabetes 33±7 years, time since kidney transplant 7±6 years) were switched from a cyclosporin-based immunosuppression to sirolimus and tacrolimus. No significant changes were observed in body weight, blood pressure, serum creatinine, fasting lipid or hemoglobin A1c levels. Six patients underwent successful islet cell transplantations (four off insulin after the second transplant and two on reduced insulin doses after the initial transplants), and 7 are currently on the waiting list. Five patients were found ineligible for islet cell transplantation, 1 opted for a pancreas transplantation, 2 could not tolerate sirolimus, one developed acute rejection and is back on dialysis, and one patient had sudden death. The percentage of patients with blood pressure at goal (130/80 mmHg) was 35% before and 52% after change in immunosuppression without any obvious changes in hypertensive regimens, with 70% of patients on anti-hypertensive drugs. Forty-three percent of patients had LDL cholesterol at goal (100 mg/dl) and 87% had LDL < 130 before changing immunosuppression, with 52% and 95%, respectively after the change. Thirty percent of patients were on lipid-lowering drugs at baseline and 50% while on sirolimus and tacrolimus. In conclusion, switching type 1 diabetic patients with renal allografts from a cyclosporin-based immunosuppressive regimen to sirolimus and tacrolimus is not associated with worsening renal function or cardiovascular risk factor profile, although there was an increased need for lipid-lowering drugs. This immunosuppression regimen was tolerated by most patients and allowed successful islet cell transplantation in the six patients that have been transplanted so far.

Abstract# 806 Poster Board #-Session: P262-II

INSULIN INDEPENDENCE IN ISLET AFTER KIDNEY (IAK) TRANSPLANT RECIPIENTS USING A SIROLIMUS/ TACROLIMUS-BASED STEROID-FREE IMMUNOSUPPRESSIVE REGIMEN. Thierry Berney,[1] Axel Andres,[1] Domenico Bosco,[1] Reto Baertschiger,[1] Pascal Bucher,[1] Christian Toso,[1] Leo H. Bühler,[1] Philippe Morel.[1] *[1]Department of Surgery, Geneva University Hospitals, Geneva, Switzerland.*

The Edmonton protocol has significantly increased the rate of insulin independence after islet transplantation. This was achieved in a population of patients with brittle diabetes and preserved kidney function. The purpose of this study is to present the results of a series of patients with type 1 diabetes with an established kidney graft, who received islet after kidney (IAK) transplants with an immunosuppressive regimen similar to the Edmonton protocol.

Five patients (4 females/1male) with a median age of 45 (30-54) received a first percutaneous islet infusion between August 2002 and April 2003. They had been transplanted with a kidney for terminal diabetic nephropathy 3.5 to 23 years earlier (median 13), and had a creatinin clearance 32-69 ml/min (median 46) at the time of islet transplantation. They received a total 10,045-18,134 islet equivalent (IEQ)/kg body weight (median 12,559) isolated from 2 donors each. Immunosuppression was switched to sirolimus (12-15 ng/ml) and tacrolimus (4-6 ng/ml) with daclizumab induction at islet transplantation, with slow wean of any steroids since waitlisting.

All five patients became insulin-independent. Three patients are still insulin-free 8, 9 and 11 months after transplantation, and 2 went back on insulin 3 and 8 months post-transplant. Islet function was associated with improved metabolic control in all patients, as assessed by a steady decrease of HbA1c (median 8% pre-Tx vs 6.6% at latest follow-up) and fructosamine (median 346 vs 277). One patient lost islet and kidney graft function 9 months post-transplant after immunosuppression withdrawal for pneumonitis. One patient developed transplant glomerulopathy. Two additional patients developed microabuminuria. Immunosuppression side effects included dyslipidemia (N=4), mouth ulcers (N=3), polyarthritis (N=1) and pneumonitis (N=1). The steroid-free, sirolimus/tacrolimus-based immunosuppressive regimen was successfully applied to a series of IAK recipients rceiving a total islet mass > 10,000 IEQ/kg. Particular attention to kidney function must be paid in this particular patient population.

Abstract# 807 Poster Board #-Session: P263-II

IMPARED RENAL FUNCTION AFTER ISLET TRANSPLANT ALONE (ITA) OR ISLET-AFTER-KIDNEY (IAK) TRANSPLANTATION USING A SIROLIMUS-TACROLIMUS-BASED REGIMEN. Axel Andres,[1] Christian Toso,[1] Philippe Morel,[1] Sandrine Demuylder-Mischler,[1] Domenico Bosco,[1] Pascal Bucher,[1] Zoltan Mathe,[1] Reto Baertschiger,[1] Leo H. Buhler,[1] Thierry Berney.[1] *[1]Visceral and Transplantation Surgery, Geneva University Hospitals, Geneva, Geneva, Switzerland.*

Introduction: The Edmonton protocol, a steroid-free immunosuppressive regimen including sirolimus, low-dose tacrolimus and dacluzimab has greatly improved the outcome of islet transplantation. This regimen is believed to exibit limited nephrotoxicity. The aim of our study was to assess its real impact on renal function after ITA and IAK procedures.

Materials ans methods: From July 2002 to April 2003, 10 patients (female/male: 5/5, median age:41 years) received their first islet infusion in 5 ITA and 5 IAK procedures, the latter 12.6 years (3.5-22.7) after kidney transplant. A median of 12885 IEq/Kg were injected in one, two or three infusions (2, 6 and 2 cases). Eight patients achieved insulin-independance, 5 being currently free of insulin. Data were prospectively collected over a median follow-up period of 10 months. Renal function was monitored by measured or calculated creatinin clearance (CCl), proteinuria, micro-albuminuria (MA) and, when indicated, by renal biopsies.

Results: Four patients (2 ITA, 2 IAK) have experienced a marked decrease of CCl (>20%) by 6 months post-transplant. One additional patient (IAK) has decreased CCl by the same extent at the end of follow-up. MA increased in 7 cases.Two patients (1 IAK, 1 ITA) already had proteinuria at the time they were put on the waiting list, including 1 IAK patient with already impaired kidney function (CCl = 36). The two ITA patients who decreased CCl were the older of the series (age 56 and 58). Two of 3 IAK patients who decreased CCl had an established graft for > 15 years. One experienced humoral rejection 5 months after the first islet infusion. There was no obvious relationship between the decrease of CCl and insulin-independance or immunosupression blood levels.

Discussion: In our experience, the impairment of renal function is not uncommon after ITA and IAK using an Edmonton-inspired immunosuppression protocol. Age over 55 years, a long established kidney graft or a borderline renal function appear as potential risk factors.

Abstract# 808 Poster Board #-Session: P264-II

PANCREAS TRANSPLANTATION PROVIDES EXCELLENT LONG TERM INSULIN-FREE OUTCOMES FOR PATIENTS WITH TYPE 2 DIABETES MELLITUS (DM). D. Nath,[1] A. Gruessner,[1] R. Kandaswamy,[1] R. Gruessner, D. Sutherland,[1] A. Humar.[1] *[1]Surgery, U of MN, Mpls., MN.*

Background: Pancreas txs have demonstrable efficacy in enabling recips with type 1 DM to achieve insulin independence. The basis of treatment lies on the allograft providing insulin in patients with an absolute deficiency. What is less clear is the ability of pancreas txs to improve glucose control in type 2 diabetic patients who produce insulin, but are unable to use it effectively. The objective of this study was examining the effectiveness of pancreas txs to provide long-term glucose control in patients with type 2 DM.

Methods: A retrospective review of all patients with type 2 DM post-pancreas tx at our center between 1994 and 2002 was done. We used guidelines from the American Diabetes Association to appropriately classify patients with type 2 DM (vs. type 1 DM).

Results: A total of 17 recips that fit the study criteria were identified. The mean age at diabetes onset was 35.7 yrs (range=19-48). Most patients had 1 or more secondary complications related to their diabetes: retinopathy (94%), neuropathy (76%), and nephropathy (65%). The mean age of recips at time of tx was 52.5 yrs (range=38-65); mean duration of diabetes was 16.8 yrs. At the time of tx 4(24%) were on oral hypoglycemics alone. The remaining 13(76%) were on insulin therapy, with a mean daily dose of 64 units. Prior to initiating insulin therapy, these patients were on oral hypoglycemics for a mean time period of 9 yrs.

Of the 17 txs, 7(41%) were SPK, 4(24%) were PAK, and 6(75%) were PTA. Most recips were male (65%). There was 1 perioperative death due to aspiration. All other recips became euglycemic and had a functioning graft at 1-yr posttx (patient and graft survival=94%). With a mean follow-up of 3 yrs since tx, patient survival is 14/17 (82%). The 2 additional deaths during the follow up were due to sepsis (n=1) and suicide (n=1). Both deaths were just after 1-yr posttx, and both had a functioning graft at the time of death. Of the 14 recips currently alive, 12 remain euglycemic without insulin or oral hypoglycemics. One patient (6%) was initiated on oral hypoglycemics 2 yrs post-tx. Another patient was initiated on insulin 2 yrs posttx with a daily dose of 38 U; this patient had an episode of acute rejection 1-yr posttx, which rendered the graft nonfunctional.

Conclusion: These findings suggest that pancreas txs can provide excellent glucose control in recips with type 2 DM. All recips with a technically successful tx were rendered euglycemic. Long-term results were comparable to those seen with tx in type 1 diabetics.

Abstract# 809 Poster Board #-Session: P265-II

PERSISTENT HYPERGLYCEMIA FOLLOWING PANCREAS TRANSPLANTATION. Patrick G. Dean,[1] Yogish C. Kudva,[2] Timothy S. Larson,[3] David J. Rea,[1] Mark D. Stegall.[1] *[1]Department of Surgery, Division of Transplant Surgery, Mayo Clinic College of Medicine, Rochester, MN; [2]Department of Medicine, Division of Endocrinology, Mayo Clinic College of Medicine, Rochester, MN; [3]Department of Medicine, Divison of Nephrology, Mayo Clinic College of Medicine, Rochester, MN.*

Introduction. Persistent hyperglycemia following pancreas transplantation is commonly attributed to graft loss, but may occur despite an otherwise well-functioning pancreas allograft. The aim of this study was to define the incidence and possible causes of persistent hyperglycemia after pancreas transplantation.

Methods. We retrospectively studied all patients (n=88) undergoing pancreas transplantation at our institution between 1/2001 and 1/2003. Persistent hyperglycemia with a functioning pancreas graft was defined as: 1) the need for exogenous insulin therapy to achieve a non-diabetic fasting plasma glucose (<126 mg/dl) and 2) evidence of graft function, i.e. an increase in serum C-peptide from pretransplant levels and a normal allograft ultrasound. Data were analyzed using Student's t-test and the Chi-square test and are expressed as median (25%-75% inter-quartile range).

Results. Median follow-up was 25 months (18-30 months). Actual 1-year patient survival was 100%. Of the 88 grafts, 4 were lost to immediate post-operative thrombosis, 2 to late thrombosis, and 5 to late patient death with a functioning graft. Of the remaining 77 patients, 57 (74%) remained normoglycemic, 6 (8%) were hyperglycemic for less than 1 month and 14 (18%) required insulin longer than 1 month despite evidence of graft function. Hyperglycemia occurred in 23% (16/70) of Type 1 diabetics and 57% (4/7, p=0.0489) of Type 2 diabetics. Other factors associated with hyperglycemia included: high pre-transplant insulin dose, high BMI and increased rejection episodes (p<0.0001, p=0.0005 and p=0.0002, respectively). With respect to insulin dose, hyperglycemia developed in 100% (6/6) of patients requiring >100 U insulin/day pretransplant, in 83% (10/12) requiring > 75 U/day and in only 15% (10/65) of those requiring ≤ 75 U/day (p< 0.0001). Despite hyperglycemia, glucose control significantly was improved after transplant. The median hemoglobin A1c pretransplant for patients developing hyperglycemia was 8.0 (6.7-8.7) compared to 6.6 (5.5-7.4) following transplant (p=0.0294). There were no significant differences between the groups with regard to age, gender, pre-transplant hemoglobin A1c, steroid doses or tacrolimus concentrations at 1 month and 1 year.

Conclusions. Hyperglycemia in patients with a functioning pancreas allograft is primarily due to the high insulin requirements. However, glycemic control is significantly improved after pancreas transplantation.

Abstract# 810 Poster Board #-Session: P266-II

SAFETY AND EFFICACY OF PANCREAS TRANSPLANTATION ALONE IN TYPE 1 DIABETIC PATIENTS: EFFECTS ON METABOLIC PARAMETERS AND LATE DIABETES COMPLICATIONS. Ugo Boggi,[1] Fabio Vistoli,[1] Alberto Coppelli,[1] Rosa Giannarelli,[1] Tiziana Vanadia Bartolo,[1] Michele Aragona,[1] Chiara Croce,[1] Marco Del Chiaro,[1] Alberto Piaggesi,[1] Stefano Del Prato,[1] Franco Mosca,[1] Piero Marchetti.[1] [1]*Regional Referral Center for Treatment of Pancreatic Diseases, Tuscany Region and University of Pisa, Pisa, Italy.*

Background: Pancreas transplantation alone (PTA) in Type 1 diabetic patients (T1D) is still matter of debate as for its safety and efficacy. Hereby we report our single centre experience on PTA in T1D, mainly focusing on metabolic outcome and effects on vascular diabetes complications. **Methods**: Between April 2001 and September 2003 a consecutive series of 32 PTA was performed according to the technique of portal-enteric drainage (PED). Recipients' characteristics were: age, 39 ± 10 yrs; body mass index, 23.4 ± 1.4 kg/m²; duration of diabetes, 24 ± 8 yrs. Donors were selected according to standard criteria irrespective of HLA match, although the best HLA matching was considered at the time of graft allocation. Immunosuppression consisted of quadruple regimen including basiliximab (n= 29) or ATG (n= 3), steroids, MMF and tacrolimus. **Results**: After a mean cold ischemia time of 697 min. (range: 517-965 min.) all pancreata functioned rapidly. No technical failure occurred. Three grafts were lost in the early post-transplant period due to hyperacute/accelerate rejection. A relaparotomy was required in 5 cases (15.6%). After a mean follow-up period of 16.0 months (range: 2.9-31.8 months) 2 further recipients were diagnosed with rejection episodes that were reversed with steroid boluses. Actuarial 1-year, 2-year and 31-month patient and graft survival rates are 100% and 93.5%, respectively. During the follow-up the following clinical results were obtained and compared to the respective pre-transplant data: solid insulin-independence (HbA1c: 5.5 ± 0.3% vs 9.4 ± 1.1%, $p < 0.01$); significant ($p < 0.05$) improvement of total and LDL-cholesterol concentrations and arterial blood pressure values; significant ($p < 0.05$) improvement of some echocardiographic parameters (left ventricular ejection fraction, left ventricular mass index, E-wave/A-wave ratio, isovolumetric relaxation time); improvement/stabilization of diabetic retinopathy in 94% of patients; significant reduction of proteinuria (from 1.4±2.5 g/24h to 0.7±1.9 g/24h, $p<0.05$), improvement of autonomic and/or peripheral neuropathy in 87% of patients. **Conclusions:** PTA in T1D is safe and effective; restored normoglycemia has beneficial effects on cardiovascular risk factors and late diabetic complications.

Abstract# 811 Poster Board #-Session: P267-II

RESULTS WITH PANCREAS TRANSPLANT IN RECIPIENTS WITH A HISTORY OF MAJOR LOWER EXTREMITY AMPUTATIONS. Abhinav Humar,[1] Raja Kandaswamy,[1] James Harmon, Joseph Melancon, Miguel Tan, Ty Dunn, Rainer W. G. Gruessner,[1] David E. R. Sutherland,[1] Angelika C. Gruessner.[1] [1]*Surgery, University of Minnesota, Minneapolis, MN.*

Background: Diabetes is the leading cause of non-traumatic lower extremity amputations in North America. Many centers exclude diabetic patients with a previous major amputation for a pancreas transplant. We looked at results after pancreas transplant in this group of patients.

Results: Between 1994-2003, pancreas transplants were performed in 24 recipients with a history of a major lower extremity amputation (either a below knee or above knee amputation). During that same time 913 transplants were performed in recipients without an amputation. Demographics for the 2 groups are shown in the table. Recipients with an amputation have a significant history of other vascular problems such as TIA, blindness, or peripheral bypass. Surgical complications with the surgery were not significantly different compared to non-amputation recipients. Total relaparotomy rate was 20.1% in those without amputation and 12.5% in those with (p=0.35). Other surgical complications are shown in the table. Death censored graft survival difference was also not significantly different between the 2 groups. At 3 years posttransplant, it was 67% in those with amputations vs. 63% in those without (p=0.65). Patient survival was, however, significantly lower in those with amputations: at 1-year posttransplant, 80% in those with amputation vs. 95% in those without (p= 0.01). Of the 24 recipients, 4 have died – all within the first 6 months posttransplant. Causes of death include MI (1), sepsis (1), suicide (1), malignancy (1), and unknown (1). Of these deaths, 2 were in the early perioperative period.

Conclusions: While patients with amputations are higher risk, pancreas transplants can be performed without an increase in surgical complications, and reasonable graft survival rates. A successful pancreas transplant in these recipients can often have a dramatic impact on quality of life.

Demographics

Characteristic	No Amuptation	Amputation	p value
n	913	24	
Recipient age	41.5	44.8	0.001
% Male	52.2	66.7	0.01
% Bladder drained	80.7	66.7	0.08
Years diabetic	26.5	31.1	0.001
% retransplants	16.0	8.3	0.08
% blind	8.7	20.8	0.04
Hx of peripheral bypass	1.7	12.5	0.002
Hx of TIA	3.7	12.5	0.03

Surgical Complications

Complication	No amputation	Amputation	p value
Abdominal infection	12.6	4.2	0.21
Bleeding	17.9	20.8	0.91
Leak	4.9	4.2	0.86
Thrombosis	7.2	4.2	0.56
Pancreatitis	6.2	4.2	0.67

Abstract# 812 Poster Board #-Session: P268-II

MULTIVARIATE ANALYSIS OF THE INFLUENCE OF DONOR AND RECIPIENT CMV SERO-PAIRING ON OUTCOMES IN SIMULTANEOUS KIDNEY-PANCREAS TRANSPLANTATION: THE SOUTH-EASTERN ORGAN PROCUREMENT FOUNDATION EXPERIENCE. Robert J. Stratta,[1] Leroy R. Thacker,[2] Aimee K. Sundberg.[3] [1]*Surgery, Wake Forest Univ, Winston-Salem, NC;* [2]*SEOPF, Richmond, VA;* [3]*Pharmacy, Wake Forest Univ, Winston-Salem, NC.*

Introduction: Diabetic patients undergoing simultaneous kidney-pancreas transplantation (SKPT) have a relatively high rate of CMV seronegativity at the time of transplant, placing them at greater risk for primary CMV exposure. The purpose of this study was to determine if donor (D) and recipient (R) CMV sero-pairing at the time of SKPT subsequently influences outcomes in a large cohort of patients with long-term follow-up.

Methods: Between 1/1/97 and 12/31/99, 1025 pancreas transplants were performed at SEOPF member institutions and reported to the registry, including 746 SKPTs. CMV serology and survival data were available in 740 SKPTs, including 723 primary transplants. For purposes of this study, retransplants were excluded and 4 groups were defined based upon D and R CMV sero-pairing: D+/R-, N=203 (28%); D+/R+, N=206 (28%); D-/R+, N=156 (22%); and D-/R-, N=158 (22%). Patient and graft survival for the study groups were computed by Kaplan-Meier estimates and tests of equality of survival curves were performed utilizing both the Log-Rank and Wilcoxon test statistics. A multivariate Cox proportional hazards model was fit to adjust for variables known or suspected to impact patient and graft survival. Logistic regression was used to examine the effect of CMV sero-pairing on rejection.

Results: A total of 56% of Ds were CMV+ and 50% of Rs were CMV-. D serostatus was not, but R serostatus was, a significant independent risk factor for patient and kidney, but not pancreas, graft survival in the uncensored analysis. The 5 year survival rates were: Patient (90% R- vs 80% R+, p=.01); kidney (81% R- vs 71% R+, p=.008); and pancreas (73% R- vs 64% R+, p=.12). When examining the CMV D/R groups in both univariate and multivariate fashion and adjusting for other covariates, CMV sero-pairing was not an independent risk factor for either death, graft loss, or rejection in both censored and uncensored analyses (significant risk factors included donor age and cold ischemia time). However, when considering CMV sero-pairing as a binary variable (D-/R- vs all other D/R groups), the 5 year uncensored survival rates were as follows: Patient (92% D-/R- vs 83%, p=.03); kidney (85% D-/R- vs 74%, p=.006); and pancreas (80% D-/R- vs 65%, p=.01).

Conclusion: CMV seronegativity is present in half of diabetic patients at the time of SKPT, and protective CMV seronegative matching confers a long-term survival advantage.

Abstract# 813 Poster Board #-Session: P269-II

GANCICLOVIR VS. VALGANCICLOVIR FOR CMV PROPHYLAXIS IN SOLITARY PANCREAS TRANSPLANT RECIPIENTS. David J. Rea,[1] Yogish C. Kudva,[2] Timothy S. Larson,[3] Mark D. Stegall.[1] *[1]Department of Surgery, Division of Transplantation, Mayo Clinic College of Medicine, Rochester, MN; [2]Department of Internal Medicine, Division of Endocrinology, Mayo Clinic College of Medicine, Rochester, MN; [3]Department of Internal Medicine, Division of Nephrology, Mayo Clinic College of Medicine, Rochester, MN.*

Introduction. CMV is a major source of morbidity especially in heavily immunosuppressed solitary pancreas recipients. The aim of this study was to compare CMV prophylaxis with ganciclovir (GAN) vs. valganciclovir (VAL) in pancreas recipients.

Methods. From 1/01 to 6/03, 79 patients underwent pancreas after kidney transplant (PAK, n=48) or pancreas transplant alone (PTA, non-uremic diabetics, n=31) with thymoglobulin induction (1.5 mg/kg/d x 10d) and maintenance immunosuppression with tacrolimus, mycophenolate mofetil and prednisone. The first 21 patients received oral GAN prophylaxis (1 gram three times a day) while the subsequent 58 patients received oral VAN (900mg daily). Prophylaxis continued 3 months and follow up was an average of 20 months (range 6 – 34 months).

Results. 9 patients (11%) developed CMV infection/disease. The CMV rate was similar for GAN and VAL treatment (14% vs. 10%, p=0.48 by Kaplan-Meier). There was only one CMV infection during prophylaxis — on VAL at 72 days after a PAK transplant. All remaining infections occurred after 3 months of prophylaxis. The acute rejection (AR) rate was 11% and AR showed a trend toward predicting CMV disease (29% in those with AR vs. 10% in those without AR, p=0.13). CMV rate was the highest in the D+/R- group (3/17, 18%) and the D-/R+ group (2/11, 18%) but this was not statistically different from the D-/R- and D+/R+ groups (10% and 6% respectively, p=0.59 for all groups). CMV rates were not affected by recipient gender, recipient age, donor age or transplant type. Donor gender approached statistical significance as a predictor (17% male donors vs. 3% females, p=0.08). Three patients had CMV viremia, 4 patients had only organ involvement (colitis, pancreatitis, retinitis and small bowel disease), and 2 had combined viremia with organ involvement. Treatment was either with additional oral VAL or intravenous GAN. The cost of prophylaxis with VAL was significantly less than GAN ($800 vs. $5281).

Conclusions. VAL and GAN are equally safe and efficacious as primary CMV prophylaxis after solitary pancreas transplantation with thymoglobulin induction. The data suggest that avoidance of rejection may be one of the most important means of preventing CMV disease in these patients.

Abstract# 814 Poster Board #-Session: P270-II

MODE OF DIALYSIS DOES NOT INFLUENCE POST-OPERATIVE INTRA-ABDOMINAL INFECTION RATES IN SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION. Robin D. Kim,[1] Kenneth Qiu,[1] Lesley Adcock,[1] Ian McGilvray,[1] David Grant,[1] Paul Greig,[1] Mark S. Cattral.[1] *[1]Surgery, University of Toronto, Toronto, ON, Canada.*

Peritoneal dialysis (PD) is associated with changes in the intraabdominal milieu which may predispose simultaneous pancreas-kidney (SPK) transplantation patients to postoperative intraabdominal infections. However, conflicting data exist regarding the impact of PD on the infection rates and patient/graft survival. In this study we compared the outcome SPK recipients who were receiving either hemodialysis (HD) or peritoneal dialysis (PD) prior to transplantation.

Patients and Method: Demographic characteristics (age, duration of diabetes), mode of dialysis, method of exocrine drainage (bladder vs enteric) were correlated with postoperative intraabdominal infection. Patient and graft overall survival for 1) each dialysis group and 2) the group with or without infection based on Kaplan-Meier analysis were compared using log-rank test.

Results: Between November 1995 to August 2003, 120 patients underwent 120 SPK transplantations, of whom 44 were receiving PD and 76 HD preoperatively. The mean follow-up was 42 ± 27 and 39 ± 27 months in PD and HD groups, respectively (p=ns). One-year patient, kidney, and pancreas survival rates were 95%, 95%, 93% and 97%, 97%, 92% in the PD and HD groups, respectively (p=ns). Intraabdominal infection requring an intervention (surgery, percutaneous drainage) developed in 5 PD patients and 6 HD patients (p=ns). Demographic characteristics (age, p=0.47; duration of dialysis, p=1.00; method of exocrine drainage, p=0.72) did not signficantly impact on infection rates. Neither patient nor graft survival rates were affected by the development of an intraabdominal infection.

Conclusion: The mode of dialysis does not influence the rate of intraabdominal infection or patient survival following SPK. Furthermore, the development of an intraabdominal infection does not adversely impact patient survival. Peritoneal dialysis is a safe mode of dialysis for patients awaiting SPK transplantation.

Abstract# 815 Poster Board #-Session: P271-II

OUTCOMES FOR AFRICAN-AMERICANS (AA) UNDERGOING SIMULTANEOUS KIDNEY PANCREAS TRANSPLANTATION (SPK) IS EQUIVALENT TO CAUCASIANS (C) WITH THYMOGLOBULIN (THY) INDUCTION. Carlton J. Young,[1] Clifton Kew,[2] Sharon Hudson,[1] Michael Gallichio,[1] Arun Chandrakantan,[2] Bruce Julian,[2] Mark Deierhoi,[1] Robert Gaston.[2] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL; [2]Medicine, University of Alabama at Birmingham, Birmingham, AL.*

Historically, AA have had inferior graft survival (GS) following SPK compared to C. With the advent of new immunosuppressive agents, we wanted to determine if GS rates have improved for AA. **Methods:** From 1/1/98 to 6/30/03, 72 patients (AA, n=19, C, n= 53) underwent bladder drained (BD) SPK with either THY or Daclizumab (D) induction. Two groups were retrospectively studied: Group 1 (G1) (n=48, AA =14) THY/Tacrolimus (T)/ Mycophenolate Mofetil (MMF)/ Prednisone (P); and Group 2 (G2) (n=24, AA=5), D/T/MMF/P. THY (1.5 mg/kg) was given intraoperatively, plus six consecutive days with a solumedrol taper. D (1 mg/kg) was given intraoperatively and on POD 5 with a solumedrol taper.Maintenance immunosuppression: T (trough, 7-10ng/ml); MMF (2 gm/day); and P 10 mg/d. Donor and recipient demographics (age, gender, ethincity, ABDR match, and cold ischemic time) were similar between groups. **Results:** Overall patient, renal, and pancreas 1-yr survival was 100%, 99%, and 98%, respectively. There were no differences bewteen the groups.

Table 1: 1 and 3 year GS by Group

	Patient		Renal		Pancreas	
	1 yr	3 yr	1 yr	3 yr	1 yr	3 yr
G1 (n=48)	100%	96%	98%	94%	97%	90%
G2 (n=24)	100%	100%	100%	88%	100%	85%

AA had overall equivalent pt. (p=0.59), renal (p=0.43), and pancreas survival (p=0.07) as C. However, pancreas survival for AA v. C was poorest in G2 (p=0.01).

TABLE 2: GS AA V. C By Group

	Renal			Pancreas		
	1 yr	3 yr	p	1 yr	3 yr	p
AA G1 (n=14)	100%	100%		88%	88%	
C G1 (n=34)	97%	92%	0.41	100%	91%	0.43
AA G2 (n=5)	100%	67%		100%	—	
C G2 (n=19)	100%	93%	0.13	100%	93%	0.01

AA pancreatic losses in G2 were due to rejection at 13 and 15 months post-transplant. UTI, 26.5%, was most the common infection. Infectious complications did not differ among groups (p=0.46). There was a trend to fewer AR in G1 (28%) v. G2 (39%) (p=0.10). There were no leaks, pancreatitis, or venous thrombosis leading to graft loss. **Conclusions:** 1. G1 AA renal and pancreas outcomes were equivalent to C. 2. G2 AA renal outcomes compared to C trended toward significance. 3. G2 AA pancreas survival was significantly worse compared to C. 4. THY appears to be more efficacious than D in AA undergoing SPK.

Abstract# 816 Poster Board #-Session: P272-II

IMPAIRED RENAL VASODILATORY RESERVE AND KIDNEY HIGH ENERGY PHOSPHATES (HEPs) METABOLISM IN TYPE 1 DIABETIC (T1DM) UREMIC PATIENTS AFTER KIDNEY-ALONE (K) BUT NOT KIDNEY-PANCREAS TRANSPLANTATION (KP). Paolo Fiorina,[1] Gianluca Perseghin,[1] Francesco De Cobelli,[2] Marta Bruno Ventre,[1] Gabriella Mazzolari,[1] Chiara Gremizzi,[1] Giorgio Torri,[3] Valerio Di Carlo,[4] Alessandro Del Maschio,[2] Antonio Secchi.[1] *[1]Medicine, San Raffaele Scientific Institute, Milan, Italy; [2]Radiology, San Raffaele Scientific Institute, Milan, Italy; [3]Anesthesiology, San Raffaele Scientific Institute, Milan, Italy; [4]Surgery, San Raffaele Scientific Institute, Milan, Italy.*

In T1DM patients with uremia which underwent kidney transplantation, both impaired glucose homeostasis and hypertension may significantly affect graft survival. Aim of our study was to assess whether vascular of metabolic markers or kidney disfunctions were detectable non-invasively *in vivo* in the transplanted patients depending on whether they received K or KP transplantation. 20 KP and 15 K patients matched for anthropometric features and plasma creatinine but with different HbA1c levels (6.4±0.7% and 8.6±0.6% in KP and K respectively, P<0.05) underwent two procedures: 1) evaluation of renal blood flow with Magnetic Resonance Quantitative-flow (MR-Qflow) in the resting state and after i.v. L-arginine infusion to assess renal vasodilatory reserve 2) assessment of high energy phosphates (HEP) in the resting state using localized ^{31}P-MR-Spectroscopy of the transplanted kidney. Both MR-Qflow and MRS protocols were performed using a 1.5T system (Gyroscan Intera; release 8; Philips). Basal blood flow was similar in KP and K and it increased significantly (+12.0±8.1%) after L-arginine administration in KP (p=0.02), but not in K (-1.3±8.6%). At the same time a trend for a reduced inorganic phosphate (Pi)/βATP ratio was also evident in the K (1.17±0.14) in comparison with KP (1.62±0.30). In summary, L-arginine-dependent renal vasodilatory response was demonstrated in KP patients using MR-Qflow technique and it resulted to be blunted in K patients. Altered HEPs metabolism in the transplanted organ was also evident in K in comparison with KP patients using ^{31}P-MRS. In conclusion, correction of diabetes in KP along with that of uremia was shown to be associated with better vascular and metabolic features of the transplanted kidney than in K; both MR-Qflow and MRS techniques may be useful tools to reveal early alterations of renal function in the transplanted organs.

Abstract# 817 Poster Board #-Session: P273-II

LATE ANASTOMOTIC LEAKS IN PANCREAS TRANSPLANT (PTx) RECIPIENTS—CLINICAL CHARACTERISTICS AND PREDISPOSING FACTORS . D. Nath,[1] A. Gruessner,[1] R. Kandaswamy,[1] R. Gruessner,[1] D. Sutherland,[1] A. Humar.[1] *[1]Surgery, U of MN, Mpls., MN.*

Background: Anastomotic leaks after PTx are seen in 3-5% of recips, usually occur early posttx, and are typically due to technical factors. However leaks may occur late after tx. We studied such events to determine predisposing factors and management.

Methods: The study population consisted of all PTx recips that were diagnosed with a late leak, defined as one occurring more than 3 months after the tx. Excluded were recips with an early leak or a leak immediately after enteric conversion.

Results: Between 1994-2002, a total of 25 PTx recips were identified that had a late leak (incidence=3.5%). Mean recip age was 40.3 yrs; mean donor age 31.3 yrs. Category of tx was as follows: SPK (n=5, 20%), PAK (n=10, 40%), and PTA (n=10, 40%). The majority of patients were bladder drained (n=23, 92%); only 2 were enteric drained. The mean length of time from tx to presentation of the leak was 20.5 months (range=3.5-74). A direct predisposing event occurring in the 6 weeks preceding the leak was identified in 10 (40%) of the recips. These factors included: a biopsy proven episode of acute rejection (n=4, 16%; diagnosed at a mean of 17.8 days prior to the leak), a history of blunt abdominal trauma (n=3, 12%; which occurred at a mean of 3.3 days before the leak), and CMV infection (n=3,12%; diagnosed at a mean of 19.3 days before the leak). Non-operative management (Foley catheter placement with or without percutaneous abdominal drains) was the initial treatment in 14(56%) of the recips. This was successful in 9(64%) of these cases; the other 5 required surgical repair after failure of conservative management at a mean of 10 days after placement of the Foley. 11 of the recips had surgical intervention as their initial treatment: repair in 9 and pancreatectomy due to severe peritonitis in 2. After appropriate management of the initial leak (conservative or operative), 5(20%) of the recips had a recurrent leak at a mean time of 5.6 months after the initial leak. All 5 of these patients ultimately required surgical repair. Blunt abdominal trauma or acute rejection was noted in the antecedent period leading up to the recurrent leak in 2 of these recips.

Conclusions: Late pancreatic leaks are not uncommon and may be more common with bladder drained grafts. Almost half of the cases have some obvious preceding event such as acute rejection, abdominal trauma, or CMV infection that predisposes to the leak. For stable patients with bladder-drained grafts, non-operative treatment will be successful in two-thirds of the cases.

Abstract# 818 Poster Board #-Session: P274-II

DELAYED PANCREAS GRAFT FUNCTION IN SPK RECIPIENTS – ITS IMPACT ON KIDNEY GRAFT FUNCTION. Abhinav Humar,[1] James V. Harmon,[1] Ty Dunn,[1] J. Keith Melancon,[1] Miguel Tan,[1] Raja Kandaswamy,[1] Rainer W. G. Gruessner,[1] Arthur Matas,[1] David E. R. Sutherland,[1] Angelika C. Gruessner.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Background: Delayed graft function (DGF) after kidney transplant is a well-defined entity with documented risk factors. DGF of the pancreas graft is a less well defined entity with poorly known risk factors. It is unclear if risk factors influencing kidney graft function would similarly impact pancreas graft function, or vice versa. We looked at kidney graft function outcomes in a group of SPK recipients with DGF of the pancreas graft.

Methods: DGF of the pancreas graft was defined as the need for ≥ 10 units of exogenous insulin at time of discharge after pancreas transplant. Of 184 technically successful primary, cadaver SPK transplants, 154 (84%) had immediate graft function (IGF) of the pancreas and 30 (16%) had DGF. The incidence of DGF of the kidney (defined as the need for dialysis in the 1st week posttransplant) in those with IGF of the pancreas was 17.5%, vs. 20.0% in those with DGF of the pancreas (p=ns). Mean donor age in the DGF pancreas group (vs. IGF pancreas group) was higher (38.4 vs. 32.6 years, p=0.03), and there was a higher proportion of enterically drained grafts (73.3% vs. 55.8%, p=0.07). Other demographic characteristics were similar between the 2 groups. Outcomes including kidney and pancreas graft survival and mean serum Cr are shown in the table. Kidnay graft survival rates were similar in the 2 groups but serum Cr at 6 months and 1 year were higher in the group with DGF of the pancreas. Pancreas graft survival rates were significantly lower in the group with DGF of the pancreas (p=0.05). Acute rejection rates tended to be higher in the DGF group, though this did not reach statistical significance (p=0.22).

Conclusions: Recipients with DGF of the pancreas after SPK transplant had inferior kidney graft function at 1 year posttransplant with higher mean serum Cr. This suggests that factors impacting on the function of the pancreas are also impacting on function of the kidney.

Outcomes		IGF of Pancreas	DGF of Pancreas	p
n		154	30	-
Kidney Graft Survival	(1yr)	92	90	0.80
	(3yrs)	87	86	0.80
Pancreas Graft Survival	(1yr)	88	73	0.05
	(3 yrs)	84	69	0.05
Serum Cr	(Discharge)	2.03	1.96	0.83
	(6 months)	1.45	1.90	0.003
	(1 year)	1.43	1.66	0.05
Acute Rejection	(6 months)	16	34	0.22
	(1 year)	19	34	0.22

Abstract# 819 Poster Board #-Session: P275-II

IMPACT OF PANCREAS AFTER KIDNEY TRANSPLANTATION ON KIDNEY ALLOGRAFT SURVIVAL. Ajay K. Israni,[1,2] Kevin C. Mange,[1,2] Kathleen Propert,[2] Mary Leonard,[2,3] Harold I. Feldman.[1,2] *[1]Medicine, University of Pennsylvania, Philadelphia, PA; [2]Center for Clinical Epidemiology & Biostatistics, University of Pennsylvania, Philadelphia, PA; [3]Pediatrics, University of Pennsylvania, Philadelphia, PA.*

In 2002, over 363 pancreas after kidney transplants (PAK) have been performed in patients with end stage renal disease due to Type I diabetes. The aim of this study was to assess the impact on PAK on kidney allograft survival. We conducted a nonconcurrent cohort study of patients all waitlisted for a simultaneous pancreas-kidney transplant (SPK), but transplanted with a kidney alone (KA) from a cadaveric or a living donor between 1/1/9010/31/02. We used data from United States Scientific Registry of Transplant Recipients. A Cox proportional hazards model was fit incorporating PAK as a time-varying covariate. This model evaluated the impact of PAK on kidney allograft loss (defined as death with a non-functioning kidney allograft, return to dialysis or relisting for a kidney transplant). A secondary outcome was the composite of kidney allograft loss or patient death due to any cause.

Results: 1,132 individuals who were wait-listed for an SPK were transplanted with a KA. 865 (76%) received a KA from a cadaveric donor and 267 (24%) received a KA from a living donor. The mean follow-up time for these KA transplants was 2557 days. 153 (14%) of KA recipients underwent a PAK. Kidney allograft loss occurred in 410 patients (35%) of patients. Univariate analysis detected no association of PAK with kidney allograft loss; hazard ratio = 0.75 (95 % C.I. 0.48–1.27, p=0.32). After adjustment for donor factors (age and race), recipient factors (age, race, gender), transplant related factors (preemptive KA transplantation, HLA mismatch), donor type (cadaveric or living donor of the KA), immunosuppression (use of induction therapy, initial calcineurin inhibitor used, mycophenolate mofetil use) and stratifying by transplant centers, PAK was associated with a higher rate of kidney allograft loss which was not statistically significant (Table). In adjusted models, PAK was associated with a trend to a higher rate of the combined outcome of kidney allograft loss or patient death.

Conclusion: Among those eligible for an SPK and receiving a KA, our study failed to confirm a benefit of a pancreas after a kidney transplant. Whether the trend to an elevated risk of allograft loss or patient death in PAK is in the perioperative period only, needs further exploration.

Multivariable Survival Analysis of PAK vs non-PAK

	Hazard Ratio	95 % C.I.	p-value
Kidney Allograft Loss	1.64	0.81 - 3.31	0.167
Kidney Allograft Loss or Patient Death	1.59	0.96 - 2.65	0.074

PEDIATRIC LIVER TRANSPLANT: ADVANCES IN TECHNIQUE AND IMMUNOSUPPRESSION

Abstract# 820 Poster Board #-Session: P276-II

LONG-TERM NEUROLOGIC OUTCOMES AND COMPLICATIONS IN PEDIATRIC PATIENTS TRANSPLANTED FOR FULMINANT HEPATIC FAILURE (FHF). Abhinav Humar,[1] Brooke Glessing,[1] Marci Knaak,[1] Brenda Durand,[1] Elizabeth Larson,[1] William D. Payne,[1] Khalid Khan,[2] Sarah-Jane Schwarzenberg,[2] Harvey Sharp.[2] *[1]Surgery, University of Minnesota, Minneapolis, MN; [2]Pediatrics, University of Minnesota, Minneapolis, MN.*

Background: Long-term neurologic recovery and complications in children receiving a liver transplant for FHF are not well described. The purpose of this analysis was to determine if there were long-term neurologic sequelae once these patients had recovered from their liver transplant. We also tried to determine the impact of this on long-term schooling in these children.

Results: We studied outcomes in pediatric patients receiving a successful liver transplant for FHF. Only patients transplanted after 1990 were included in this analysis. Excluded were recipients who lost their graft or died within the first 3 months after transplant. A total of 10 recipients were identified that fit the study criteria. Mean age at time of transplant was 8.2 years (range = 1-18). There were no documented neurologic abnormalities in these patients prior to the onset of FHF. Cause of FHF was due to Hep A (1), autoimmune (1), acetaminophen (1), and unknown (7). Seven of the 10 recipients had severe encephalopathy (Gr III or IV) at the time of transplant. Mean waiting time from presentation to transplant was 8.8 days (range = 2-23 days). With a mean follow up of 6.3 years (range 1-12 years), all 10 recipients are alive with functioning grafts. Neurologic complications in the 10 recipients include persistent seizures in 2 of the recipients – in 1 recipient the seizures are well controlled medically , but the other recipient has ongoing severe seizure disorder and is now globally aphasic as a result of these. Of the other 8, 1 has major developmental and language delays and another recipient has mild cognitive impairment on formal neuropsychological testing. Another 3 recipients have had major behavior issues requiring psychological intervention. With regards to schooling – 5 are at an age appropriate grade level, 2 are 2 grade levels behind, 1 is 1 grade level behind, 1 does not attend school due to neurologic impairment, and another left school after 2 years due to behavioral problems.

Conclusions: Long-term major and minor neurologic problems are not uncommon in pediatric patients transplanted for FHF. These should be anticipated and adequately addressed in the long-term management of these patients.

Abstract# 821 Poster Board #-Session: P277-II

ORTHOTOPIC LIVER TRANSPLANTATION IN INFANTS UNDER 5 KILOGRAMS. Greg M. Tiao,[1] Maria Alonso,[1] John Bucuvalas,[1] Jorge Bezerra,[1] Nada Yazigi,[1] James Heubi,[1] William Balistreri,[1] Frederick Ryckman.[1] *[1]Pediatric Liver Care Center, Cincinnati Childrens Hospital and Medical Center, Cincinnati, OH.*

The success of pediatric liver transplantation (OLTxp) has improved greatly since its widespread application in the 1980's however, it remains a technically challenging procedure, especially in the very small recipient. We report our experience in infants who weighed less than 5 kilograms at the time of OLTxp.

Methods: A retrospective review of the medical records of all children who underwent OLTxp who weighed less than 5 kg, transplanted from 1987 – 2003 was performed.

Results: 17 patients were identified. The mean age at the time of OLTxp was 5.5 ±3.1 months with a range from 19days to 1.2 years. The mean weight was 4.21±0.76kgs with the smallest child weighing 2.5kgs. The indication for liver transplantation was acute liver failure 41%, biliary atresia 35%, chronic liver disease 18% and vascular malformation 6%. At the time of transplantation, 65% of the patients were Status I, 24% were Status II and 11% were Status III. 10 patients received cadaveric reduced size grafts, 4 received cadaveric whole organs and 3 received living related left lateral segments.

Vascular complications occurred in 2 patients (hepatic artery thrombosis (HAT, n=1), portal vein thrombosis (n=1)). The patient with HAT expired. Four patients died from sepsis. Two patients required re-transplantation for chronic rejection. Post-transplant lymphoproliferative disease (PTLD) occurred in 24% of the patients and was managed by a reduction in immunosuppression.

The one and five year survival of these patients was 76% and 65% respectively. In contrast, our overall series (n=268 patients) one and five year survival was 87% and 82% respectively.

Conclusion: Liver transplantation can be performed successfully in very small infants but at a higher mortality rate. More of these children required urgent transplantion. Sepsis, not technical complications was the most common cause of poor outcome. Technical complications occur but not at a greater rate than that experienced in all other patients undergoing transplantation.

Abstract# 822 Poster Board #-Session: P278-II

THE PRESENCE OF MULTIPLE BILE DUCTS IN THE LIVER GRAFT INCREASES THE INCIDENCE OF BILIARY COMPLICATIONS IN PEDIATRIC LIVER TRANSPLANTATION. Paolo Salvalaggio,[1] Kishore Iyer,[1] Peter Whitington,[1] Estella Alonso,[1] Riccardo Superina.[1] *[1]Surgery and Hepatology, Division of Organ Transplantation, Childrens Memorial Hospital, Chicago, IL.*

Background. Post-transplant biliary complications occur in 15-35% of the pediatric liver transplant recipients. The presence of multiple bile ducts in the graft has been rarely studied as a risk factor for biliary complications in pediatric liver transplantation. ***Aims***. To review the risk factors for the occurrence of biliary complications and to investigate the impact of the presence of multiple ducts in causing biliary complications in pediatric liver transplantation. ***Methods***. 106-children (39-whole allografts, 1-RL, 15 LL, 51 LLS) who underwent primary liver transplantation were included in this study. Patients were divided into two groups: those with a single bile duct (n=80) and those with multiple bile ducts (n=26). For accurate analysis, the number of bile ducts was considered as the number of biliary anastomoses, as described in the operative note. Biliary complication was defined as any deviation from the expected postoperative course caused by a problem in the biliary anastomoses. ***Results***. Mean follow-up was 39.8±20.8 months. 31-patients presented with biliary complications (29.2%). 15-patients presented with biliary leaks (14.1%) and 16-patients (15.1%) with postoperative biliary strictures (6 early, 10 late). By univariate analysis, only ABO blood group incompatibility between D/R (p=0.03), the occurrence of hepatic artery thrombosis (p=0.02) and the presence of multiple bile ducts (p=0.05) were considered risks factors for the development of biliary complications. There was no difference in age, weight, cold ischemia time, presence of hepatic artery thrombosis, donor source (cadaveric vs LR), type of graft (whole, reduction, split, LR) and anatomical unit (LLS, LL or RL) between the groups. However, patients with multiple bile ducts had almost double the risk (RR=1.8) for the development of biliary complications, particularly biliary leaks (RR=1.8). The presence of multiple bile ducts did not affect 1-year patient (89.9% for single bile duct group and 76.9% for multiple bile ducts group, p=0.09) and graft survival (81% for single bile duct group and 73% for multiple bile ducts group, p=0.25) between the groups.

Conclusions. The presence of multiple bile ducts is an independent risk factor for biliary complications after pediatric liver transplantation. The risk of biliary complications is not associated with the size, anatomical segment or source of graft, but with the number of bile ducts and the complexity of the biliary reconstruction.

Abstract# 823 Poster Board #-Session: P279-II

BILIARY RECONSTRUCTIONS IN ISOLATED LIVER TRANSPLANT (ILTx) FOR INTESTINAL FAILURE ASSOCIATED LIVER DISEASE (IFALD). Khalid Sharif,[1] Susan V. Beath,[1] Patrick J. McKiernan,[1] Darius F. Mirza,[2] Anthony David Mayer,[2] Carla Lloyd,[1] Deirdre A. Kelly,[1] Jean de Ville de Goyet.[1] *[1]The Liver Unit, Birmingham Childrens Hospital, Birmingham, United Kingdom; [2]The Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Background: Infants with IFALD may develop rapidly progressively liver disease on parenteral (PN) nutrition some of whom have the potential for gut adaptation. In these children isolated liver transplant (ILTx) may be life saving but biliary reconstruction may be technically challenging, because of the size and limited length of intestine. **Aim:** To review outcome of duct to duct and duct to intestine biliary anastomosis in children who underwent ILTx. **Subjects & Methods:** Retrospective review of all children with IFALD who underwent ILTx in a single centre between 1998 to 2003. Patients & grafts surviving more than 1 month were included in the study. **Results:** 9 children (6 male) median (range) age 11.8 (5.5-14.7) months with residual bowel length 30-80 cm underwent ILTx. Biliary reconstruction: Duct to Duct (Group I, n=4); Duct to Intestine without Roux loop (Group II, n=5). Surgical complications, biochemistry, radiology, histology, patients and graft survival were compared and summarised in table.

Table

	Group 1 (n=4) Median(range)	Group II (n=5) Median(range)
Recipient age (months)	9.6 (9.4-14.7)	12.2 (5.5-14.6)
Recipient wt (kg)	8 (5.8-8.9)	7.5 (5-9)
Donor wt (kg)	25 (10-60)	65 (62-80)
Type of graft	Reduced 3, Full size 1	Split 4, Reduced 1
Cholangitis (liver biopsy)	Nil	One
Transient duct dilatation	3/4	2/5
Interventions for biliary complications	nil	1/5, re-exploration for bile leak
Acute rejection (<6months)	1/4	1/5
Follow Up (months)	9.5 (2-30)	21 (35-65)

Summary: In group II, (2/5) showed air in the biliary tree; one required re-exploration for bile leak and one had cholangitis with mild fibrosis at 5 year with normal liver function tests. Patient and graft survival in both groups was 100% with normalization of liver function test within 3 months of ILTx and similar incidence of complications. The median time to discharge was 71 days with 7/9 patients requiring partial PN at the time of discharge.

Conclusion. Both types of biliary reconstructions are technically possible in children with IFALD undergoing ITLx with good medium term outcome

Abstract# 824 Poster Board #-Session: P280-II

SPLIT LIVER TRANSPLANTATION: LOCAL SHARING BETWEEN PEDIATRIC AND ADULT TRANSPLANT CENTERS. Patrick J. Healey,[1,2] Adam E. Levy,[2] James D. Perkins.[2] *[1]Department of Surgery, Children's Hospital & Regional Medical Center, Seattle, WA; [2]Department of Surgery, University of Washington, Seattle, WA.*

Background. Split liver transplantation (SLT) results in the creation of two donor liver grafts, each suitable for transplantation, from a single adult-sized deceased donor liver. In SLT of adult and pediatric recipient pairs, transection along the plane of the falciform ligament creates a left lateral segment (II-III) graft for the child and an extended right lobe (I, IV-VIII) graft for the adult recipient. The purpose of this study is to review our experience with locally sharing deceased donor livers split for transplantation at a pediatric and an adult transplant center.

Methods. All deceased donor liver grafts (n=10) split for use in pediatric and adult recipients and shared locally at our centers from November 1992 to September 2003 were retrospectively reviewed. Children received the segment II-III split graft at the pediatric center and the adult received the segment I, IV-VIII graft at the adult center. Ex situ splitting (n=8) was performed at the pediatric center, and the remaining extended right lobe graft was repackaged in cold storage and transported to the adult center. In situ splitting was performed in 2 cases and the segmental grafts were transported directly to the individual transplant centers for transplantation. Donor clinical and procurement data, recipient demographic and transplant data was collected. Outcome data including actuarial graft and patient survival, and the incidence of biliary and vascular complications were recorded. Followup was available for all recipients and ranged from 3 months to 11 years.

Results. Twenty patients underwent SLT with one month, one year, and three year post-transplant patient survival measuring 90%, 85%, and 85%, respectively. Graft survival was identical to patient survival. Two children died in the first week post-transplant, and one adult recipient died at six weeks post-transplant from myocardial infarction. Portal vein thrombosis occurred in one pediatric recipient, and hepatic artery thrombosis occurred in 2 recipients, one child and one adult. Biliary complications were most common, occurring in 6/20 recipients (30%), of whom most (5/6) were children.

Conclusions. We conclude that excellent patient and graft survival outcomes can be achieved with the coordinated local sharing of split liver grafts between pediatric and adult transplant centers. Biliary complications were the most frequent morbidity. We would encourage the increased application of local sharing between pediatric and adult liver transplant centers, and also consider opportunities for increased regional sharing.

Abstract# 825 Poster Board #-Session: P281-II

IMPACT OF GRAFT SIZE MISMATCHING ON OUTCOME IN PEDIATRIC SPLIT LIVER TRANSPLANTATION. Marco Spada,[1] Alessandro Aluffi,[1] Matteo Cescon,[1] Michela Guizzetti,[1] Alessandro Lucianetti,[1] Domenico Pinelli,[1] Giuliano Torre,[2] Bruno Gridelli,[3] Michele Colledan.[1] *[1]General and Transplantation Surgery, Ospedali Riuniti, Bergamo, Italy; [2]Pediatrics, Ospedali Riuniti, Bergamo, Italy; [3]Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, IsMETT, Palermo, Italy.*

Purpose: although split liver transplantation for pediatric patients is increasingly accepted, few data are available on graft size mismatch impact on the outcome.

Methods: two-hundred and thirty-two cases of pediatric primary, isolate, orthotopic liver transplantation were reviewed for graft size matching. One-hundred and seventy-one cases (74 %) were split liver transplants. Twenty-nine cases were urgent, receiving intensive care preoperatively. Graft weight was aveilable in 145 cases. Patients were categorized in four groups by graft-to-recipient weight ratio (GRWR): small grafts (SG; GRWR ≤ 1.5 %, 27 cases), medium grafts (MG; 1.5% < GRWR ≤ 3.5%, 62 cases), large grafts (LG; 3.5% < GRWR ≤ 6%, 47 cases), and extra large grafts (XLG; GRWR > 6%, 9 cases).

Results: SG were associated with larger and older recipients, but not with older donors; LG and XLG from extra-large donors were more used in urgent cases. Post-transplant bilirubine clearence was delayed in SG. Post-transplant surgical complications requiring relaparotomy were more frequent in SG, while no differences were observed in vascular, biliary and infectious complications and acute rejecton rates among groups. Unrelated death-censored graft survival in XLG (actuarial 75% at 1 year, and 63 % at 4 years) was significantly lower compared to that in SG(82% and 80%), MG (90% and 88%), and LG (84% and 82%), while differences was not significant considering elective cases (83%, 91%, 97%, and 75% at 1 year in SG, MG, LG, and XLG, respectively). Patients survival were similar among groups (actuarial 92%, 94%, 85%, and 81% at 1 year in SG, MG, LG, and XLG, respectively). A strong relationship was observed between GRWR and donor-to-recipient weight ratio (r=0.741).

Conclusion: Pediatric split liver transplantation can be safely performed with a wide range of GRWR. GRWR can be fairly predicted by means of donor-to-recipienent weight ratio.The use of SG led to an higher incidence of complication requiring relaparotomy, while the use of XLG resulted in a significant lower graft survival. The analysis of elective cases showed that SG and XLG can be used obtaining results comparable to those obtained with MG and LG.

Abstract# 826 Poster Board #-Session: P282-II

A SINGLE CENTER'S 34 YEARS OF EXPERIENCE WITH PEDIATRIC LIVER TRANSPLANTATION (LTx) FOR ALPHA-1-ANTITRYPSIN DEFICIENCY (AATD). J. Keith Melancon,[1] Harvey Sharp,[1] Angelika C. Gruessner,[1] Lakshmi Kanth,[1] A. Humar,[1] William D. Payne,[1] Rainer W. Gruessner.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Background: AATD is one of the most common metabolic liver diseases with excellent short-term outcome. We performed our first LTx for AATD in 4/69 and studied long-term medical and social outcome parameters in 3 immunosuppressive eras.

Methods: Between 4/96 and 11/03, 39 pediatric patients underwent LTxs for AATD. Of those, 34 were primary and 5 were retransplants. The median age at LTx was 8.4 years (range, 8 mos-17 yrs). The three immunosuppressive eras were: Era A (pre-CSA) 1969-1987, Era B (CSA) 1988-1995, and Era C (Tac) 1996-2002. Patient and graft survival were studied according to Kaplan-Meier. All survivors were interviewed and a quality of life assessment was performed.

Results: Currently, 68% of our patients are alive and well with functioning grafts. Best results were obtained in Era C, but 10-year patient survival in Era A was already 45%. (Table 1)

Table 1

	Era A (13 patients)	Era B (12 patients)	Era C (9 patients)
1 year survival	68%	95%	100%
10 year survival	45%	86%	n/a
Current survival	31%	83%	100%

Quality of life assessment for Era A patients (followup 15-34 yrs) showed that 80% had graduated from high school and of these, 90% had obtained a college or technical degree. Despite the use of steroids, normal growth was achieved in 90% of Era A patients. (Table 2)

Table 2

	Era A	Era B	Era C
Excellent Health	95%	95%	95%
Education on track	80%	90%	100%
Socially Active	95%	95%	100%
Normal Growth	90%	95%	95%
Stated contented	95%	95%	100%

Conclusions: LTx in children with AATD provides excellent long-term patient and graft survival. With a max followup of 34 yrs, we observed normal growth, social activity, and quality of life in ≥ 80% of the patients.

Abstract# 827 Poster Board #-Session: P283-II

INCREASED PREVALENCE OF AUTOANTIBODIES AFTER PEDIATRIC LIVER TRANSPLANTATION AND ITS CLINICAL IMPACT. Yaron Avitzur,[1] Annie Fecteau,[1] Vicky Lee Ng.[1] *[1]Paediatric Academic Multi-Organ Transplantation (PAMOT) Program, Hospital for Sick Children, Toronto, ON, Canada.*

Background: Elevated levels of serum autoantibodies (AAB) after orthotopic liver transplantation (OLT) can be associated with late graft dysfunction. However, the impact of isolated elevation of AAB on otherwise stable pediatric recipients is unknown. This study aimed to evaluate the prevalence, clinical significance, and risk factors of isolated elevation of AAB in children after OLT.

Methods: Children more than 6 months post-OLT with no history of hepatitis type C or autoimmune disease were prospectively recruited. A single blood specimen to determine levels of ANA, anti SM, anti LKM 1, and pANCA was drawn. Past medical history, physical exam and laboratory data were obtained to identify potential risk factors and to evaluate clinical significance of presence or absence of serum AAB.

Results: Sixty-eight children, median age of 9 (range 1.6-18.2) years and median time since OLT of 5 (range 0.6-15.1) years were consented for the study. Eighteen (26%) patients had at least one positive (titer≥1/20) AAB test with ANA being the most common (n=10). AAB prevalence increased over time with positive AAB in 39% of the patients >5 years post OLT vs. 14% of the patients <5 years post OLT. Time since OLT was identified as the only risk factor for development of AAB (RR 3.3, 95% CI 1.2-9). Patient demographics, reason for OLT, immunosuppressive regimens and infection history did not increase the risk for a positive AAB test. Patients with positive AAB test did not have increased risk for liver dysfunction, occurrence of chronic or acute rejection, or other autoimmune phenomenon.

Conclusions: The prevalence of AAB increases over time in survivors of pediatric OLT. Isolated elevation of AAB was not a risk factor for graft dysfunction or onset of de-novo autoimmune phenomenon and did not require immediate clinical intervention. Longer prospective follow-up is needed to determine if the presence of AAB can forecast patients at risk for late graft dysfunction.

Abstract# 828 Poster Board #-Session: P284-II

SUCCESSFUL ABO INCOMPATIBLE PEDIATRIC LIVER TRANSPLANTATION UTILIZING SELECTIVE PLASMAPHERESIS. Thomas G. Heffron,[1] David Welch,[2] Todd Pillen,[2] Gregory A. Smallwood,[3] Renee Romero.[4] *[1]Department of Surgery, Emory University School of Medicine, Atlanta, GA; [2]Liver Transplantation, Children's Healthcare of Atlanta, Atlanta, GA; [3]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [4]Pediatric Medicine, Emory University School of Medicine, Atlanta, GA.*

Body: Background: It has been reported that ABO incompatible grafts during liver transplantation results in lower patient and graft survival as well as an increase in incidence and severity of rejection.

Aim: The aim of this study is to report our experience of emergent transplantation of 11 pediatric recipients utilizing ABO incompatible, mismatched grafts while using selective plasmapheresis with an induction protocol using daclizumab with tacrolimus, mycophenolate and steroids.

Methods: From July 1998 to November 2003, 115 liver transplants in 101 pediatric pediatric recipients were prospectively followed for outcomes of children receiving an ABO incompatible allografts emergently. Additional intravenous methylprednisolone (10-20 mg/kg per dose) was administered when evidence of hemolysis was confirmed by increased AB antibody titers on serial isohemagglutinins assay. If the titers did not respond to methylprednisolone, plasmapheresis was instituted. Total plasma exchange and splenectomy were not used for pre-transplant induction treatment.

Results: ABO incompatible liver allograft (n=11) consisted of group pairings which included blood type B donors to A liver recipient (n = 4), A to B (n = 2), A to O (n=4), and AB to A (n =1). Two of eleven (18%) children with ABO incompatible allografts had three rejection episodes compared with 48/91 (53%) children with 111 rejection episodes in control (p=0.03). The incompatible liver group was similar in age (8.1 6.2 years vs. 6.5 6.2 years; p = NS), time to first rejection (135 178 days vs. 340 396 days; p = NS), with the contrrol group having longer follow-up (573 372 days vs. 930 568 days, p = 0.013). Only one patient unresponsive to steroids received plasmapheresis when anti-B titers increased to 1:1024 and t.bilirubin increased to 8.4 mg/dl. Plasmapheresis was instituted for 10 days, 80% total blood replacement for three days and 150% replacement for 7 days. One child lost their allograft following a percutaneous transhepatic cholangiogram due to a intra-hepatic hematoma. Patient actuarial survival for ABO matched children vs ABO incompatible children was 84/90(93.3%), versus 11/11 (100%) and graft survival was 84/101 (89.7%) vs 10/11 (91%) at one year.

Conclusion: ABO mismatched grafts can be used without an increase in patient/graft mortality or an increase in rejections when an enhanced immunosuppressive protocol of daclizumab/tacrolimus/mycophenolate/prednisone is used with selective plasmapheresis.

Abstract# 829 Poster Board #-Session: P285-II

LONG TERM RENAL FUNCTION IN CHILDREN AFTER LIVER TRANSPLANTATION (OLT) WITH CALCINEURIN INHIBITOR (CNI) BASED IMMUNOSUPPRESSION. Silke Wiesmayr,[1] Helmut Ellemunter,[1] Johannes Eder,[1] Alfred Königsrainer,[2] Wolfgang Steurer,[2] Raimund Margraiter,[2] Lothar Bernd Zimmerhackl.[1] *[1]Pediatrics, Leopold-Franzens-University, Innsbruck, Austria; [2]Transplant Surgery, Leopold-Franzens-University, Innsbruck, Austria.*

Introduction: Nephrotoxic side-effects of CNI are of critical importance in clinical outcome and "quality of life" after pediatric transplantation. Long-term renal function after OLT in children under CNI based immunosuppression is not well documented.

Patients and methods: Longitudinal data of 31 children (19 male,12 female) transplanted after 1990 were acquired. Mean age at transplantation was 6,3 ± 5,8 years (mean ± SD) with a range from 0,3 to 24,6 years. Mean follow-up was 5,2 ± 3,5 years (range 0,4 to 15,7). Multifactorial causes lead to the liver failure.The immunosuppressive therapy included neoral (CSA)/tacrolimus (TAC) and azathioprin/ mycophenolate mofetil with prednisone.16 patients were treated with TAC, 15 with CSA based immunosuppressive regimen. Glomerular filtration rate was estimated using the Schwartz formula (calculated GFR (ml/min/1,73 m²) = k x height (cm) / serum creatinine).

Results: Before transplantation GFR was elevated significantly with 159,5 ± 38,2 ; after OLT GFR decreased significantly in both treatment groups within one month into the normal range within the CSA (105,8±18,1) group (and similarly in the TAC (108,4±32,8group respectively (p< 0,05 pre versus post). In long term follow-up GFR remained constant with a GFR of 109,6 ± 40,7 at two years and 107,4 ± 31,4 at six years after OLT. Mathematical modeling of renal function using exponential one compartment model (y= *a**exp*b*x) did not demonstrate decrease in GFR even for very long observation times with a positive *b* for both treatment groups. This indicates again that in this patient group GFR was maintained.

Conclusion: Liver insufficiency leads to glomerular hyperfiltration pre transplantation. After OLT GFR normalizes within weeks and remains stable even in long term follow-up. Unlike renal transplantation the potential nephrotoxic side effects of CNI do not necessarily cause renal insufficiency even in long term observation time after pediatric liver transplantation. If lower trough levels of CNI in OLT versus renal transplantation or other factors are important to explain these striking differences remains to be elucidated.

Abstract# 830 Poster Board #-Session: P286-II

LONG-TERM OUTCOMES WITH ANTI-CD20 MONOCLONAL ANTIBODY IN THE MANAGEMENT OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS. I-Fen Chang,[2] Ruben E. Quiros-Tejeira,[1,3] Saul J. Karpen,[1] Lisa J. Bristow,[1] Jaymee D. Scott,[1] John A. Goss.[3] *[1]Pediatrics, Texas Children's Hospital, Houston, TX; [2]Pharmacy, Texas Children's Hospital, Houston, TX; [3]Surgery, Baylor College of Medicine, Houston, TX.*

Background: Post-transplant lymphoproliferative disorders (PTLD) are a well-known complication following organ transplantation. Pediatric liver transplant (LTx) patients are at increased risk, since many of them are Epstein-Barr Virus (EBV) sero-negative at the time of transplant. Much data confirms the positive correlation of EBV viral load and PTLD. The standard of care in managing PTLD is reduction or withdrawal of immunosuppression; however, this is associated with a high incidence of mortality due to graft rejection. Recently, rituximab, a chimeric humanized anti-CD20 monoclonal antibody (MoAb) has opened new treatment possibilities. **Purpose**: Although much experience has been accumulated on short-term effects on EBV-associated PTLD, we sought to determine the long-term treatment outcomes with anti-CD20 MoAb and baseline immunosuppression. **Methods and Results**: From 1998-2003, 5 pediatric LTx patients (median age 73 months; range: 31-105 months) were diagnosed with EBV-associated PTLD. Diagnosis was made by presence of bulky lesions or progressive disseminated disease along with proliferation of B-cells expressing the CD20 antigen. At the time of diagnosis, the mean EBV viral load measured by PCR of peripheral blood was 14,076 copies/mcg of DNA. Patients were treated with 4 weekly intravenous doses of anti-CD20 MoAb at 375 mg/m². Immunosuppression was modified to maintain a tacrolimus or cyclosporine trough concentration between 3-6 ng/ml or 50-75 ng/ml, respectively. Clinical remission of PTLD was achieved 4 weeks after completing therapy with an absence of B-cells and a decrease in EBV viral load to a mean of 73 copies/mcg of DNA. Two patients reached undetectable levels of EBV titer 4 weeks after completion of anti-CD20 MoAb, and of these, 1 patient maintained a negative EBV titer. Five months after therapy, B-cells returned to a level of >1% in all patients, and we noted that EBV titers also increased to a mean of 1,458 copies/mcg (range: 0-4246 copies/mcg) of DNA. All patients are alive and disease free after a median follow-up of 23 months (range: 10-32 months). No patient has experienced recurrence of disease, even with the resurgence of EBV viremia. **Conclusion**: Anti-CD20 MoAb along with a reduction in immunosuppression is effective in the long-term management of PTLD. However, long-term follow-up is necessary with the resurgence of EBV viremia and the potential for recurrence of PTLD.

Abstract# 831 Poster Board #-Session: P287-II

SIGNIFICANCE OF SERIAL MONITORING OF REAL-TIME EBV PCR IN PEDIATRIC LIVING DONOR LIVER TRANSPLANTAITON. Hiroyuki Furukawa,[1] Tsuyoshi Shimamura,[2] Maeng Bong Jin,[1] Tomomi Suzuki,[3] Masahiko Taniguchi,[3] Masahiro Hattori,[3] Toshiya Kamiyama,[3] Michiaki Matsushita,[3] Satoru Todo.[3] *[1]Dept. of Organ Transplantation and Regenerative Medicine, Hokkaido University School of Medicine, Sapporo, Japan; [2]Devision of Organ Transplantation, Hokkaido University Hospital, Sapporo, Japan; [3]Dept. of General Surgery, Hokkaido University School of Medicine, Sapporo, Japan.*

BACKGROUND: Quantitative analysis of the Epstein-Barr virus (EBV) genome has been recently reported to be helpful for early identification of EBV viremia which could reduce the risk of EBV infection and post-transplantation lymphoproliferative disorder(PTLD).

AIM: To demonstrate the significance of serial monitoring of EBV genome load by real-time quantitative polymerase chain reaction (PCR) after pediatric living donor liver transplantation.

METHODS: From Sep 1997 to June 2003, the EBV genome load in peripheral blood mononuclear cells was measured serially in a total of 25 consecutive pediatric recipients of living donor liver transplantation (LDLT) with a minimum of 6 months follow up. EBV PCR was measured every week for 8 weeks after LDLT and every 2-4 weeks thereafter. EBV DNA levels were expressed in copies/microg DNA.

RESULTS: Eight of 25 (28%) developed URS (n=4), diarrhea (n=3), fever (n=2), severe stomatitis (n=1), liver dysfunction (n=1), and megaroblastic anemia (n=1) with significantly elevated EBV DNA levels with peak values ranged from 531 to 8530 copies/microg DNA(mean 2843+-1971). The patients were treated by reduction or discontinuation of immunosuppressants and administration of acyclovir. The EBV DNA levels decreased in all these patients following the recovery from their symptoms. One case developed two episodes of PTLD; first episode in intestine and peritracheal lymphnodes with peak EBV DNA level of 421 copies/microg DNA POD 103, second in neck and inguinal lymphnodes with peak EBV DNA level of 2700 copies/microg DNA POD 194. Both episodes were successfully treated immunoglobulin and gancyclovir with either reduction or discontinuation of immunosuppression. Seven of 8 recipients with EBV infection and one with PTLD were EBV seronegative, who received EBV seropositive grafts. The other one with EBV infection was EBV-seronegative, receiveing EBV-seronegative graft.

CONCLUSIONS: Serial quantitative analysis of the EBV genome load by means of real-time PCR are thought to be useful for early detection and treatment of EBV infection as well as PTLD through adjustment of the immunosuppression level. EBV-seropositive to seronegative combination seems to be high risk for EBV infection and PTLD.

Abstract# 832 Poster Board #-Session: P288-II

IMMUNOSPRESSION FOR LIVER TRANSPLANT (LTx) IN INFANTS WITH SHORT BOWEL SYNDROME (SBS). Dominic Dell-Olio,[1] Khalid Sharif,[1] Carla Lloyd,[1] Sara Clarke,[2] Patrick J. McKiernan,[1] Indra V. van Mourik,[1] Deirdre A. Kelly,[1] Susan V. Beath.[1] *[1]The Liver Unit, Birmingham Childrens Hospital, Birmingham, United Kingdom; [2]Dietetic Department, Birmingham Children's Hospital, Birmingham, United Kingdom.*

Isolated LTx is indicated in infants with SBS and intestinal failure associated liver disease (IFALD) who may have a potential for intestinal adaptation. The management of LTx in these patients is complicated by the pre-existing intestinal failure and its unpredictable effects on immunosuppressant drug (IS) absorption and metabolism.

Aim: To compare IS requirements between infants following LTx either for SBS and IFALD or extra hepatic biliary atresia (EHBA) from 1998-2003.

Subjects: 7 patients with SBS (6M), median (range) age at LTx 11.2m, (5-25), median (range) weight 7.4kg (5.3 - 8.9), median (range) z-score -1.44 (-3.64 to -0.11) followed for at least 6 months after LTx. SBS was secondary to gastroschisis (3), necrotising enterocolitis (4). 15 patients EHBA (8M) matched for median (range) age 12.4m (5-23) and median weight (range) 8.2 kg (4.5-12.2), median (range) z-score -1.45 (-3.9 to 0.73). Both groups received dual therapy IS, Tacrolimus (Tac) and steroids. 2/7 SBS received Tac as capsules, 6/15 EHBA received Tac as granules. Remainder received Tac as oral suspension.

Methods: Retrospective review of clinical and demographic data. Statistical analysis by non-parametric methods. Data expressed as medians. Tac dose normalised level is expressed as the ratio of 12hr trough level:dose in mg/kg/day. Acute rejection defined as histologically proven occurring <6m after LTx.

Results:

Three months post LTx

	Tac mg/kg/day	Tac level ng/ml	Normalised Tac level	Steroids mg/kg/day
SBS (n=7)	0.19	5.8	33.1	0.34
EHBA (n=15)	0.35	7.9	23.3	0.35
p-value	ns	0.045	ns	ns

6 months post LTx

	Tac mg/kg/day	Tac level ng/ml	Normalised Tac level	Steroids
SBS (n=7)	0.12	5.0	42.4	0.31
EHBA (n=15)	0.23	8.0	36.0	0.4
p-value	ns	0.042	ns	ns

Median follow up after LTx: 16.3 months in SBS; 20.8 months in EHBA. Survival was 100% in both groups. 1/7 patients with SBS developed acute rejection compared with 10/15 EHBA (p=0.06).

Summary: The SBS patients showed lower IS requirements than the EHBA group and a lower rejection rate.

Conclusion : Isolated LTx in SBS is associated with an excellent medium term outcome and a good allograft tolerance. The anticipated problems with the bioavailability of oral IS were not evident.

Acknowledgement: We are grateful to Dr S Protheroe, Dr. D. Wilson, Dr. D. Dalzell, Professor C.J. Taylor for referring their patients and Dr. M.S. Murphy for sharing care.

Abstract# 833 Poster Board #-Session: P289-II

BONE MINERAL STATUS IN CHILDREN AGED OVER 3 YEARS UNDERGOING LIVER TRANSPLANTATION. Helen M. Evans,[1] Sharon M. Bant,[1] Nicola J. Crabtree,[2] William D. Fraser,[3] Deirdre A. Kelly,[1] Patrick J. McKiernan,[1] Nicholas J. Shaw.[4] *[1]The Liver Unit, Birmingham Children's Hospital, Birmingham, West Midlands, United Kingdom; [2]Department of Nuclear Medicine, University Hospital of Birmingham, Birmingham, West Midlands, United Kingdom; [3]Department of Clinical Chemistry, The University of Liverpool, Liverpool, Merseyside, United Kingdom; [4]Department of Endocrinology, Birmingham Children's Hospital, Brimingham, West Midlands, United Kingdom.*

Background: Children with chronic liver disease are at risk of reduced bone mineral density (BMD) which may worsen in the months following liver transplantation (OLT). Probable aetiological factors include vitamin D (vit D) malabsorption, immobility and immunosuppressive drugs.

Aim: To assess bone mineral health and vit D status in children over 3 years undergoing OLT.

Methods: Children with cholestatic liver disease underwent dual energy X-ray absorptiometry (DXA) at the time of listing for OLT and bone mineral density was compared to normative values for children over 3 years. Volumetric bone density (BMAD) was calculated for the lumbar spine to adjust for body size. Bone ions, parathyroid hormone (PTH) and vit D metabolites were also measured.

Results: 12 children (6 M; median age 9.4 yrs (3.0 to 14.6 yrs)) underwent assessment at a median of 1.5 months pre OLT (0 to 6.9 months). All were ambulant and only 1 previously had fractures. 10 were receiving vit D supplements and 7 supplemental feeding. Median height and weight z-scores were –0.7 (-4.8 to 3.0) and –0.2 (-3.3 to 1.8) respectively. Ionised calcium, phosphate and magnesium were low in 4, 7 and 3 children respectively but no child had a raised serum PTH. Although 9/10 and 5/10 had low 25-OH vit D2 and 25-OH vit D3 respectively, 1,25-$(OH)_2$ vit D was normal in all cases. Median BMD z-score for L2 to L4 lumbar spine was -1.2 (-2.5 to 0.02) and for total body was -1.0 (-1.61 to -0.34). Median BMAD z-score was -0.7 (-3.0 to -1.3).

Conclusions: In children aged over 3 undergoing OLT, bone mineral density and vitamin D and PTH status were better than anticipated. Optimal bone health can be achieved in children with chronic liver disease prior to OLT using vitamin D supplementation and ensuring optimal nutrition.

Abstract# 834 Poster Board #-Session: P290-II

GLUCOSE INTOLERANCE IN LONG-TERM PEDIATRIC LIVER TRANSPLANT SURVIVORS. Nada Yazigi,[1] Fredrick Ryckman,[1] Maria Alonso,[1] Gregory Tiao,[1] William Balistreri,[1] John Bucuvalas.[1] *[1]Pediatric Liver Care Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Little is known about de novo glucose intolerance (GI) after pediatric liver transplantation (LT). Given the potential impact of GI on long term survival and quality of life, it is important to identify those children at increased risk for GI, and the effects of GI on morbidity.

Purpose: To determine the prevalence of de novo GI as defined by the need for insulin treatment within 30 days of transplantation (insulin group), and to define the prevalence of GI at one year post-LT as defined by fasting blood sugar (FBS) >126mg/ml or insulin therapy. Secondary aims were to identify the predictors of GI in children undergoing liver transplantation, and assess the impact of GI on graft rejection (acute cellular (AR) and ductopenic (CR)) and mortality.

Methods: We conducted a single center retrospective cohort study of 123 children who underwent 126 LT from 7/96 to 7/02. All patients but one received Tacrolimus and corticosteroids. Demographic and clinical data were obtained from a prospectively maintained LT database. Independent variables were primary diagnosis, age at LT, UNOS status at the time of transplant. We performed univariate analyses to identify independent predictors of diabetes, and compare outcomes.

Results: Three patients received a second transplant during this period. Twenty one patients required insulin treatment within 30 days post-LT; at one year post-LT, 3 had recurrent or continuous insulin requirement, and one had a high FBS. In the non-insulin group, 6/102 required insulin with steroid cycles, and 2 had high FBS at one year. In univariate analysis, early GI correlated with a higher mortality rate (15/102 v/s 5/21). There was no difference in the incidence of AR (55/102 v/s 11/21), or CR (3/102 v/s 0/21). Risk factors included advanced age (mean age 9.3 years v/s 1.5 years), immune and metabolic liver disorders, re-transplantation (8/102 v/s 7/21), acute presentation (UNOS status one 31/102 v/s 11/21).

Conclusions: GI is an uncommon complication in children who are long-term survivors of LT. Early insulin requirement highlights a high risk group. Follow up on long term complications is also needed in those who do improve past the acute period, or maintain GI, particularly in regards to their kidney function, lipid status and cardio-vascular risk.

Abstract# 835 Poster Board #-Session: P291-II

THE IMACT OF PRE-TRANSPLANT NUTRITION ON GROWTH AND DEVELOPMENT IN CHILDREN WITH BILIARY ATRESIA AWAITING LIVER TRANSPLANT. Kathleen P. Falkenstein.[1] *[1]Solid Organ Transplant, AIDupont Hospital, Wilmington, DE.*

The purpose of this study was to examine the effects of nutrition, oral vs. nasogastric, on growth and development in children with biliary atresia who are waiting for a liver transplant. Also examined was how the chronic illness impacted on the family. The subjects were a convenience sample of 24 children with biliary atresia who were awaiting liver transplant. Twelve of the children received nutrition by mouth only. The other twelve received oral as well as nasogastric feedings.

At the time of referral for transplant evaluation, which served as baseline for this study, the ages of the children ranged from 2 months to 30 months. The mean age of those who received oral feedings was 10.6 months and was 4.6 months among those who also received nasogastric feedings.

A prospective design was implemented to follow the children from their transplant evaluation to six months post evaluation. Anthropometrics growth measurements of length, standard deviation scores for height (SDS), weight, head circumference, midarm circumference (MAC), tricep skinfold thickness (TSF), and nutritional assessment were recorded bimonthly. The children served as their own controls.

Developmental evaluation using the Mullen Scales for Early Learning, were performed at the baseline evaluation and six months later. A family assessment, using the Stein Impact of the Illness of the Family Scale, was used to evaluate the family's level of stress at six months post baseline evaluation.

Repeated measures analysis of variance were performed separately on the oral and nasogastric feeding groups to evaluate changes in body length, SDS, weight, head circumference, MAC, TSF and nutritional intake (calorie per kilogram of body weight). Both groups demonstrated significant gains over time in height, weight, head circumference, and midarm circumference. The results of a paired t-test did not show a significant developmental change among the children in the oral feeding group. However, the children in the nasogastric group, demonstrated a significant decline in expressive language, and visual reception raw scores. Based on the results of an independent t-test, utilizing the Stein Impact of the Illness of the Family scale there were no significant differences in the level of stress between the two feeding groups.

Abstract# 836 Poster Board #-Session: P292-II

THE USE OF RAPAMYCIN AND AZATHIOPRINE IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS. Nanda Kerkar,[1] Christina Dugan,[1] Carolina Rumbo,[1] Nancy Krieger,[1] Gabriel Gondolesi,[1] Benjamin Shneider,[1] Sukru Emre.[1] *[1]Pediatric Liver/Liver Transplant, RMTI, Mount Sinai Medical Center, New York, NY.*

Maintenance immunosuppression for pediatric liver transplant recipients in our unit is with tacrolimus and prednisolone. We retrospectively reviewed the circumstances for using rapamycin (rapa) or azathioprine (aza) in selected patients. Of the 9 patients on rapa; 3 had autoimmune hepatitis (AIH), 3 developed 'de novo' AIH. Five of these 6 children had previously been on aza or mycophenolate mofetil (MMF), but were switched to rapa (cross over) as their AIH was not controlled. A diagnosis of 'de novo' AIH was made in the presence of interface hepatitis on liver biopsy, antibodies (antinuclear antibody, smooth muscle antibody, liver kidney microsomal antibody) in the serum, and presence of increased IgG following transplant in children who did not have AIH before transplant. Two were on rapa as they were transplanted for malignancy. The 9th child had rapa added when she developed late hepatic artery stenosis after an episode of rejection. The dose of rapa was titrated to maintain levels of 5-8mcg/dl.

In 15 other children aza was added: 6 'de novo' AIH, 6 with AIH before liver transplant, 2 who were transplanted elsewhere with aza as part of primary regime, and 1 who was converted to cyclosporine and aza when she developed tacrolimus toxicity. Aza doses were typically titrated to yield 6-thioguanine levels >235 pmoles per 8 x 10^8 RBC.

table1

Diagnosis	Subjects	Rapamycin	Azathioprine
	Number/Female	n=9, 6F	N=15, 11F
	Age median (range)	5 yrs(0.4 - 14.7)	1.6 yrs (o.5-18.7)
AIH	Responders	3 (cross over in all)	6
	Non-responders	0	0
De novo AIH	Responders	3 (cross over in 2)	5
	Non-responders	0	1*
Other		3	3
Adverse events		1-cellulitis	3 - UTI, pneumonia. stomatitis
Graft function at last follow-up	Normal	7	13
	Abnormal	2(1)*	2*

Poor adherence*

The only non-responder was a child with severe non-adherence issues. One of the children with abnormal graft function in the rapa group had several other complications including chronic rejection and has had rapa withdrawn on suspicion of lymphoproliferative disease. In conclusion, it is possible to achieve stable graft function in liver transplant recipients with a diagnosis of AIH before and after liver transplantation by using rapa or aza. In our experience, rapa has been successful in cases where aza was not, especially in AIH and 'de novo' AIH (cross over patients above). Adverse events have been minimal and monitoring drug levels is essential for optimal drug dosage. Adherence remains an important issue for long term stable graft function.

Abstract# 837 Poster Board #-Session: P293-II

PEDIATRIC LIVER TRANSPLANTATION WITHOUT THE USE OF MICROSURGICAL TECHNIQUES. Thomas G. Heffron,[1] David Welch,[2] Todd Pillen,[2] Gregory A. Smallwood,[3] Rene Romero.[4] *[1]Department of Surgery, Emory University School of Medicine, Atlanta, GA; [2]Liver Transplantation, Children's Healthcare of Atlanta, Atlanta, GA; [3]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [4]Pediatric Medicine, Emory University School of Medicine, Atlanta, GA.*

Background: Hepatic artery thrombosis (HAT) after pediatric liver transplantation has been attributed to lack of the use of an operating microscope, small recipient size and/or weight, interposition grafts, lack of the postoperative use of anticoagulants (eg. heparin or low molecular weight dextran).

Aim: The purpose of this review is to report our experience focusing on the interrelationships between risk factors, surgical technique and the incidence pediatric hepatic artery thrombosis.

Methods: From the period 02/01/1997 to 06/01/2003, all liver transplants were prospectively tracked for HAT. All hepatic arterial anastomoses were performed utilizing 3.5-6 magnification loupes. All patients underwent intraoperative ultrasound with Doppler flow studies after allograft revascularization daily for three postoperative days. Anticoagulation consisted of aspirin in all patients for the first three months post transplant with or without alprostadil (PGE_1) for the first seven post-op days.

Results: 141 consecutive liver transplants were preformed in 125 pediatric patients. 75 grafts (53%) in 69patients were whole organ transplants while 66 grafts (47%) in 56patients were partial livers. Of partial grafts, 22 left segments and 1 right lobe were from living donors. Children receiving whole livers were older (9.47 ± 6.76 years vs. 3.25 ±3.59 years; p = 0.001) and weighted more (35.1 ±26.7 kg vs. 16.2 ±13.2 kg; p = 0.001). In the whole liver group13 liver grafts (18.8 %) were transplanted into children less than 10 kg. while in the partial liver group 21 (37.5 %) were less than 10 kg. Seven iliac jump graft arteries were anastomosed using an interposition technique from the infrarenal aorta to the donor artery. while 136 allografts underwent donor hepatic artery reconstruction, end to end. In the study period, 3/141 (2.1%) grafts developed HAT, partial liver grafts 3/66 (4.5%), while whole liver grafts were 0/75 (0%). Of the three patients with HAT, two were successfully revascularized in the first 24 hours. Therefore 1/141(.71%) liver grafts were lost to HAT. No patient death was associated with HAT. Overal actuarial survival at one and two years was 95.7% and 93.0% respectively.

Conclusions: HAT may be minimized in the pediatric transplantation population without the use of intraoperative microscopes, heparin, urokinase, or dipyridamole. Surgical loupes, anticoagulation utilizing aspirin with alprostadil were adequate to minimize HAT in the pediatric population.

TISSUE INJURY II

Abstract# 838 Poster Board #-Session: P294-II

INITIAL SELECTIN BLOCKADE OF T CELL ACTIVATION AND SIGNALLING COMBINED WITH LOW-DOSE SIROLIMUS/CsA IMMUNOSUPPRESSION PREVENTS CHRONIC REJECTION OF KIDNEY ALLOGRAFTS. Martin Gasser,[1] Miriam S. Lenhard,[2] Michael Grimm,[2] Martin Grimm,[2] Gray D. Shaw,[3] Wayne W. Hancock,[4] Arnulf Thiede,[1] Nicholas L. Tilney,[5] Ana Maria Waaga-Gasser.[2] *[1]Dept. of Surgery, University of Wuerzburg, Wuerzburg, Bavaria, Germany; [2]Dept. of Surgery, Molecular Oncoimmunology, University of Wuerzburg, Wuerzburg, Bavaria, Germany; [3]Genetics Institute, Cambridge, MA; [4]Dept. of Pathology, The Children's Hospital, Philadelphia, PA; [5]Dept. of Surgery, Surgical Research Lab, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

We examined the efficacy of recombinant soluble P-selectin glycoprotein ligand (rPSGL-Ig) ± sirolimus (SRL) ± cyclosporine (CsA) in treating the accelerated chronic rejection (CR) of renal allografts from brain dead (BD) F344 donors transplanted into LEW recipients.

Materials and Methods: rPSGL-Ig (50µg iv) was administered to the donor 3hrs after BD and immediately to the host after transplantation to inhibit initial cellular activity. As shown previously, this strategy inhibits T cell activation and signalling. SRL±CsA was given to immunosuppress subsequent antigen-dependent responses. Grafts from all recipient groups (n=8-12/Gp) were examined histologically at 150 days. Activated CD4+CD25+ T lymphocytes and T cell signalling (signal 1 and 2: TCR/MHC class II and CTLA-4/B7-1/B7/2/CD28) were assessed using double immunostaining. Gene expression of IL-2, IL-10, IFN-γ, TNF-α, TGF-β, and ICAM-1 were measured with RNAse protection assay and Real Time PCR. Recipient groups included: Gp1=isografts; Gp 2=BD donor allografts in rats treated with CsA 1mg daily x 10days; Gp 3=SRL 0.4mg daily x 21; Gp4=SRL(as above)+rPSGLIg (as above); Gp5=SRL+CsA; Gp6=SRL+CsA+rPSGL-Ig; Gp7=SRL 0.1mgx4-13 days+CsA+rPSGL-Ig.

Results: At 150 days after transplantation, Gp1 grafts showed only minor (1/4+) signs of CR (tubular atrophy, interstitial fibrosis, and vascular obliteration). CR of Gp 2 allografts was moderate (2/4+) and extensive in Gp 3 allografts (3/4+). The addition of rPSGL-Ig protected the allografts of Gp4 hosts substantially (1/4+). Severe tubular

injury (4/4+) occurred with high dose SRL+CsA treatment (Gp5), regardless of rPSGL-Ig (Gp6). However, grafts of Gp7 (low dose SRL+CsA+rPSGL-Ig) resembled isografts with inhibited gene expression (IL-2, IFN-γ, and TGF-β), sporadic CD4+CD25+ cells, and signal 1+2.
Conclusions: Organs from recipients treated with high dose SRL±CsA showed severe chronic changes, regardless of the effects of rPSGL-Ig on initial T cell activity. In contrast, selectin blockade acts synergistically with low dose SRL+CsA to prevent CR of renal allografts from BD donors over the long-term.

Abstract# 839 Poster Board #-Session: P295-II

THE EFFECT OF HEPATIC ISCHEMIA/REPERFUSION INJURY ON EXPRESSION OF RAE-1 AND H60 GENES IN MICE LIVER. Feng Cheng, You-ping Li, Jing-qiu Cheng, Li Feng, Ying He, Zi-zhen Yie. *Lab of Transplant Engineering and Immunology, West China Hospital, Sichuan University, Chengdu, 610041, Sichuan, China.*

Documented reports have demonstrated that ischemia/reperfusion injury (IRI) might promote the development of chronic graft dysfunction (CGD). However, the mechanism has not been well-defined. It has been found that MHC class I chain-related antigen A (MICA) and B (MICB), the ligands for NKG2D (an activating receptor of natural killer cells), could be induced through cellular stress, and were closely related with CGD. The purpose of this study is to investigate whether IRI could upregulate expression of RAE-1 and H60 (MICA/B homologues) genes in mice and then trigger CGD by activating NK cells. **Methods:** Male Balb/c mice weighing 22 to 25*g* were anesthetized with sodium pentobarbital, then a midline laparotomy was performed and an atraumatic clip was used to interrupt the arterial and the portal venous blood supply to the left lob of the liver. After 90 minutes of partial hepatic ischemia, the clip was removed initiating hepatic reperfusion. Sham control mice underwent the same protocol, but without vascular occlusion. The sham and ischemic lobs were taken at intervals of 1, 2, 3, 5, 7, 10, 15, 20, and 30 days postoperation for analysis. Total RNA was extracted from liver tissue and quantified by UV spectrophotometer. RNA (1 μg) was reverse transcribed and amplified by real-time quantitative PCR. All samples were detected in triplicate, and the change folds of RAE-1 and H60 mRNA were shown by the ratios of ischemic sample mRNA/sham sample mRNA.**Results:** Compared with the mRNA level of sham control, IRI caused a decrease of RAE-1 mRNA level in ischemic liver from day 1 to day 3 after surgery, while the RAE-1 mRNA levels increased by day 5 to day 30. Starting on day 7 and persisting to day 30, RAE-1 mRNA levels were increased by 2.5 to 5 times in the ischemic liver over the sham control. On the other hand, IRI caused a 2-6 fold increase in H60 mRNA level from day 1 to day 20 over the sham control, however, H60 mRNA level was decreased by day 30 after surgery. The change patterns of RAE-1 and H60 mRNA levels caused by hepatic IRI were apparently different.**Conclusion:** This study first report that hepatic IRI increased RAE-1 and H60 mRNA levels in ischemic mouse liver over the sham control with different change patterns, though the mechanism was unknown. The upregulated expression of these NKG2D ligands might activate NK cells and play a significant role in innate immunity associated with transplantation and then promote CGD, and these molecules may become the new interfering targets for prevention.

Abstract# 840 Poster Board #-Session: P296-II

NEW INSIGHTS INTO A ROLE FOR VEGF AND ITS RECEPTORS IN LIVER ISCHEMIA-REPERFUSION INJURY. Yoshikazu Tsurui,[1] Masayuki Sho,[1] Yukiyasu Kuzumoto,[1] Satoru Akashi,[1] Hisanori Kashizuka,[1] Naoya Ikeda,[1] Mizuno Takashi,[1] Daniel J. Hicklin,[2] David M. Briscoe,[1] Yoshiyuki Nakajima.[1] *[1]First Department of Surgery, Nara Medical University, Kashihara, Nara, Japan; [2]ImClone Systems Inc., New York, NY; [3]Department of Internal Medicine, Children's Hospital, Boston, MA.*

Background: VEGF, a major angiogenesis factor, is also a pro-inflammatory cytokine and plays a critical role in a variety of physiological and pathological immune response. It is well established to be induced by hypoxia, suggesting that its expression will be characteristic of transplantation. However, little is known of its role in post-transplantation ischemia/reperfusion (I/R) injury. In this study, we investigated the expression and function of VEGF and its receptors (flt-1 and flk-1) in I/R injury. Methods and Results: Seventy percent partial hepatic ischemia was performed for 75 minutes in male C57BL/6 mice. First, we evaluated the local expression of VEGF and receptors in the liver using quantative real-time PCR. VEGF expression was significantly up-regulated after 2h of reperfusion following 75 minutes of ischemia, while flt-1 and flk-1 expression was down-regulated after reperfusion. Next, we administrated anti-flk-1 (DC101) and anti-flt-1 (MF-1) monoclonal antibodies (mAb, 1mg of each) 30 minutes before reperfusion. Interestingly, mAb treatment significantly inhibited hepatic injury compared to control at 6 h of reperfusion (sAST: P=0.0068, sALT: P=0.034, sLDH: P=0.006). Histological analysis also revealed the protective effect of targeting VEGF receptors on hepatic damage. Massive cellular infiltration and extensive hepatic cellular necrosis was observed in control mice, while the lobular architecture was relatively preserved and there was less necrosis in mice treated with mAb of VEGF receptors. Since VEGF may function in vivo via alterations in leukocyte trafficking, we also evaluated intragraft expression of chemokines and adhesion molecule in control and antibody-treated grafts using real-time PCR. We found that blockade of flt-1 and flk-1 significantly down-regulated the local expression of the VEGF-regulated pro-inflammatory chemokine MCP-1 and the adhesion molecule E-selectin after 2h of reperfusion. Conclusion: This study demonstrates for the first time that VEGF is expressed and is functional in hepatic I/R injury. We suggest that blockade of VEGF via inhibition of its receptors may represent a novel target for the protection of the liver in the peri-transplant period.

Abstract# 841 Poster Board #-Session: P297-II

OBJECTIVE AND RAPID ASSESSMENT OF PANCREAS GRAFT VIABILITY USING 31P-NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY COMBINED WITH TWO-LAYER COLD STORAGE METHOD. Takuro Yoshikawa,[1] Yasuyuki Suzuki,[1] Masaru Kanashiro,[2] Shiri Li,[1] Tadahiro Goto,[1] Tomohiro Tanaka,[1] Keitaro Kakinoki,[1] Tetsuya Sakai,[1] Yasuki Tanioka,[1] Yasuhiro Fujino,[1] Yoshikazu Kuroda.[1] *[1]Division of Gastroenterological Surgery, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan; [2]Laboratory of Nuclear Magnetic Resonance Research Institute, National Cardiovascular Center, Suita, Osaka, Japan.*

(Background) With the current shortage of cadaver donors and the increasing number of diabetic patients on the transplant waiting list, there is a critical need to optimally use "less-than-ideal" donors for pancreas transplantation. However, there are no objective and rapid means for assessing pancreas graft viability and suitability for transplantation. In this study, we examined the possibility of graft viability assessment and post-transplant outcome prediction using 31P-Nuclear Magnetic Resonance (NMR) spectroscopy combined with two-layer cold storage method (TLM) preservation. (Methods) Segmental canine pancreas grafts were preserved with TLM for 24 hours after 0, 60 or 120 minutes of warm ischemia (Group 1, 2 or 3, respectively). After preservation, we determined intragraft phosphate metabolites non-invasively using 31P-NMR spectroscopy. Time required for this assessment was only 5 minutes. Since all grafts in groups 1 and 2 were successfully transplanted (the viable group) while all in group 3 failed to survive after transplantation (the non-viable group) based on our previous study, possibility of post-transplant outcome prediction was examined based on the comparison between these two groups. (Results) The ratios of Pi/γ-ATP and Pi/β-ATP reflected the extent of graft damage and the differences were statistically significant among groups 1, 2 and 3. Based on analyses of receiver operator characteristic (ROC) curves, the optimum cutoff levels between the viable and non-viable groups were 1.6 and 2.2 for Pi/-γATP and Pi/β-ATP, respectively. The accuracy rates of these ratios were both 83%. (Conclusion) 31P-NMR spectroscopy combined with TLM preservation could provide an objective, rapid, and possibly non-invasive mean to assess pancreas graft viability and to determine suitability of damaged pancreata for organ transplantation.

Abstract# 842 Poster Board #-Session: P298-II

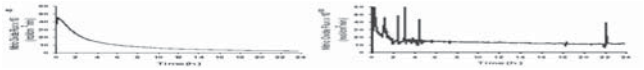
LOCAL NITRIC OXIDE DELIVERY SYSTEMS: IMPLICATIONS FOR TRANSPLANT PRESERVATION. P. Roy-Chaudhury,[1] M. Frost,[2] H. Zhang,[2] M. Batchelor,[2] E. Chang,[2] M. Meyerhoff,[2] S. Rudich.[3] *[1]Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Chemistry, Univ of Michigan, Ann Arbor, MI; [3]Surgery, Univ of Cincinnati, Cincinnati, OH.*

Nitric oxide (NO) is a short-lived chemical mediator which is a vasodilator, as well as an inhibitor of smooth muscle cell proliferation, platelet adhesion and aggregation. NO could therefore be an ideal agent to prevent stenosis and thrombosis in dialysis access grafts, as well as to help ameliorate the deleterious effects of cold storage/ischemia-reperfusion injury.

Purpose: To develop a local delivery system for NO that could be used in PTFE dialysis grafts and to use this as a model for developing technology to help prevent preservation, storage, and reperfusion injury during transplantation.

Methods: NO releasing polymers were prepared using diaminoalkyl trimethoxylsilane-N_2O_2 (DACA/N_2O_2-SR) for dip coating onto standard PTFE graft material. To prepare grafts releasing a range of NO fluxes, PTFE tubing was dipped into the silicone rubber-diazeniumdiolate solution. NO flux was measured in real time via chemiluminescence.

Results: A wide range of steady state NO fluxes, ranging from 1-20 x 10^{-10}mol/cm²min, was achieved by varying the number of dip coats and the concentration of the diazeniumdiolate compound. This followed an intial burst effect of NO release over the first 30 mins of as high as 40-50 x 10^{-10}mol/cm²min (Figure). [Basal rate of NO release from stable endothelial cells is 1 x 10^{-10}mol/cm²min; while the rate of release from activated endothelial cells is 4 x 10^{-10}mol/cm²min.] At 24 hrs, only 4-11% of the total amount of NO available had been released. Analysis of the NO release curve suggests that steady state NO release is obtainable for at least 10-14 days.



Conclusions: Local delivery of NO is a very favorable and logical approach to harness the beneficial effects that NO has on stenosis and thrombosis, without the problems of short half life and systemic toxicity. The local application of NO may find utility as a means to improve organ function following cold preservation and reperfusion.

Abstract# 843 Poster Board #-Session: P299-II

VERY EARLY T CELL INFILTRATION AND ACTIVATION IN ISCHEMIA-REPERFUSED KIDNEYS IN THE ABSENCE OF ALLOANTIGEN. Chu-chun Chien,[1] Manchang Liu,[2] Hamid Rabb.[2] *[1]Medicine, Chang-Gung Memorial Hospital & University, Taoyuan, Taiwan; [2]Medicine, Johns Hopkins University, Baltimore, MD.*

Recent data in kidney, liver and lung has implicated a direct modulatory role for T cells in the organ response to ischemia-reperfusion injury (IRI). The underlying mechanisms for this important response are largely unknown. Early T cell activation in vivo, particularly in the absence of alloantigen, is not established. Furthermore, conventional immunohistochemistry has not revealed a significant T cell influx early into reperfused tissue. We hypothesized that T cell activation and infiltration occured very early after reperfusion and enhanced teniques are required to detected this. We have established a tissue collagenase digestion technique followed by sequential lymphocyte isolation techniques to isolate T cells from kidney tissue. We used a murine model of 30 min of warm ischemia followed by one hour of reperfusion at which time kidneys were harvested. Isolation of T cells was performed from normal mouse kidneys, sham IRI kidneys that had anesthesia and surgery but no renal artery clamping, and IRI kidneys. This was followed by quantification of T cell infiltration/kidney, and then flow cytometric evaluation of the T cell activation markers CD69 (early activation marker) and CD25 (later activation marker). T cell counts in kidney increased from normals ($6.3x10^5$, n=6) and sham IRI mice that underwent surgery ($6.7x10^5$, n=6) to renal IRI mice ($13.8x10^5$, n=5)($p<0.01$). A significant increase in CD3CD69 double positive cells ($14.7x10^4$, n=6) was seen in renal IRI mice, but not in sham IRI mice ($5.3x10^4$, n=6), compared to the normal ($3.8x10^4$, n=6) ($p<0.01$). The numbers of CD3CD25 double positive cell did not differ. Similar quantification at later time points and further phenotyping of these IRI- activated T cells is underway.

This is the first demonstration of very early T cell activation and infiltration into post-ischemic kidneys in the absence of alloantigen. Mechanisms underlying this response are unknown, but could involve "Signal 3" on T cells. Early T cell activation during IRI could explain why ischemic organs are more susceptible to rejection. Interventions directed against T cell infiltration and activation could improve allograft injury for both cadaveric as well as live donor transplants.

Abstract# 844 Poster Board #-Session: P300-II

THE SYNERGISTIC EFFECT OF CARBON MONOXIDE AND BILIVERDIN FOR KIDNEY AND CARDIAC ISCHEMIA/ REPERFUSION INJURY. Atsunori Nakao,[1] Joao Seda Neto,[1] Shinichi Kanno,[1] Kei Kimizuka,[1] Robert J. Bailey,[1] Augustine M. K. Choi,[2] Fritz H. Bach,[3] Leo E. Otterbein,[2] Noriko Murase.[1] *[1]Surgery, University of Pittsburgh, Pittsburgh, PA; [2]Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA; [3]Harvard Medical School, Boston, MA.*

Heme oxygenase (HO) has been shown to provide potent protection in numerous models of cellular stress. Both carbon monoxide (CO) and biliverdin (BV), products of heme degradation by HO, have been shown to suppress ischemia/reperfusion (I/R) injury. We hypothesized that co-treatment of CO and BV would show enhanced protective effects against I/R injury following prolonged cold preservation and transplantation. **Methods**: Syngenic heterotopic heart transplantation (HTx) and orthotopic kidney transplantation (KTx) were performed in Lewis rats with 24 hrs UW cold preservation. Recipients received CO inhalation (20 ppm) for 24 hours and/or BV (50 mg/kg, ip) at -2 hrs and immediately after reperfusion. **Results (Table)**: While monotherapy with CO or BV did not alter the survival of heart grafts, combination of CO and BV significantly reduced myocardial injury (lower CPK levels), inhibited proinflammatory mediator activation (e.g. TNFα), and improved graft function (Langendorff apparatus), resulting in improved graft survival of 80% from 0% in untreated recipients. Following KTx, there was a significant improvement of creatinine clearance (CCR) with combination therapy. Protective effects of CO and BV were mediated via different mechanisms, since CO, but not BV, effectively improved renal cortical blood flow. Whereas, BV was more effective than CO in inhibiting lipid peroxidation (lower tissue malondialdehyde [MDA] level) with maintainance of the reductive capacity. **Conclusions**: These data demonstrated that the co-therapy with CO and BV was more effective than monotherapy in protecting HTx and KTx against I/R injury. Enhanced effectiveness appeared to be provided by the different mechanisms of CO and BV to exert protection against I/R injury. The study may also suggest that the potent cytoprotection of HO-1 attributes to the different actions of byproducts of heme catabolism to mediate cytoprotection against oxidative stress.

	Heart transplantation				Kidney transplantation	
	graft survival	s-CPK	graft MDA	graft TNFα	blood flow	CCR
(14d)	(3h, IU/l)	(1h, μM/mg)	mRNA (3h)	(1h, ml/min)	(ml/min, 28d)	
normal	100%	152	0.04	1.0±0.7	63.3±4.1	1.6
no treat	0%	12997	0.31	8.1±1.4	21.3±6.5	0.08
CO	10%	9521	0.24	7.0±0.6	34.4±5.8*	0.27
BV	30%	9656	0.17*	5.9±0.6	25.3±5.7	0.22
BV/CO	80%	5449*	0.17*	5.2±0.2*	37.2±5.9*	0.64*

*p<0.05 vs no treat

Abstract# 845 Poster Board #-Session: P301-II

CYCLOSPORINE-INDUCED RENAL INJURY IS ASSOCIATED WITH INCREASED IMMUNOGENICITY. Chul Woo Yang,[1] Can Li,[1] Sun Woo Lim,[1] Bo Kyung Sun,[1] Bum Soon Choi,[1] Yong Soo Kim,[1] Byung Kee Bang.[1] *[1]Internal Medicine, Kangnam St.Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea.*

Growing experimental and clinical evidence supports the concepts that injury to allograft activates immune system. Cyclosporine(CsA)-induced nephropathy is non-immunologically associated with persistent low-grade ischemic injury. But, it has not been tested whether CsA-induced renal injury is associated with activation of immune system. Based on above findings, we hypothesized that CsA-induced nephropathy may increase immunogenicity by activating immune system. To define this hypothesis, we evaluated the expression of HLA class II Ag, Toll-like receptor (TLR) and heat shock protein (HSP)70 expression in normal and CsA-treated rat kidneys. Sprague-Dawley rats were used. Chronic CsA nephropathy was induced by administering CsA (15 mg/kg, S.C.) for 4 weeks, and control rats were treated with vehicle (olive oil, 1mg/kg, S.C.) for 4 weeks. Renal function and histological findings (striped fibrosis, interstitial inflammatory cells infiltration, arteriolopathy) were measured. HSP70 and HLA class II antigen expression was detected with immunoblot and immunohistochemistry, and TLR-4 mRNA and protein was detected with RT-PCR and immunohistochemistry. Compared to the vehicle, CsA-treated rat kidneys showed dramatic increase of HSP70 on renal tubular cells on outer medulla as well as cortical tubular cells. TLR-4 mRNA was significantly increased in CsA-treated rat kidney, and localization of TLR-4 protein revealed increased immunoreactivity on proximal tubular cells. HLA class II antigen expression was also increased on renal tubular cells and dendrite cells in outer medulla in CsA-treated rat kidneys as compared with vehicle-treated rat kidneys.The results of our study suggest that CsA treatment increases HSP70 protein, and this activates immune system by increasing MHC class II antigen via TLR. This finding provides new concept that CsA-induced renal injury is associated with activation of immune system, and this may increase rejection episode in allograft.

Abstract# 846 Poster Board #-Session: P302-II

NON-SELECTIVE BLOCKADE OF CYCLOOXYGENASES-1 AND –2 AMELIORATES THE HYPOXIC EFFECTS ON ENDOTHELIAL CELLS. Maria A. Da Gloria,[1] Marcos A. Cendeze,[1] Alvaro Pacheco-Silva,[1] Niels O. S. Camara.[1] *[1]Nephrology, Federal University of Sao Paulo, Sao Paulo, Brazil.*

Deprivation of oxygen induces a cascade of events culminating in cell activation, lipid peroxidation and DNA fragmentation that ultimately lead to cell death. Toxic metabolites of oxygen are the major mediator of these events, generated in part by the action of cyclooxygenases (COX). COX-1 is the ubiquitous form and –2 the inducible one. Both are implicated in the generation of superoxide, peroxides and other metabolites of oxygen. We investigated the effect of non-selective blockade of COX-1 and –2 on the endothelial response to hypoxia. Mouse immortalized endothelial cells were submitted to 30 minutes of oxygen deprivation by nitrogen gas exchange. Acridine orange/etidium bromide dyes and lactic dehydrogenase enzyme (LDH) were used to monitor cell viability. mRNA of COX-1 and –2 was amplified and semi quantified before and after hypoxia, in cells treated or not with indometacin. RANTES expression was also investigated as a marker of endothelial cell activation. Nitrogen infusion into cell flask induced a substantial hypoxic effect with decreased of 50% in pO2. LDH increased in those cell submitted to hypoxia compared to control (35.97 to 22.2%, $p<0.05$). Thirty minutes of hypoxia also induced a decreased in cell viability. COX-2 was up regulated after hypoxia. Indometacin treatment decreased both COX-1 and COX-2 expression. Cells treated with indometacin did not decrease the viability after hypoxia and down regulated the expression of RANTES. Blocking COX up regulation, that is present early in the event of hypoxia, can ameliorate endothelial injury. This blockade has also effect on RANTES production, one chemokine implicated in chemoattraction of leukocytes.

Abstract# 847 Poster Board #-Session: P303-II

GENE EXPRESSION PROFILING AFTER PROLONGED COLD ISCHEMIA IN RAT KIDNEY TRANSPLANTS FOLLOWING INDUCTION OF HO-1. Katja Kotsch,[1] Paulo N. A. Martins,[2] Annelie Dernier,[1] Uwe Janssen,[3] Bernhard Gerstmayer,[3] Birgit Sawitzki,[1] Stefan G. Tullius,[2] Hans-Dieter Volk.[1] *[1]Institute of Medical Immunology, Universitätsmedizin Charité, Humboldt-University, Berlin, Germany; [2]Department of General-, Visceral- and Transplant Surgery, Universitätsmedizin Charité, Humboldt-University, Berlin, Germany; [3]Memorec Biotec GmbH, Cologne, Germany.*

Introduction: Ischemia/Reperfusion (I/R) injury is an independent risk factor for long-term outcome of renal allografts. However, current knowledge about the molecular mechanisms underlying renal I/R injury is limited so far although the induction of cytoprotective genes like HO-1 has been shown to improve graft function. Therefore, efficient strategies to prevent I/R injury require a better understanding of the molecular processes occuring during cold ischemia and following pretreatment strategies. We were interested in the gene expression profile of 820 immune-related genes using cDNA microarrays in rat kidney allografts undergoing prolonged cold ischemia with or without the induction of HO-1.

Material and Methods: In a well established F344 to Lewis rat kidney transplant model, F344 donors were either pretreated with the selective inductor of HO-1, cobalt-protoporphyrin (CoPP), or remained untreated. Kidneys were engrafted following 20 min or 24 hrs cold ischemic time. Recipients were sacrificed 12 hrs later and mRNA was extracted. A customized cDNA microarray with 820 rat related target genes was used for analysis. Native F344 kidneys served as controls.

Results: Cold ischemia of 20 min. and 24 hrs induced differential expression of 78 genes and 114 genes, respectively. A short ischemic time of 20 min leads to the induction of adhesion molecules (e.g. ICAM1), or apoptosis related genes (e.g. FAS). Prolonged ischemia of 24 hrs results in the upregulation of a higher number of genes (e.g. stress proteins like HSP70 and HSP105), stronger up-regulation (2-10 times higher than 20 min) and in accelerated chronic graft dysfunction compared to 20 min controls. CoPP pretreatment could not abrogate enhanced expression of HSP70, but in contrast, leads to the inhibition of macrophage-related markers (e.g. CD68), cell cycle associated genes (p53) and transcription factors (STAT1).

Conclusion: Microarrays provide a powerful tool to uncover the multitude of molecular events occuring during I/R contributing to early allograft dysfunction. We could already demonstrate that upregulation of HO-1 by a single donor treatment with CoPP improves graft function long-term significantly. Our recent study suggests that CoPP treatment protects allografts against I/R injury within the first 12 hrs by interacting at the transcriptional level with selective but not all inflammation related markers.

Abstract# 848 Poster Board #-Session: P304-II

THE DYSREGULATION OF PI3-KINASE/AKT PATHWAY CONTRIBUTES TO THE ISCHEMIA/REPERFUSION INJURY DURING GRAFT PRESERVATION BY UW SOLUTION. Xian-Liang Li,[1] Kwan Man,[1] Kevin Tak-Pan Ng,[1] Chris Kin-Wai Sun,[1] Chung-Mau Lo,[1] Sheung-Tat Fan.[1] *[1]HBP Division, Department of Surgery, Centre for the Study of Liver Disease, The University of Hong Kong, Hong Kong, China.*

Objectives: PI3-kinase/Akt pathway is so-called cell survival pathway, insulin can stimulate this pathway. However, our previous study showed that insulin currently used in UW solution were harmful to the preserved rat liver grafts. We want to clarify the roles of PI3-Kinase/Akt pathway on the ischemia/reperfusion injury during graft preservation in UW solution by stimulation with insulin or inhibition with LY294002.

Methods: The rat liver grafts were preserved in UW solution with the addition of insulin or LY294002 for different peroids. The downstream proteins of PI3-kinase/Akt pathway were detected by Western-blot. The ischemia injruy in the preserved grafts were compared in terms of apoptosis and necrosis between the two groups. The reperfusion injury were investigeted by comparing the survival rates, apoptosis, and necrosis of the implanted grafts. **Results:** Akt was activated by insulin at the beginning of graft preservation with the phosphorylation at Thr308 and Ser 473 and the expression levels of phospho-Akt were decreased gradually. The higher expression levels of Bad, phospho-GSK-3β, and caspase-12 in insulin group contributed to stimulate the caspase-3/7 and cleaved caspase-3/7, leaded the cell apoptosis and necrosis. LY294002 inhibited the expression of the PI3-kinase/Akt pathway by downregulating the expression of phospho-Akt at Thr308 and Ser 473. The downregulation of Bad, phospho-GSK-3β, and caspase-12 could be found in LY294002 group in which the liver grafts were shown less ischemia injury in terms of apoptosis and necrosis after 9 hours' preservation. For the implanted grafts, the one-week survival rates were significant increased in LY294002 group than that of insulin group.Severe reperfuson injury could be found in that of massive confluent necrosis and apoptosis at the 24 hours after operation in the grafts preserved in UW solution with insulin for 9 and 24 hours. **Conclusions:** PI3-Kinase/Akt pathway was dys-regulated and related to ischemia/reperfusion injury during graft preservation. Insulin exacerbated ischemia injury through activation of caspase-12 , phosphorylation of GSK-3β, and dephosphorylation of Bad during graft preservation. Inhibition of PI3-Kinase/Akt pathway might be beneficial to long-term graft preservation.

Abstract# 849 Poster Board #-Session: P305-II

PROTECTIVE EFFECT OF YM AGAINST WARM ISCHEMIA REPERFUSION INJURY IN MURINE KIDNEY. Li Feng,[1] Youping Li,[1] Feng Cheng,[1] Shengfu Li,[1] Li Zhang,[1] Zizhen Ye.[1] *[1]Lab of Transplant Engineering and Immunology, West China Hospital, Sichuan University, Chengdu, Sichuan, China.*

Ischemia/reperfusion (I/R) injury has been established as a non-immunologic risk factor for the development of chronic graft nephropathy following renal transplantation. We showed previously that a chemically identified injection, YM, flavonoid extracted from Chinese herbs, attenuated the short and long-term consequences of cold I/R injury in the pig renal allografts with CsA synergetically. Since warm I/R is potentially more damaging than cold storage, this study was undertaken to determine if YM would attenuate warm I/R injury in mice.

Methods. C57BL/6 males were subjected to 50 min of left renal (with right renal resection) ischemia as three groups (n=8/group). Group I-sham operated animals; group II-non-treatment animals (saline, i.p.), 30 min before I/R; group III-YM (25 mg/kg, i.p.), 12 hours and 30 min before I/R. Mice were sacrificed 24 hours post-reperfusion. Serum creatinine and blood urea nitrogen were measured. Myeloperoxidase activity of kidney was measured for assessment of neutrophil infiltration. Morphology changes of kidneys were evaluated by histological analyses. Renal expression of TNF-alpha and ICAM-1 was studied using reverse transcription-polymerase chain reaction (RT-PCR) and immunohistology.

Results. I/R caused a 6-fold increase in creatinine and urea nitrogen levels 24 hours post-reperfusion in group II. YM reduced the increase by 50%. The saline treated mice demonstrated an increased infiltration of neutrophils and widespread loss of brush border (dramatically decreased PAS staining). YM-treated mice had only patchy necrosis and dramatically decreased neutrophil plugging. YM significantly reduced kidney Myeloperoxidase activity (5.6 ± 0.8 vs. 15.2 ± 2.4 U/g wet tissue). Immunohistochemical staining revealed that the upregulation of TNF-alpha and ICAM-1 was greatly diminished by YM ($p<0.01$). Using RT-PCR, the enhanced TNF-alpha and ICAM-1 mRNA expression was decreased in the YM treated animals, compared with controls.

Conclusion. We observed that administration of YM resulted in morphological and functional protection of renal warm I/R injury. These observations show that YM reduced mice renal warm I/R injury at least partially via decreasing neutrophil infiltration and the inhibition of upregulation of TNF-alpha and ICAM-1. This recent experiment proves that pharmacological preconditioning with YM may be beneficial for attenuating renal warm I/R injury.

Abstract# 850 Poster Board #-Session: P306-II

δ-OPIOID AGONISTS, AS HIBERNATION INDUCTION TRIGGERS, ATTENUATE THE MACROPHAGE RESPONSE TO LIPOPOLYSACCHARIDE STIMULATION. Thomas L. Husted,[1] Alex B. Lentsch,[1] Steven M. Rudich.[1] *[1]Surgery, University of Cincinnati, Cincinnati, OH.*

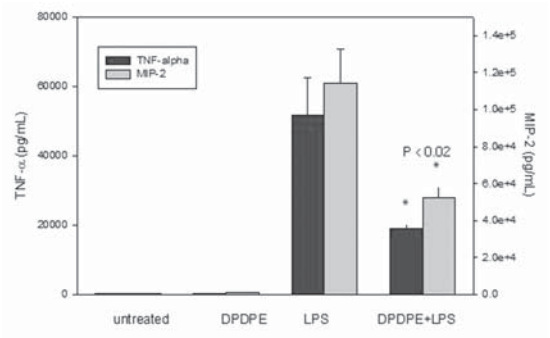
Background: Ischemia reperfusion (I/R) injury and cold storage preservation are significant sources of hepatic injury and dysfunction following liver transplantation. Activation of hepatic macrophages are a key component of I/R injury. Hibernation induction triggers, as well as their surrogates, δ-opioid agonists, have been shown to ameliorate I/R injury in several animal model systems.

Purpose: To determine if the synthetic δ_1-opioid agonist, [$D^{2,5}$Pen]-enkephalin (DPDPE), could attenuate proinflammatory cytokine production by macrophages after activation with lipopolysaccharide (LPS).

Methods: The murine macrophage cell line, RAW 264.7, were cultured to confluence. After 4 hrs pre-treatment with DPDPE, cells were exposed to 0.1mg/mL LPS for 4 hrs. After a further 4 hours in culture, media was assessed by ELISA for production of tumor necrosis factor-a (TNF-a) and macrophage inflammatory protein-2 (MIP-2).

Results: Macrophages expressed baseline levels of TNF-α of 206 ± 46 pg/mL and MIP-2 of 546 ± 105 pg/mL. Pretreatment with DPDPE had no effect. LPS treatment caused marked production of TNF-α (51590 ± 10823 pg/mL) and MIP-2 (114341 ± 18072 pg/mL). However, with DPDPE pre-treatment, there were 2.5 – 3 fold reductions in the production of TNF-α and MIP-2 ($p < 0.02$).

Conclusions: The δ_1-opioid specific agonist DPDPE suppressed the proinflammatory cytokine response of a murine macrophage cell line to LPS. The data suggest that δ_1-opioid agonists may protect against hepatic I/R injury by reducing the production of proinflammatory mediators by hepatic macrophages.



Abstract# 851 Poster Board #-Session: P307-II

SINGLET OXYGEN ENERGY (SOE) ILLUMINATION DURING COLD ISCHEMIA IMPROVES THE PRESERVATIVE EFFECT OF THE UW SOLUTION ON HIGH ENERGY PHOSPHATES IN ISCHEMIC RAT HEARTS. Daniel J. Lukes,[1] Ulrika Skogsberg,[1] Anna Nilsson,[1] Andreas Lundgren,[1] Ann Lindgård,[2] Olivier Rakotonorainy,[2] Bassam Soussi,[2] Michael Olausson.[1] *[1]Department of Surgery and Transplantation, Sahlgrenska University Hospital, Goteborg, 413 45 Vastra Gotaland, Sweden; [2]The Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska University Hospital, Goteborg, 413 45 Vastra Gotaland, Sweden.*

Purpose: Singlet oxygen energy (SOE) has been demonstrated to be is a potent inhibitor of reactive oxygen species (ROS) *in vitro and in vivo* and can mitigate the negative consequences of cold ischemia (CI) on heart transplants and skeletal muscle. We investigated if SOE illumination in rat hearts during CI could improve the preservative effect of the University of Wisconsin solution (UW) on high-energy phosphate (HEP) levels.

Material and methods: The hearts of 24 Lewis rats weighing 220 g were explanted with standard technique using cold (+ 4 ° C) UW solution. They were subsequently immersed in UW. In half of the grafts, SOE was produced by illuminating the hearts for 10 minutes each 30 minutes period with photons at λ634 nm with the Valkion® equipment. After 2 or 4 hours of ischemia, the hearts were snap frozen in liquid nitrogen before freeze – drying. The samples were then minced to powder and the nucleotides extracted using a 1.5 M perchlorid acid solution containing 1 mM EDTA. The samples were then analyzed with *in vitro* ^{31}Phosphorus Magnetic Resonance Spectroscopy (^{31}P MRS) at 11.75 T and the relative concentrations in mmol/g dry weight of phosphocreatine (PCr), inorganic acid (Pi) and beta-adenosine triphosphate (β-ATP) were obtained. Their relative concentrations were obtained by integrating their peak areas and comparing to the internal standard phenylphosphonic acid (PPA). The phosphorylation ratio, PCr/β-ATP, a known correlate to biochemical and functional outcome, was calculated.

Results: After 2 hours of CI the group were SOE was induced had a higher PCr/β-ATP ratio, 0.79 ± 0.36 vs. 0.31 ± 0.07 ($p < 0.05$). After 4 hours of CI the difference in absolute numbers remained, but failed significance, 0.58 ± 0.16 vs. 0.42 ± 0.19 (ns).

Conclusions: Reduction of ROS through SOE illumination at λ 634 nm during CI (+ 4°C) improves the preservative effect of the UW solution on HEP in moderately ischemic rat hearts. This might represent a new treatment modality in organ preservation and warrants further investigation.

Abstract# 852 Poster Board #-Session: P308-II

RINGER'S ETHYL PYRUVATE SOLUTION IMPROVES LIVER ISCHEMIA-REPERFUSION INJURY IN RATS. Allan Tsung,[1] Takashi Kaizu,[1] Atsunori Nakao,[1] Brain Bucher,[1] Mitchell P. Fink,[1] Noriko Murase,[1] David A. Geller.[1] *[1]Surgery, University of Pittsburgh, Pittsburgh, PA.*

Introduction: Hepatic ischemia occurs in the settings of trauma, transplantation, and elective liver resections. Reactive oxygen species (ROS) have been shown to play a major role in organ ischemia reperfusion (I/R) injury. Pyruvate, a key intermediate in cellular metabolism, is an effective scavenger of ROS. The purpose of this study was to test the hypothesis that ethyl pyruvate, a stable pyruvate derivative, is effective in preventing hepatic I/R injury.

Methods: Lewis rats underwent 60 min of partial warm hepatic ischemia by clamping of the hepatic artery and portal vein of the median and left lobes. Ethyl pyruvate dissolved in lactated Ringer's solution (REPS) or lactated Ringer's solution (LR) alone were given by IV injection 1 hr prior to ischemia, immediately before ischemia, and just prior to hepatic reperfusion. Serum and tissue samples were obtained at 3, 6, and 24 hrs post reperfusion to measure liver enzyme levels, histopathology, and inflammatory mediators.

Results: Liver enzyme levels at 3 and 6 hr and histology at 24 hr post-reperfusion showed significantly decreased liver damage in the REPS-treated rats compared to LR-treated animals. Serum TNF-a, IL-1β, IL-6 levels, and iNOS protein expression at 3 hr post reperfusion were significantly decreased in the REPS-treated animals. Likewise, treatment with REPS instead of LR downregulated the expression of iNOS, TNF-a, IL-6, and ICAM-1 mRNA levels in the liver. Compared with controls, treatment with REPS also decreased hepatic neutrophil infiltration and hepatic apoptosis as determined by TUNEL staining and caspase 3 activity.

Group	ALT-3 h	ALT-6 h	ALT-24 h	Necrosis (HE)
Control	67 ± 24	57 ± 8	51 ± 2	0
I/R + LR	2520 ± 213	1806 ±329	474 ± 67	+++
I/R + REPS	845 ±157*	813 ± 165*	260 ± 33	+

Data are mean ± SEM, n=4-8 per group, * indicates $p<0.05$ vs LR

Conclusions: Ringer's ethyl pyruvate solution (REPS) has a protective effect on hepatic ischemia-reperfusion injury, mediated in part by down-regulation of inflammatory mediators. Strategies utilizing this additive to LR solution should be considered in clinical settings of ischemic liver injury to decrease organ damage.

Abstract# 853 Poster Board #-Session: P309-II

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN KIDNEYS FROM BRAIN DEAD DONORS USING OLIGONUCLEOTIDE ARRAYS. Theo A. Schuurs,[1] Frans Gerbens,[2] Joost A. B. van der Hoeven,[1] Petra Ottens,[1] Krista A. Kooi,[2] Henri G. D. Leuvenink,[1] Robert M. W. Hofstra,[2] Rutger J. Ploeg.[1] *[1]Surgery, University Hospital Groningen, Groningen, Netherlands; [2]Medical Genetics, University of Groningen, Groningen, Netherlands.*

Brain death has been shown to affect hormone regulation, inflammatory reactivity and hemodynamic stability. Previously, we and others have observed that brain death results in progressive organ dysfunction and immune activation. Moreover, in the transplant model, kidneys, livers and lungs retrieved from brain-dead (BD) rats were subject to increased primary non-function and deteriorated graft survival. However, the mechanism(s) by which brain death leads to these processes remain yet unclear. To further unravel these mechanisms we have now performed DNA microarray studies with pooled RNA isolated from kidneys from normo- and hypotensive 6 hours BD rats, corresponding with optimal and marginal BD donors, respectively, and used RNA from living donor kidneys as control. Oligonucleotide arrays were manufactured using the Sigma/Genosys Rat Oligonucleotide Library harbouring 4854 unique rat sequences. A 2-fold change in expression was regarded as the cut-off point of a gene being differentially expressed. In kidneys from normotensive donors 72 genes were identified that were either up (64) or down (8) regulated, whereas 91 genes were differentially expressed (67 up and 24 down regulated) in hypotensive BD donor kidneys. Most of the differentially expressed genes from the normotensive group (87%) were recognized in the hypotensive group as well. From a selected number of genes (e-selectin, MCP-1, KC, Egr-1, KIM-1, HO-1, Hsp70 and Aqp-2) expression changes were confirmed ($p<0.05$) using semi-quantitative RT-PCR. Moreover, genes were found which, in previous studies, had been identified as being differentially expressed (e.g. Il-1beta, Il-6). Analyses of the data enabled us to categorize most of the genes in different functional groups: Inflammation/Coagulation, Cell Division/Fibrosis and Defense/Repair. Also genes encoding transcription factors and proteins involved in signal transduction were identified. Summarizing, the use of DNA microarrays has clarified parts of the process of brain death. It appears that not only deleterious processes as inflammation

and fibrosis occur in brain dead donor kidneys but genes involved in protection and repair processes are activated as well. This insight allows us to use specific cytoprotective interventions in the brain dead donor to better maintain or even repair organ viability and protect against ischemia/reperfusion injury.

Abstract# 854 Poster Board #-Session: P310-II

EFFECT OF BRAIN DEATH AND NON-HEART-BEATING KIDNEY DONATION ON RENAL FUNCTION AND INJURY. Mark M. E. D. van den Eijnden,[1] Henri G. D. Leuvenink,[1] Petra J. Ottens,[1] Nils A. 't Hart,[1] Wim van Oeveren,[2] Aurora M. Morariu,[2] Harry van Goor,[3] Rutger J. Ploeg.[1] *[1]Surgery, University Hospital Groningen, Groningen, Netherlands; [2]BioMedical Engineering, University of Groningen, Groningen, Netherlands; [3]Pathology and Laboratory Medicine, University Hospital Groningen, Groningen, Netherlands.*

Background. Ischemic injury to the renal allograft prior to implantation is considered as the major cause of primary non and never-function and delayed graft function. Evidence has been put forward that brain dead and non-heart-beating (NHB) donor organs are of poorer quality compared to living donors. The purpose of this study was to evaluate renal function and injury of brain dead and NHB kidneys.

Methods. Fisher F344 male rats were either maintained brain death for 4 h or subjected to cardiac arrest with the resulting warm ischemia for 45 min (NHB). Living rats served as controls. After donornephrectomy, kidneys were flushed with cold University of Wisconsin solution and subsequently reperfused at 37°C with oxygenated Krebs-Ringer bicarbonate solution using the isolated perfused kidney model. Renal function and injury were assessed by monitoring urine production, glomerular filtration rate, sodium and potassium reabsorption, glucose metabolism and reabsorption, as well as release of alanine aminopeptidase (AAP), alkaline phosphatase (AP), N-acetyl-β-D-glucosaminidase (NAG), and lactate dehydrogenase (LDH). Also, kidneys from all donor groups were stained for AAP and AP.

Results. Renal dysfunction and injury were most pronounced in NHB kidneys reflected by a significantly reduced urine production (71±43 vs. 312±64 µl/min/gram living; $p<0.05$), by anaerobic glucose metabolism resulting in increased lactate formation (11.4±1.3 vs. 7.0±1.2% living, $p<0.05$), and by significant higher luminal release of LDH (0.22±0.02 vs. 0.13±0.03 U/min/gram living, $p<0.05$) and NAG (6.9±1.1 vs. 1.8±0.5 U/min/gram living, $p<0.05$). Brain dead kidneys showed an increased urine production (588±65 µl/min/gram; $p<0.05$ vs. living) and were functionally abnormal in potassium reabsorption showing a net excretion of potassium (-18.5±6.8 vs. 22.2±8.5% living; $p<0.01$), as a result of ATP depletion. Histochemical staining of AAP and AP showed nephron cell injury by loss of brush border membranes in kidneys from all donor groups, however, to a much larger extent in the brain dead and NHB groups compared to living controls.

Conclusions. Both, brain death and NHB kidneys have a considerable detrimental effect on renal function and renal injury. Despite that it did not suffer from the non-physiological state of brain death, the ischemically NHB donor kidney was functionally inferior to the brain dead donor kidney.

Abstract# 855 Poster Board #-Session: P311-II

PROLONGED COLD ISCHEMIA IN RAT CARDIAC ALLOGRAFTS PROMOTES ISCHEMIA REPERFUSION INJURY AND THE DEVELOPMENT OF GRAFT CORONARY ARTERY DISEASE IN A LINEAR FASHION. Masashi Tanaka,[1] Raya D. Terry,[1] Golnaz K. Mokhtari,[1] Grant Hoyt,[1] David T. Cooke,[1] Anthony D. Caffarelli,[1] Theo Kofidis,[1] Robert C. Robbins.[1] *[1]Department of Cardiothoracic Surgery, Stanford University, Stanford, CA.*

Prolonged cold ischemia is suggested to exacerbate ischemia reperfusion (I/R) injury and graft coronary artery disease (GCAD). We investigated the effects of cold ischemia for different periods on cardiomyocyte apoptosis and inflammatory response during I/R injury and the degree of GCAD in rat cardiac allografts. **Methods:** PVG rat ($RT1^c$) hearts subjected to cold ischemia for 30, 60, 90, 120, 150 min (n = 6 each group) were heterotopically transplanted into ACI rats ($RT1^a$). Grafts were procured after 4 hours of reperfusion and analyzed for superoxide generation by the spin-trapping method; for myeloperoxidase activity, TNF-α, IL-1β, and MCP-1 production by ELISA; for cardiomyocyte apoptosis by TUNEL and by caspase-2, -3, -8, and -9 activities. Additional animals (n = 8 each group) received cyclosporine A 7.5 mg/kg/day for 10 days as chronic rejection models. Indices of GCAD were determined at 90 days. **Results:** A positive linear correlation was found between cold ischemia time, I/R injury, and GCAD. Superoxide generation, myeloperoxidase activity, TNF-α, IL-1β, and MCP-1 production, cardiomyocyte apoptosis, and caspase-2, -3, -8, and -9 activities increased with ischemia time, peaked at 120 min, and plateaued at 150 min. The percentage of luminal narrowing, the intima-to-media ratio, and the percentage of diseased vessels increased with increased ischemia time, peaked at 120 min, and plateaued at 150 min. All tested variables in both the acute and chronic phases were significantly increased with 120-min ischemia in comparison to 30-min ischemia ($p < 0.01$). **Conclusions:** These data indicate that the degree of cardiomyocyte apoptosis and inflammatory response in the cardiac allografts during I/R injury depends on the duration of cold ischemia. More importantly, prolonged cold ischemia correlates with advanced GCAD.

GCAD indices at different ischemia times

	% luminal narrowing	Intima-to-media ratio	% diseased vessels
30 min	13.4 ± 3.1	0.09 ± 0.02	28.7 ± 7.6
60 min	28.6 ± 5.1*	0.20 ± 0.04	39.2 ± 15.8
90 min	44.0 ± 3.9*	0.32 ± 0.04*	66.6 ± 2.8*
120 min	50.3 ± 4.2*	0.49 ± 0.05*	71.0 ± 11.1*
150 min	51.5 ± 2.9*	0.50 ± 0.05*	72.6 ± 8.4*

* $p < 0.01$ vs 30 min ischemia

Abstract# 856 Poster Board #-Session: P312-II

GERANYLGERANYLACETONE INCREASES RENAL EXPRESSION OF HEAY SHOCK PROTEIN 70 AND PROTECTS KIDNEY FROM ISCHEMIA/REPERFUSION INJURY. Masayoshi Miura,[1] Keita Minami,[2] Ken Morita,[2] Yoshihiko Watarai,[2] Katsuya Nonomura.[2] *[1]Department of Renal Transplantation, Sapporo City General Hospital, Sapporo, Hokkaido, Japan; [2]Department of Renal and Genitourinary Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan.*

Geranylgeranylacetone increases renal expression of heat shock protein 70 and protects kidney from ischemia/reperfusion injury.

Introduction and Objectives: Heat shock proteins (HSPs) are known as molecular chaperons that protect cells from various stress. Geranylgeranylacetone (GGA), an antiulcer drug, has been shown to increase expression of HSPs in various cells of humans and other mammals. The aim of this study was to test the effects of GGA on induction of HSPs in kidney and renal ischemia/reperfusion injury.

METHODS. Male C57BL/6 mice of 8-12 weeks old were orally administered either GGA 600 mg/day (GGA group, n=8) or vehicle (control group, n=8) for 7 consecutive days. These mice were subjected to 27 min of warm ischemia following right nephrectomy. Another group of mice underwent sham surgery after GGA treatment (sham group). Twenty-four hours after reperfusion the mice were euthanized and the serum levels of creatinine (Cr) and urea nitrogen (UN) were measured. Expression of HSPs (25, 40, 60, 70, and 90) in renal homogenates was measured by immunoblotting assay and was semi-quantitated using NIH Image. Renal parenchymal injury was assessed using histology score (0: none to 4: severe) for each tubulus (10 microscopic field per slide, 3 slides per kidney, x 200 magnification) in hematoxylin and eosin stained sections.

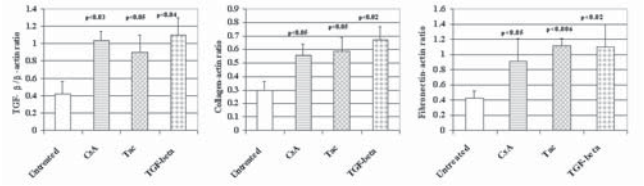
RESULTS: The expression of HSP70 was significantly higher in the GGA group when compared to the control group ($p<0.05$). The expression of HSP 25, 40, 60, and 90 was comparable in both groups. Serum Cr and UN levels were significantly lower in the GGA group when compared to the control group (Cr 0.56±0.45 mg/dL vs. 1.45±0.40 mg/dL, $p<0.05$, UN 89.8±14.5 mg/dL vs. 146±10.9 mg/dL, $p<0.05$). Average histology score was significantly lower in the GGA group in comparison with the control group (2.84±0.49 vs. 3.62±0.18 , $p<0.05$).

CONCLUSIONS: Based on these results we conclude that GGA induces the expression of HSP70 in kidney and protects kidney from ischemia/reperfusion injury.

Abstract# 857 Poster Board #-Session: P313-II

CONTRASTING EFFECT OF TGF-BETA, CYCLOSPORINE, TACROLIMUS, AND SIROLIMUS ON EXPRESSION OF PRO-FIBROGENIC GENES AND iNOS IN RENAL CELLS. Ashwani K. Khanna,[1] Matthew S. Plummer,[1] Galen M. Pieper.[2] *[1]Medicine (Nephrology), Medical College of Wisconsin, Milwaukee, WI; [2]Surgery (Transplantation), Medical College of Wisconsin, Milwaukee.*

Proximal tubular epithelial cells (PTE) are integral part of renal histological changes observed in organ transplant recipients treated for long-term with immunosuppressive agents. These changes could be reflected in molecular changes in these cells. We studied the effect of CsA, Tacrolimus (TAC), Sirolimus (SRL) and TGF-beta on mRNA expression of TGF-beta, collagen, fibronectin, CTGF and iNOS. Methods: PTE cells were treated with different concentrations of these agents and at 4 h, at which maximal expression for these genes was observed. Renal cells were treated with 50 ng/ml of Tac and SRL and 250 ng/ml of CsA and TGF-β protein (5 ng/ml). The semiquantitative analysis of mRNA expression was performed using RT-PCR. Results: A significant increase in TGF-β mRNA expression in PTE cells treated with CsA ($p<0.03$) tacrolimus ($p<0.05$) and TGF-β ($p<0.04$) are shown in Fig. A. A significant increase in collagen mRNA expression in PTE cells treated with CsA ($p<0.05$), TAC ($p<0.05$) and TGF-β ($p<0.02$) are shown in Fig. B. A significant increase in fibronectin mRNA expression in PTE cells treated with CsA ($p<0.03$), tacrolimus ($p<0.006$) and TGF-β ($p<0.02$) are shown (Fig. C). These agents failed to induce mRNA expression of fibrogenic molecules in TGF-β deleted PTE cells. We also studied the effect of CsA, tacrolimus (Tac), sirolimus (SRL) on iNOS and CTGF mRNA expression in these cells. The expression of iNOS mRNA was significantly inhibited by these agents whereas CTGF was increased. Conclusions: These results demonstrate that TGF-β mediates nephrotoxic effects of both CsA and tacrolimus by inducing expression of pro-fibrogenic molecules in renal cells. These results also for the first time demonstrate the effect of immunosuppressive drugs on CTGF mRNA expression in renal cells in relationship to iNOS expression, allowing the use of specifc modulators to prevent nephrotoxicity.



Abstract# 858 Poster Board #-Session: P314-II

IMORTANCE OF TUMOR NECROSIS FACTOR CLEAVAGE PROCESS IN REIMPLANTATION LUNG INJURY. Taichiro Goto,[1] Akitoshi Ishizaka,[2] Mitsutomo Kohno,[1] Makoto Sawafuji,[1] Kohichi Kobayashi.[1] *[1]Department of Surgery, Keio University, Tokyo, Japan; [2]Department of Medicine, Keio University, Tokyo, Japan.*

Despite the well-known pro-inflammatory effects of tumor necrosis factor (TNF), role of TNF cleavage process in the pathogenesis of lung injury following lung transplantation remains unclear. Although TNF plays a critical role in certain physiological defensive responses, it causes severe cellular damage in the host when produced in excess. TNF has two forms with apparently different biological activities, a membrane associated form and a soluble form generated from the membrane-bound protein by proteolytic cleavage with TNF converting enzyme (TACE). We hypothesized that TACE inhibition might prevent TNF-induced tissue injury while preserving benefits of TNF, such as host defense. In this study, we used TACE-inhibitor (TACEI), Y41654, to elucidate a role of TNF cleavage process in acute inflammation using a rat model of lung transplantation. Inbred male Lewis rats were subjected to left lung isotransplantation. Donor lungs were kept in Euro-Collins solution with or without 1 mg/ml Y41654 (TACEI and control groups, respectively) (n=10 for each group) for 6 hours. Then, the left lung was transplanted into recipient rat and reperfused for 4 hours. The animals were injected intravenously with 125I-labeled albumin, as a marker of pulmonary albumin leakage, 3 hours after the initiation of reperfusion. Pulmonary 125I-albumin leakage was assessed by using the concentration ratio of lung tissue to plasma (T/P ratio) and that of BAL fluid to plasma (B/P ratio), which were used as parameters of pulmonary endothelial and alveolar septal damage, respectively. The TACEI group showed significantly lower T/P and B/P ratio than the control group. Neutrophil accumulation in the alveolar space and other histopathological findings after lung isotransplantation were significantly attenuated in the TACEI group. Additionally, significantly lower levels of MCP-1, CINC-1, HMGB-1(High Moblity Group Box -1) and soluble E-cadherin and decreased neutrophil elastase activity were observed in BAL fluid from the TACEI group. We conclude TNF cleavage process plays a critical role in the development of post-transplantation lung injury in rat.

Abstract# 859 Poster Board #-Session: P315-II

STAT3 CONFERS RESISTANCE AGAINST HYPOXIA/ REOXYGENATION-INDUCED OXIDATIVE INJURY IN HEPATOCYTES VIA Mn-SOD. Keita Terui,[1,2] Sanae Haga,[1] Shin Enosawa,[1] Naomi Onuma,[2] Michitaka Ozaki.[1,3] *[1]Department of Innovative Surgery, National Research Institute for Child Health and Development, Setagaya, Tokyo, Japan; [2]Department of Pediatric Surgery, Chiba University Graduate School of Medicine, Chuo-ku, Chiba, Japan; [3]Department of Food and Health Science, Okayama University Graduate School of Medicine and Dentistry, Shikata, Okayama, Japan.*

Ischemia/reoxygenation (I/R) is still a serious concern in organ transplantation. I/R confers oxidative stress to the cell and induces apoptotic cell death. Signal transducers and activators of transcription-3 (Stat3) is one of the most important molecules involved in the initiation of liver development and regeneration, and recently known to protect cells from various pathogens. In order to investigate the hepatoprotective effects of Stat3, we examined whether Stat3 protects against hypoxai/reoxygenation (H/R)-induced injury in primary hepatocytes (PHC) of rats.

[Methods] PHC were prepared from SD rat (250g, male) by collagen-perfusion method, and seeded at 3 x 10(6) cells per 10-cm dish. Adenovirus and cytokine were added 2 days and 1 hr, respectively prior to the H/R insult.

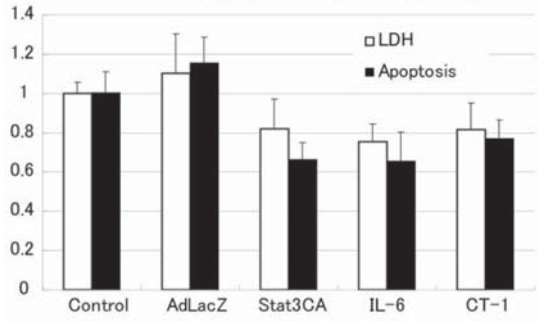
[Results] Interleukin-6 (IL-6) and cardiotropin-1 (CT-1), that function mainly through Stat3 activation, protected cells from H/R-induced apoptosis, which was reversed by over-expression of dominatly negative form of Stat3. Adenoviral over-expression of constitutively activated form of Stat3 (S3-C) or N-acetyl-L-cysteine (NAC) reduced H/R-induced apoptosis (Figure) as well as generation of reactive oxygen species (ROS) in hepatocytes. Interestingly, S3-C induced Survivin and Mn-SOD (but not Cu,Zn-SOD) both in protein and mRNA levels, though S3-C did not affect the expression of other anti-oxidant proteins. Over-oxpression of Mn-SOD significantly reduced H/R-induced apoptosis by inhibiting redox-sensitive caspase-3 activity.

Stat3, activated by cytokines or over-expressed in the cell, functions to protect hepatocytes from H/R-induced cell injury in a redox-dependent manner by up-regulating Mn-SOD and inactivating caspase-3.

Considering its strong mitogenic and anti-oxidant/apoptotic properties, Stat3 seems to be a good therapeutic target for preventing injury and promoting regeneration in liver transplantation.

Figure

IL-6, CT-1 and overexpressed Stat3 decreases apoptosis after HR stress.



Abstract# 860 Poster Board #-Session: P316-II

WHICH COLLOID IS PREFERRED IN A NEW PRESERVATION SOLUTION FOR MACHINE PERFUSION OF THE LIVER? Maud Bessems,[1] Benedict M. Doorschodt,[1] Olga Hooijschuur,[1] Arlene K. van Vliet,[1] Thomas M. van Gulik.[1] *[1]Department of Surgery, Surgical Laboratory, Academic Medical Center, Amsterdam, Netherlands.*

Introduction. Machine perfusion (MP) proves to be beneficial in the preservation of the liver. Currently the modified University of Wisconsin solution (UW-G) is used as the MP preservation solution of choice. However, this solution may not contain a sufficient amount of substrates for the decreased liver metabolism at 4 degrees. Therefore we have developed Polysol, a MP preservation solution based on a colloid, containing the necessary nutrients for the liver. In a previous study we have shown that Polysol results in equal or even better quality liver preservation when compared to UW-G. We sought to optimize Polysol by substituting the colloid Hydroxyethylstarch (HES), which causes microvasculatory obstructions, is expensive and difficult to obtain. We therefore compared HES with the colloids Dextran and Polyethyleneglycol (PEG).

Methods. In an isolated perfused rat liver model, hepatocellular damage and liver function were assessed during reperfusion after 24 hours hypothermic MP using P-

HES, P-Dextran or P-PEG. To determine hepatocellular damage ALT and LDH levels were measured during 60 minutes of normothermic reperfusion with oxygenated KHB. Liver function was assessed using oxygen consumption, bile production and ammonia clearance. Control livers were preserved for 24 hours by MP using UW-G.

Results. Compared to the control (UW-G) MP with either P-HES, P-Dextran or P-PEG resulted in significantly less hepatocellular damage, higher flow, bile production and oxygen consumption during reperfusion. MP with P-Dextran resulted in less hepatocellular damage (U/L) as compared to MP with P-HES: ALT (t=40) 2.17±0.98 vs 3.50±3.51, respectively. Livers perfused with P-PEG also sustained less hepatocellular damage as compared to P-HES: ALT (t=40) 2.20±1.30 vs 3.50±3.51 respectively. Oxygen consumption (mmHg) was increased after MP with P-Dextran as compared to P-HES: (t=10) 447.80±53.10 vs 399.28±30.64. Neither these differences, nor differences in ammonia clearance or bile production were significant.

Conclusions: 24 hours MP of livers using UW-G results in more extensive hepatocellular damage and reduced liver function when compared to MP using either Polysol-HES, Polysol-Dextran or Polysol-PEG. MP using Polysol-Dextran or Polysol-PEG results in equal or even less hepatocellular damage when compared to MP using Polysol-HES. Therefore substituting HES in Polysol with either Dextran or PEG can be considered feasible.

Abstract# 861 **Poster Board #-Session: P317-II**

COLD ISCHEMIA-REPERFUSION CONTRIBUTES TO THE DEVELOPMENT OF CHRONIC REJECTION VIA LOSS OF MITOCHONDRIA IN CARDIAC ALLOGRAFTS. Stefan Schneeberger,[1] Oliver Renz,[1] Peter Obrist,[2] Julia Heizinger,[1] Hugo Meusburger,[1] Gerald Brandacher,[1] Walter Mark,[1] Raimund Margreiter,[1] Andrey V. Kuznetsov.[1] *[1]Dept. of General- and Transplant Surgery, University Hospital, Innsbruck, Austria; [2]Dept. of Pathology, University Hospital, Innsbruck, Austria.*

Purpose: The effect of cold ischemia-reperfusion as a non-immunologic factor in the pathogenesis of chronic rejection was evaluated in a rat cardiac allograft model. Methods: Heart transplantations were performed in the Lew to F344 rat model with (CRI) or without (CR) 10h of cold ischemia (CI) at 0-1 °C prior to transplantation. Both group were compared with corresponding syngeneic control groups. After evaluation of graft function hearts were excised at 2, 10, 40 and 60 days after transplantation. Degree of vasculopathy was investigated by HE histology. Mitochondrial function was estimated *in situ* by high-resolution respirometry of permeabilized myocardial fibers. Enzymes activities (citrate synthase, Complex I; LDH) were assessed in myocardial homogenates. Results: Graft function (cardiac score) declined with duration of reperfusion from normal function (score 4) to score 1.6±0.5. This decrease was faster in the CRI when compared to the CR group and was nearly absent in isografts. The main histopathology of allografts undergoing chronic rejection was arterial myointimal proliferation, mononuclear cell infiltration and fibrosis. After 60 days, CR but not syngeneic groups revealed graft vasculopathy and fibrosis, both significantly more evident in CRI (2,1±0,8 vs 1,6±1,0). Mitochondrial respiration declined with all substrates used, indicating the general loss of mitochondria rather than specific defect of respiratory chain complexes. This decline was significantly more pronounced in CRI group. Consistently, activity of the mitochondrial matrix enzyme citrate synthase was relatively stable in syngeneic grafts, but decreased substantially (up to 20% of syngeneic controls) in both experimental groups and correlated well with cardiac score. Similarly to mitochondrial respiration, these enzymatic changes were more evident in CRI. In contrast, the activity of mitochondrial respiratory chain enzyme complex I equally declined in all groups and did not match with cardiac score. Activity of LDH similarly decreased to 50% of syngeneic controls in CRI and CR. Conclusion: Severe mitochondrial loss and cell injury were evident in CR. In addition to an allogeneic response, prolonged CI substantially contributes to the progression of chronic rejection and loss of organ function and is associated with mitochondria-related mechanisms.

Abstract# 862 **Poster Board #-Session: P318-II**

NITRIC OXIDE MODULATES RENAL VASCULAR FUNCTION IN THE BRAIN DEATH DONOR. Terezia B. Andrasi,[1] Anna Blazovics,[2] Pal Soos,[2] Jorg Gastmeier,[1] Gabor Szabo,[3] Hans-Detlev Saeger.[1] *[1]Department of Visceral, Thoracic and Vascular Surgery, Carl Gustav Carus University Clinic, Dresden, Germany; [2]Department of Internal Medicine, Semmelweis University, Budapest, Hungary; [3]Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany.*

Vascular endothelial dysfunction occurs in the kidney graft coming from marginal brain death donors and may be responsible for a low success rate after transplantation. Given that nitric oxide is a potent endothelial cell survival factor we hypothesise that stimulating NO synthesis with L-arginine could modulate vascular integrity and hence affect the progression of alloreactivity after transplantation.

Brain death was induced in 16 dogs for 6 hours. Immediately after the inflation of the intracranial balloon, the treated group (n=6) received 40 mg/kg bolus followed by 3 mg/kg/min infusion of L-arginine for 30 min. Renal vascular function, hemodynamic and biochemical parameters were determined.

During BD progressive renal dysfunction was observed that coincided with a significant vasoconstriction, increase in renal venous nitrite (4.9 ±0.8 vs. 2.6±0.1, $p<0.05$) and MPO levels (1.43±0.04 vs. 2.43±0.23, $p<0.001$), and reduced vasodilatation of renal artery to Ach. Administration of L-arginine counteracted the significant renal vasoconstriction induced by 6 hour BD (RVR=0.92±0.06 vs. 1.38±0.003 in controls, $p<0.05$) and maintained renal $ExtO_2$ in physiological range (17.5±4.6% vs. 25.4±2.9% in controls, $p<0.05$). Moreover, L-arginine prevented the rise of MPO (1.69±0.19, $p<0.05$ vs. controls) and nitrite levels (3.3±0.5, $p<0.05$), and finally maintained endothelium dependent vasodilatation at pre-BD values ($p<0.05$ vs. controls).

The findings suggest that renal vascular dysfunction associated with altered NO metabolism occurs in brain death at a late stage. L-arginine protects renal vascular function and therefore seems to be an appropriate option to improve graft survivial in the group of marginal organ donors.

Antigen Presentation and Alloimmunity

Abstract# 863

MONOCYTES ENGULF ENDOTHELIAL MEMBRANES VIA A SCAVENGER RECEPTOR, AND SCAVENGER RECEPTOR BLOCKADE PREVENTS MONOCYTE ACTIVATION. He Xu,[1] Xiao-Jie Zhang,[1] Allan D. Kirk.[1] *[1]Transplantation Branch, NIDDK/NIH, Bethesda, MD.*

Human monocytes up-regulate co-stimulatory molecules during allogeneic cell-mediated responses. We have previously shown that monocytes engulf membranes from allogeneic endothelial cells (EC) facilitating monocyte activation. In this study we investigated whether this uptake is via direct cell to cell contact, whether it is receptor dependent, and whether receptor blockade can prevent up-regulation of monocytes-derived co-stimulatory molecules.We used human peripheral blood mononuclear cell (PBMC) responders, and cultured human EC or EC labeled with PKH-26 as targets in a PBMC-EC co-cultures. A PBMC-EC transwell co-culture was used to determine whether this up-take was cell contact dependent. PBMC were collected following co-incubation with PKH-26 labeled or unlabeled EC monolayers, and analyzed by FACS to detect PKH-26/CD14/CD40/CD80/CD86/HLA-DR positive monocytes. In additional experiments, polyguanylic acid (poly (G)), a scavenger receptor ligand, was added into the PBMC-EC co-cultures at different concentrations to block scavenger receptor. $CD14^+/CD86^+/HLA\text{-}DR^+$ monocytes were shown to phagocytize live EC membranes as indicated by PKH-26 positivity. Additionally, PKH-26 positive monocytes up-regulated CD40 and CD80 following 72 hour-co-incubation. There were no PKH-26 positive T cells observed from the co-cultures. In contrast, $CD14^+/CD86^+/HLA\text{-}DR^+$ monocytes did not become positive for PKH-26 when co-cultures were separated by transwell membrane. Scavenger receptor blockade with poly (G) demonstrated a dose-dependent inhibition of live-EC membrane up-take by monocytes. Poly (G) at 1 mg/ml completely inhibited EC membrane up-take by monocytes in a 12-hour co-culture. In addition, the up-regulation of monocytes-derived CD40 and CD80 was inhibited by poly (G) at 500 µg/ml in a 72-hour co-culture. These data demonstrate that monocyte up-take of live EC membranes during their interaction with EC monolayers is contact dependent. Scavenger receptor blockade by poly (G) completely prevents monocyte EC membrane up-take, and block subsequent monocyte activation and co-stimulatory molecule expression. These data suggest that monocyte up-take of allogeneic membranes is via the poly(G) scavenger receptor, and that therapeutic intervention targeting these receptors may limit allorecognition and antigen presentation.

Abstract# 864

INDUCTION OF "INFECTIOUS TOLERANCE" BY DENDRITIC CELLS DERIVED FROM TOLERANT ALLO-CARDIAC TRANSPLANT RECIPIENTS. Dameng Lian,[1] Mu Li,[1] Thomas E. Ichim,[1] Bertha Garcia,[1,2] Robert Zhong,[1,2,3] Wei-Ping Min.[1,2,3] *[1]The Multi-Organ Transplant Program, London Health Sciences Centre, London, ON, Canada; [2]Transplantation, Lawson Health Research Institute, London, ON, Canada; [3]Departments of Surgery, Microbiology and Immunology, and Pathology, University of Western Ontario, London, ON, Canada.*

BACKGROUND: It is established that transplant tolerance can be adoptively transferred from tolerant recipients to a naïve recipient by T cells, a phenomena termed "infectious tolerance". However, it remains unknown if tolerance can be adoptively transferred by tolerogenic dendritic cells (Tol-DC). The present study aims to determine whether Tol-DC isolated from tolerant mice can transfer graft-specific tolerance to naïve recipients. **METHODS:** BALB/c mice receiving C57BL/6 cardiac allografts were treated with LF 15-0195, a novel analogue of 15-deoxyspergualine at a dose of 2 mg/kg for 20 days, and the long-term survivors (>100 days) were defined as tolerant recipients. Tol-DC were isolated from tolerant recipients by anti-CD11c mAb-conjugated MACS beads. Tol-DC were intravenously injected into syngeneic (BALB/c) recipients followed by allogeneic (C57/BL6>Balb/c) heterotopic heart transplantation. **RESULTS:** The Tol-DC isolated from tolerant recipients demonstrated an immature phenotype as exemplified by diminished expression of MHC II, CD40, and CD86. Functionally, Tol-DC failed to stimulate allogeneic T cell response in MLR. Furthermore, the addition of Tol-DC ex vivo isolated from tolerant recipients inhibited ongoing MLR, suggesting Tol-DC play a regulatory role. To further investigate whether Tol-DC may function as regulatory cells in vivo, ex vivo isolated Tol-DC ($5x10^6$ cells/mouse) from tolerant recipients were adoptively transferred into naïve syngeneic recipients. The allografts in recipients that received Tol-DC survived significantly longer (MST 45 ± 8 days) compared to the survival of recipients without Tol-DC transfer (MST 13 ± 1 day). **CONCLUSIONS:** This study is the first demonstration of infectious tolerance by adoptively transferring Tol-DC from tolerant allograft recipients.

This study was partially supported by Heart and Stroke Foundation of Canada, Roche Organ Transplantation Research Foundation, The Kidney Foundation of Canada, and by MOTS Research Fund in London Health Sciences Centre.

Abstract# 865

PAR-1 AND PAR-2 ACTIVATION DIFFERENTIALLY MODULATE TH-RESPONSE INDUCED BY DENDRITIC CELLS (DC). P. Pontrelli,[1] G. Grandaliano,[1] M. Ursi,[1] A. Blasi,[1] V. Petruzzelli,[1] L. Roca,[1] E. Ranieri,[1] L. Gesualdo,[2] F. P. Schena.[1] *[1]Div. of Nephrology, DETO, Univ. of Bari; [2]Chair of Nephrology, Univ. of Foggia, Italy.*

There is an increasing body of evidence that coagulation factors can modulate the immune response, although their ability to influence the Th-1/Th-2 bias is unknown. DC are antigen presenting cells that modulate Th1/Th2 balance. IL-12 produced by DC turn on the Th1 response, while IL-10-producing DC promote Th2 bias. The aim of the present study was to evaluate whether monocyte(M)-derived DC express PAR-1 and 2 (thrombin and FXa receptors, respectively) and to investigate the effect of their activation on DC phenotype and IL-12 and IL-10 expression by real time PCR. M were isolated by Ficoll-Hypaque gradient. M-derived DC were obtained incubating M for 5 days with AIM V medium+IL-4 and GM-CSF.

The flow cytometric analysis of the two receptors, confirmed PAR-1 expression in M (15.1±7.2MFI) and demonstrated an increase in this receptor levels in immature (i)DC when compared with M (35.7±1.1MFI; p=0.05) and a subsequent marked reduction in mature DC (mDC 9.6±1.7MFI, p<0.01 vs M and iDC). Moreover, there was an increase in PAR-2 expression from M (14.1±4.9MFI), to iDC (21.3±0.8MFI; p=0.26 vs M) and mDC (30.4±17.6MFI; vs M p=0.03). As demonstrated by RT-PCR, both PAR-1 and PAR-2 gene expression remained unchanged in M, iDC and mDC, suggesting that the modulation observed in flow cytometry was due to post-trascriptional events or changes in protein trafficking. The latter hypothesis was confirmed by the observation that total PAR-1 and PAR-2 proteins, evaluated by western blot, were increased in iDC and mDC. Thrombin caused a time-dependent increase in IL-12 p40 gene expression, with the maximal effect at 6 hours (2.7 fold over basal), while strikingly reducing IL-10 mRNA abundance. On the contrary, DC stimulated with PAR-2-activating peptide showed a time-dependent increase in IL-10 expression with a peak at 6 hours (7 fold over basal) and no effect on IL-12 p40 mRNA. In addition, PAR-2 stimulation significantly reduced both CD86 and CD54 expression on iDC and mDC, as demonstrated by flow cytometry. Finally, both thrombin and FXa induced a significant shape-change in iDC with an increase in the length of the dendrites, as demonstrated by confocal microscopy.

In conclusion, our data would suggest a role for PAR-1 in the modulation of Th-1 response by DC while PAR-2 seems to induce Th2 response. This observation in the setting of transplantation, may suggest a potential pathogenic link between the innate and acquired immunologic mechanisms of graft damage.

Abstract# 866

FREQUENCIES OF ALLOREACTIVE B LYMPHOCYTES IN RENAL TRANSPLANT PATIENTS WITH HISTORIC AND CURRENT SENSITIZATION TO HLA ANTIGENS. Mary S. Leffell,[1] Dessislava Kopchaliiska,[1] Robert A. Montgomery,[1] Andrea A. Zachary.[1] *[1]Medicine and Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.*

While the frequencies of alloreactive T cells can be defined by various methods, there has not been a comparable assessment of B lymphocytes. We developed a procedure for labeling HLA-specific B cells with HLA tetramers, based on the specificity of surface immunoglobulin receptors for epitopes on HLA molecules. The specificity of this approach was verified in patients with either historic or current sensitization to two commonly mismatched HLA antigens, HLA-A2 and B7. **Methods:** Anti-coagulated blood samples were obtained from 23 sensitized patients; 12 to A2 and 11 to B7. Controls included healthy, non-sensitized males, patients sensitized to other HLA antigens, and patients with A2 and/or B7 in their own phenotype. B cells were enriched either by depletion of T cells with anti-CD2 magnetic beads or by positive selection with anti-CD19 beads. Enriched B cells were stained with 10µl of tetramers: HLA-A*0201MART 1-PE, HLA-A*0201GAG-PE, and/or HLA-B*0702p24-APC (Beckman Coulter) at 4° for 45 minutes. Cells were also labeled with PE or FITC-anti-CD3 and CD19 (BD Biosciences/Pharmingen). After washing and fixation, two or three color analysis was performed on a FACSCalibur cytometer using Cell Quest software (Becton Dickinson). **Results:** The mean frequencies (%) of CD19, A*0201 and B7*0702 tetramer positive(tet+) cells among patients sensitized to A2 (6.6±6.1; P=0.002) or B7 (5.4±2.5; P=0.006) were significantly higher than those of 18 controls (1.3±0.7). The frequency of binding to non-CD19 cells ranged from 0.3 to 1.0% among both patients and controls. A*0201 tet+ frequencies tended to be higher among patients with current anti-A2 antibodies compared to those with historic, but currently non-detectable, antibodies (8.3±6.9 and 3.1±0.5, respectively; P=0.086). The frequencies of B7 tet+ cells were not significantly different between current and historic samples (5.0±2.4 v.s. 5.8±2.8, p=0.63). Sustained frequencies of B7 tet+ cells may reflect reactivity with the large B7 cross reactive antigen group (CREG). Broader reactivity with B7 CREG members was supported by respective frequencies of A*0201 and B*0702 tet+ cells of 0.9 and 5.7 in a highly sensitized patient (PRA=65) who was crossmatch negative with A2 cells but positive with B7 CREG cells. **Conclusions:** Frequencies of HLA specific B cells can identify historic sensitization when antibody is no longer detectable and can also measure the breadth of sensitization to CREG antigens.

Abstract# 867

INTERACTIONS BETWEEN INDIRECT MHAG REACTIVE CD4+ T CELLS AND DIRECT CLASS I ALLOREACTIVE CD8+ T CELLS IN A MODEL OF CHRONIC GRAFT REJECTION. David M. Richards,[1] Stacy L. Dalheimer,[1] Marshall I. Hertz,[1] Daniel L. Mueller.[1] *[1]Department of Medicine and Center for Immunology, University of Minnesota Medical School, Minneapolis, MN.*

Many experiments have demonstrated a requirement for the presence of both CD4+ and CD8+ T cell responses for efficient and complete rejection of graft tissue. Previously, we have shown that trachea grafts with both H-Y minor Ag (mHAg) differences and one MHC class I mismatch are fibrosed significantly more often than grafts with either mismatch alone. These results have suggested the hypothesis that help provided by mHAg-specific CD4+ T cells promotes chronic graft rejection by allo-class I-specific CD8+ T cells. CD4+ T cells responding to mHAg either in the draining lymph node or allograft may be responsible for producing molecules that assist in the activation, clonal expansion, and/or differentiation of directly-alloreactive CD8+ effector T lymphocytes. In order to further investigate the activation requirements for B6 (H-2b) CD8+ T cells responding directly to BALB/c (H-2d) class I MHC antigens, we examined trachea allograft rejection in either the presence or absence of CD4+ T cell help. Fully mismatched BALB/c trachea allografts became fibrosed significantly less frequently in CD4-deficient B6 recipients compared to wild-type controls. Interestingly, infiltration of allograft tissue by CD8+ T cells did not depend on CD4+ help. However, CD8+ T cells in allograft tissue were significantly less likely to express CD69 (a marker of recent Ag stimulation) in the absence of CD4+ T cells. These findings imply that CD4+ T cell help is required either for the continued activation of graft-reactive CD8+ T cells, or for the retention of Ag-specific CD8+ T cells within the allograft.The polyclonal nature of normal lymphocytes prevented a determination of the Ag-specificity of the graft infiltrating CD8+ T cells. Therefore, the adoptive transfer of 2C TCR-transgenic CD8+ T cells with direct Ld-reactivity into CD4-deficient B6 recipient mice was used to test the activation state of bona fide directly alloreactive CD8+ T cells responding to BALB/c trachea allografts. 2C CD8+ T cells were found to infiltrate BALB/c trachea allografts to a similar extent in both wild-type and CD4-deficient B6 recipients. Nevertheless, in the absence of CD4+ T cells fewer 2C CD8+ T cells expressed CD69. These results suggest that CD4+ T cell help does not influence graft infiltration by directly alloreactive CD8+ T cells, but is necessary for their optimal activation within the graft tissue and efficient graft rejection. This work was supported by NIH Training Grant T32 HL07741 (DR) and PO1 AI50162 (DM & MH).

Abstract# 868

OVEREXPRESSION OF ELAFIN, A SERINE ELASTASE – SPECIFIC INHIBITOR, PREVENTS ACUTE CARDIAC ALLOGRAFT REJECTION. Monika Zwierzchoniewska,[1] Robert C. Robbins,[1] Marlene Rabinovitch,[2] Eugenia V. Fedoseyeva.[1] [1]*Department of Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA;* [2]*Department of Pediatrics, Stanford University School of Medicine, Stanford, CA.*

Purpose of the study: Serine elastases degrade the extracellular matrix, releasing growth factors and chemotactic peptides, thereby promoting vascular cell proliferation and migration. Increased elastase activity is associated with several cardiovascular disorders and with the post-cardiac transplant coronary arteriopathy. Elafin is a serine elastase-specific inhibitor, and its overexpression in transgenic mice results in reduction of experimentally-induced arterial injury. The goal of our study was to evaluate the effect of elafin overexpression on cardiac allograft survival. For this purpose, we used elafin-transgenic mice (E-Tg), in which overexpression of the human elafin transgene is targeted to the cardiovascular system.

Methods: Elafin overexpressing transgenic mice (H-2q) and their nontransgenic littermates were either heterotopically transplanted with allogenic BALB/c (H-2d) hearts or used as donors of cardiac allografts for BALB/c recipients. No immunosuppression was used. At the time of rejection, frequencies of alloreactive and heart tissue antigen (cardiac myosin, CM)-specific T cells were monitored by ELISPOT.

Results: Our data show that in full MHC-mismatched combination, elafin overexpression in the host but not in the donor heart results in significant prolongation of transplant survival (24.5 days vs 9.0 days). Prolongation of graft survival in E-Tg recipients is associated with 1) expansion of anti-inflammatory IL-4-producing T cells directly recognizing alloMHC on donor cells (direct pathway), and 2) with increase in frequency of IFNg-producing T cells reactive to both donor MHC- and CM antigens processed and presented by host antigen-presenting cells-APCs (indirect pathway and tissue-specific immunity, respectively).

Conclusions: Our data show that overexpression of serine elastase specific inhibitor-elafin in the host abrogates acute rejection of fully allogeneic heart transplant. This suggests that selective inhibition of extracellular matrix antigen processing may favor induction of protective/regulatory immunity in the host, thus improving allograft survival.

Abstract# 869

POTENTIAL ROLE OF CD103 IN PROMOTING PANCREATIC ISLET DESTRUCTION DURING PROGRESSION TO TYPE I DIABETES. Adam W. Bingaman,[1] Donghua Wang,[1] Gregg A. Hadley.[1] [1]*Department of Surgery, University of Maryland, Baltimore, MD.*

Introduction: Pancreatic islet grafts transplanted into Type I diabetics are subject to immunologic destruction from both alloimmune and autoimmune mechanisms. Several strategies exist in which tolerance can be induced to islet allografts in the absence of autoimmunity; however, these strategies have been less successful in diabetic recipients. Thus, it is important to identify factors which may regulate autoimmune destruction of islet cell transplants. CD103 is a T-cell integrin which plays a critical role in promoting destruction of epithelial cell compartments by CD8+ T cells, and whose ligand, E-cadherin, is highly expressed by pancreatic β-cells. Recently, a critical role for CD103 in promoting islet allograft destruction by CD8+ T cells was discovered. The aim of the present study was to determine if CD103 plays an analogous role in destruction of endogenous islets during development of autoimmune diabetes.

Methods: Standard multi-color and immunohistological analyses were used to monitor CD103 expression by CD8 effector populations in a group of NOD female mice at different time points in progression to diabetes (age 7 weeks through development of hyperglycemia at 25 weeks of age).

Results: Small numbers of CD8 cells ($0.2x10^6$ cells/pancreas) were present in the pancreata of female NOD mice as early as 7 weeks of age and the total number increased progressively with age, reaching maximal numbers at the time of overt diabetes ($0.5x10^6$ cells/pancreas). While CD103 was expressed by a subset of CD8 effectors (gated $CD8^+CD44^{hi}$ cells) at all time points examined, the level of CD103 expression was maximal at 12 weeks of age, the time at which female NOD mice first develop invasive insulitis. To determine the localization of the CD8+CD103+ subset within the NOD pancreas during development of diabetes, we harvested pancreata from 12 week old pre-diabetic female NOD mice and performed immunohistochemistry utilizing anti-CD103 antibody. Interestingly, CD103+ cells within the pancreas were densely concentrated within the islets of Langerhans, the critical targets in autoimmune diabetes.

Conclusions: These data provide strong support for the hypothesis that autoreactive CD8+CD103+ effector T cells play a key role in islet destruction in Type I diabetes and are consistent with a central role for CD8+CD103+ effectors in regulating progression from peri-insulitis to invasive insulitis.

Abstract# 870

CASPASE-3 INHIBITOR PROTECTS ISOLATED HUMAN ISLET CELLS FROM APOPTOSIS RESULTING IN IMPROVEMENT OF ISLET GRAFT FUNCTIONS. Masahiko Nakano,[1] Ippei Matsumoto,[1] Toshiya Sawada,[1] Jeff Ansite,[1] Jeremy Oberbroeckling,[1] Hui J. Zhang,[1] Nicole Kirchhof,[1] Jeff Shearer,[1] David E. R. Sutherland,[1] Bernhard J. Hering.[1] [1]*Diabetes Institute, Department of Surgery, University of Minnesota, Minneapolis, MN.*

Purpose: It has been reported that apoptosis appears in human islet cells after isolation and it has a detrimental effect on islet functions. The purpose of this study is to determine whether caspase-3 inhibitor (Z-DEVD-FMK) protects human islets from apoptosis immediately after isolation. We also compare human serum albumin (HSA) and fetal bovine serum (FBS) as a protein supplement in a culture medium for human islets.

Methods: Isolated human islets from 6 cadaver donors were incubated under 4 different conditions (group A: 0.5% HSA, B: 10% FBS, C: 0.5% HSA+25μM caspase-3 inhibitor, D: 0.5% HSA+100μM caspase-3 inhibitor) for 2 days. Then 1000 IEQ (islet equivalent) incubated islets from group A and D were transplanted into diabetic nude mice. The final values of in vitro assays (mean±SD) were expressed as a percentage of the value for group A.

Results: After 2 days of incubation, the islet yields was significantly increased than in group A in both group B (126.4±25.1%) and group D (135.8±30.5%). The yield in group C (126.9±29.7%) was also more than that in group A, but the difference was not statistically significant. The apoptosis index was in 73.9±30.6% in group C and 41.1±25.7% in group D, as compared with group A. Caspase-3 inhibitor thus dramatically prevented apoptosis of isolated human islet cells in a dose-dependent manner. The insulin release stimulated by high glucose (300mg/dl) was 141.6±37.9% in group B; 119.0±29.5% in group C; and 126.5±45.3% in group D, as compared with group A. In transplant experiments, diabetes of all 6 mice with islets from group D were ameliorated by 9.0±5.3 days posttransplant. However, only 3 out of 8 mice with islets from group A became normoglycemic by 17.7±11.0 days posttransplant. Intraperitoneal glucose tolerance test performed 30 days posttransplant revealed that the glucose tolerance of mice in group D was superior to that of mice in group A. The islets grafts from group A in the hyperglycemic recipient were found to be poorly granulated with insulin but the islets from group D in the normoglycemic mouse were intact with intense insulin staining.

Conclusions: Caspase-3 inhibitor prevented apoptosis of isolated human islets and improved its functions. Ten percent FBS improved the islet yield and insulin secretion more than 0.5% HSA.

Abstract# 871

PERSISTENCE OF COLD ISCHEMIA TIME AND OF ITS NEGATIVE CONSEQUENCES ON RENAL ALLOGRAFTS. Abdulla K. Salahudeen,[1] Warren May.[1] [1]*Medicine and Preventive Medicine, University of Mississippi Medical Center, Jackson, MS.*

With the advent of immunosuppression better than ever before, it is debatable whether subjecting kidneys to prolonged cold ischemic injury for the sake of better tissue matching is justifiable. Given the negative effects of cold ischemia, *attempts* are being made in US to reduce cold ischemia time (CIT) by facilitating the local utilization of organs. We surveyed the CIT of the deceased-donor kidneys from 1987 to 2000 in the UNOS database, and assessed its effect on post-transplant dialysis requirement, discharge serum creatinine, and 1-year graft survival. Using all years in the database (1987-2000), there was a significant negative correlation between year of transplant and cold ischemia time (r= -0.18). This was also reflected in the CIT using 3 representative years of 1990, 1995 and 2000, which were 24± 11, 21± 9 and 20± 9 hrs, respectively (Mean± SD; P<0.001). Discharge serum creatinine, on the other hand, continued to be positively correlated with cold ischemia time (r=0.11) and with year of transplant (r=0.15), which for 1990, 1995 and 2000 were 2.4± 2.1, 3.0± 2.6 and 3.5± 3.2 mg/dl, respectively (P<0.001). The frequency of 1st week post-transplant dialysis in 10 years was reduced by 2% (25% in 1990, 24% in 1995 and 23% in 2000; P<0.05). Despite a smaller overall reduction in CIT, first year graft survival showed a significant improvement over the 10-year period: 84% in 1990, 86% in 1995 and 89% in 2000 (P<0.001). However, the CIT continued to persist as a significant predictor of 1-yr graft survival in a Cox model, with a one hour HRR of 1.01, even after adjusting for year of transplantation. In conclusion, this analysis supports the view that reduction in CIT over the years has been very modest, and despite significant recent improvement in 1-yr graft survival, probably due to better immunosuppression, cold ischemic injury continues to contribute to early graft dysfunction and graft loss. Drastic reduction in CIT is predicted to result in cadaveric renal graft function and survival closely comparable to live donor kidneys.

Abstract# 872

THE USE OF PERFUSION PARAMETERS IN PREDICTING OUTCOMES OF MACHINE PRESERVED KIDNEYS. Martin F. Mozes,[1] Ron B. Skolek,[1] Brian C. Korf,[1] the Kidney Subcommittee.[1]
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Body: The perfusion parameters (PP) of Perfusate Flow rates (F) and the calculated Renal Resistance index (RR) have been used to predict the function of the perfused kidney after transplantation and to make decisions regarding transplantation or discard of the kidney. Thus, understanding the correlation between PP and subsequent outcome is critical in optimizing the utilization of deceased donor kidneys.

Methods: We have analyzed these correlations for 250 consecutive perfused kidneys. Only kidneys from expanded donors were preserved by continous pulsatile perfusion. The donor criteria included age ≥60; Age 50-59 with one of the following: Hx of DM or HTN; admit S. Creat. ≥1.5 or calculated GFR ≤ 70. Doubling of S. Creat (admit to final) and DCD donors.The Waters RM-3 apparatus and Belzer MP® solution were used. Pump pressures (P) were initially set at 60mmHg. Flow rates were measured by the RM-3 flow probes and RR was calculated in the standard fashion using the formula: RR= (Psyst + Pdiast x 2) / 3 x F (ml/min). Post-transplant follow up of renal function was obtained on all 184 transplanted kidneys out to 6 months. Delayed graft function (DGF) was defined as the use of dialysis in the first week.

Results: There were 130 Immediate function (IF), 50 DGF and 4 Primary None Function (PNF)kidneys (with transplant related complications in 2/4). 66 kidneys were discarded. The mean 2 hour and 4 hour F did not differ between IF, DGF and PNF kidneys (113±37.1, 116±31.5, 103±39 and 112±35.7, 118±30, 112±41 respectively). The corresponding RR for these time frames did not differ : (.28±.1, .27±.12, .41±.22 and .27±.1, .26±.1,.37±.21 respectively). The comparison of 15 mate (same donor) kidneys with different initial function did not indicate differences in PP between the the IF and DGF kidneys.

The F and RR for the 36 kidneys where PP was (at least in part) the reason for kidney discard were significantly different (p<.001) from both the IF and DGF kidneys (75±17 and .50±.18 at 2 hrs and 75±19 and .48±.18 at 4 hours.) Although biopsy findings were a factor in the decision to discard in the majority of these 36 kidneys, using the PP parameter data of the transplanted kidneys suggests a potential for significantly decreasing the discard rate in this group.

Summary and Conclusions: Standard Perfusion Parameters (PP) cannot be reliably used to predict early renal function after transplantation. Kidneys with F >60 and RR <0.6 should not be discarded based on PP alone. More sophisticated indicators for predicting PNF are needed.

Abstract# 873

DELAYED GRAFT FUNCTION AND PRIMARY NONFUNCTION IN EXPANDED-CRITERIA-DONOR KIDNEY TRANSPLANTATION. Thomas D. Johnston,[1] Leroy R. Thacker,[2] Hoonbae Jeon,[1] Bruce A. Lucas,[1] Dinesh Ranjan.[1] *[1]Department of Surgery, University of Kentucky, Lexington, KY; [2]Southeastern Organ Procurement Foundation, Richmond, VA.*

The United Network for Organ Sharing (UNOS), with organ procurement organizations and transplant programs, has defined a class of cadaver kidney grafts for special allocation procedures to enhance utilization of those organs. The criteria defining these expanded-criteria donor (ECD) kidneys are donor age 60+ or donor age 50-59 plus two of the following: donor history of cerebrovascular accident, hypertension or creatinine > 1.5 during donor management. Kidney grafts from ECD donors carry an increased relative risk of nonfunction. The purpose of this study was to assess potential best strategies for the acceptance of ECD grafts based on further stratification based on donor age and cold ischemia time (CIT).

Methods: We queried the SEOPF database for cadaveric kidney transplants between 1/1/1997 and 8/15/2002. We defined delayed graft function (DGF) as dialysis within the first week post-transplant and primary nonfunction (PNF) as dialysis within the first week and failure in the first year. We defined "good-risk" ECD as those from donors aged 50-59 yrs and "bad risk" as from donors > 60 yrs.

Results: There were 1,312 ECD transplants and 8,451 non-ECD. Between these groups, there were no significant differences in recipient gender, ethnicity, peak and most recent PRA. Recipients of ECD kidneys were significantly older (50.9± 13.0 years vs. 44.9± 13.9, p<0.0001). There were statistically significant but very small differences in DR mismatch (0.82 for ECD vs. 0.87 for non-ECD). We found that ECD kidneys had a significantly (p<0.0001) higher incidence of PNF and DGF. PNF in ECD appeared to be uniformly distributed across CIT and while DGF was more CIT-dependent , the DGF differences between ECD and non-ECD were fairly consistent across CIT.

Conclusions:While CIT minimization may be beneficial in reducing DGF, ECD kidneys were not more sensitive to it than non-ECD. The increased risk of PNF appears to be intrinsic to ECD kidneys, independent of CIT and donor age.

	No ECD (n=8451)	ECD: age 50-59 (n=576)	ECD: age 60+ (n=736)
PNF	3.99%	8.16%	8.56%
DGF	19.39%	32.99%	30.57%
CIT (hrs)	19.27±7.95	21.36±8.36	20.96±8.63

	%DGF		%PNF	
CIT	Non-ECD	ECD	Non-ECD	ECD
0-4 hrs	10.77	20.83	2.56	8.33
5-8 hrs	12.05	17.5	2.11	7.5
9-12 hrs	14.67	28.83	2.55	6.31
13-16 hrs	15.76	30.57	3.14	8.29
17-20 hrs	17.0	31.04	4.35	8.24
21-24 hrs	24.9	32.65	5.2	11.73
25-28 hrs	27.13	31.37	4.03	5.88
29-32 hrs	24.95	36.36	4.45	10.1
33-36 hrs	29.79	38.46	6.03	9.23
37+ hrs	25.64	38.81	6.41	5.97
Overall	19.39	31.63	3.99	8.38

Abstract# 874

TRANSPLANTATION OF RENAL ALLOGRAFTS FROM HEPATITIS C SEROPOSITIVE DECEASED DONORS INTO ELDERLY HEPATITIS C SERONEGATIVE RECIPIENTS. Aloke K. Mandal,[1] Nicholas Drew,[2] Mara Tableman,[2,3] Jodi A. Lapidus.[2]
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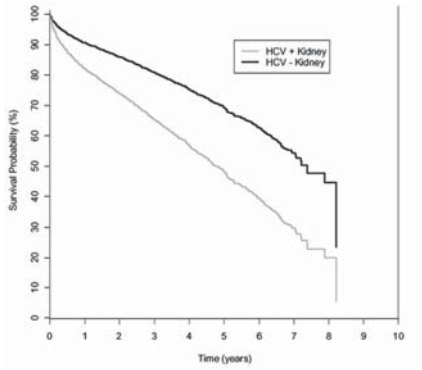
Introduction: Some transplant centers advocate transplanting kidneys from hepatitis c virus seropositive (HCV+) deceased donors into elderly HCV seronegative (HCV-) recipients. This study aims to determine the risks and benefits of such a policy. *Methods:* Registry data from the United Network of Organ Sharing was used to study HCV-, elderly (≥60 years) recipients of a deceased donor renal transplant (DDRT) on or after January 1, 1995. Follow-up continued until April 30, 2003. *Results:* Of the 12,123 elderly DDRT recipients, 11,936 obtained kidneys from HCV- donors and 187 from HCV+ donors. Recipients of HCV+ kidneys were older (p<0.001), more likely to be minorities (p<0.005), and with more HLA mismatches (p<0.001). There were no differences in donor age or cold ischemic times. Of the 232 transplant centers in the United States, 72 centers transplanted at least one kidney from an HCV+ donor during this study period. The use of HCV+ kidneys significantly decreased mean waiting times (p<0.001) and did not significantly affect death-censored graft survival—after adjusting for minority race, HLA mismatches, time on the waiting list, recipient body mass index, cold ischemic time, and donor age. On the other hand, as shown below, elderly HCV+ kidney recipients had 2.2 times the relative risk (RR) of death compared to HCV- kidneys recipients (p<0.001). Causes of death were similar in both groups of patients. After fixing for donor HCV status; recipient age ≥65 (RR 1.3), recipient history of diabetes (RR 1.3), recipient male gender (RR 1.2), and donor age ≥45 (RR 1.4) all increased the risk of death. *Conclusions:* The use of kidneys from HCV+ donors in elderly patients increases the relative risk of death by twofold. Patients who died from cardiovascular or infectious causes in either group did so at higher rates than from other causes. The risk of death can be decreased with appropriate recipient and donor selection.



Abstract# 875

DONOR ASSESSMENT: IS A POLICY CHANGE WARRANTED?

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While multiple case reports and data from the Israel Penn International Transplant Tumor Registry (IPITTR) and the United Network for Organ Sharing (UNOS) have confirmed that existence of malignancy transmission from donor to recipient, little data stratifies the risk of such an event.

Methods: 320 transplants from donors with malignancies diagnosed either pre- or post- transplantation (TXP), were examined for errors in brain death etiology. Diagnostic errors, including intracerebral hemorrhage(ICH) from undiagnosed metastatic disease and brain masses from metastatic vs.primary brain lesions, were examined along with clinical management and outcomes.

Results: 42 recipients who received organs from 29 at-risk donors with misdiagnosed primary brain deaths were examined. Mean donor age was 42.0 ± 11.6 yrs, with gender evenly divided between males and females. Eight donors (27%) had an identified history of malignancy. Post TXP identification of donor malignancies included melanoma (23%), renal cell carcinoma (RCC) (19%), choriocarcinoma (12%), sarcoma (10%), Kaposi's sarcoma (KS) (7%), and variable tumors (29%). The majority were kidney recipients (n=37), followed by liver (n=4) and lung recipients (n=1). The most common diagnostic errors were ICH (62%), tumor mass (21%), and anoxia (17%). A donor-related transmission rate of 74% (31/42) was identified among recipients with misdiagnosed brain death. Tumor transmission was associated with the following donor histologies: melanoma (n=8), RCC (n=7), choriocarcinoma (n=5), sarcoma (n=3), KS (n=3), colon cancer (n=2), lung cancer (n=2), and lymphoma (n=1). While the majority of donor-transmitted cancers were identified in the allograft (71%),64% of recipients also suffered diffuse metastatic disease. Explantation was performed in 21/42 (50%) recipients in the at risk group. Of the 31 patients with donor transmission, five-year survival was 32%. When allograft explantation was performed (17/31), a survival benefit was obtained (10/17; 59% vs. 0/14;0%; p<0.01).

Conclusions: Error in the diagnosis of donor brain death has significant and often fatal consequences. In cases of donor malignancy transmission, allograft explantation for kidney recipients or re-TXP for extra-renal recipients should be undertaken to provide a survival benefit. In an effort to reduce the potential for donor transmission of malignancy, potential donors with unclear etiologies for brain death particularly ICH should be considered for a limited brain autopsy after donation.

Abstract# 876

TRENDS OVER A DECADE OF PEDIATRIC LIVER TRANSPLANTATION IN THE UNITED STATES.

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The last decade of pediatric liver transplantation (LTx) has been characterized by significant technical advances and improved outcomes, leading us to question whether the profile of candidates undergoing LTx has changed. **METHODS:** Information regarding all pediatric (<18 yrs) LTx recipients in 1990-92 (Era1) and 2000-02 (Era2) in the UNOS database were collected and compared using Chi-squared and Wilcoxon tests. **RESULTS:** There were 1,509 pediatric LTxs performed in Era1 compared to 1,736 in Era2. While there were no changes in recipient age, gender and height and only a modest change in recipient weight, there were substantial changes for many characteristics of pediatric Ltx recipients (Table 1). Ethnicity and diagnoses leading to LTx broadened significantly. Utilization of partial grafts - reduced, split, or living donor - increased substantially while utilization of ABO-incompatible grafts decreased substantially. Nevertheless, waiting times increased. The need for retransplantation in infants and young children decreased. There was no consistent trend in laboratory parameters of disease severity (albumin, bilirubin, and creatinine) between the two eras. **CONCLUSIONS:** The national profile of pediatric LTx recipients has changed substantially during the past decade. Greater diversity in ethnicity and diagnoses of LTx children suggests a broader application of LTx as a therapeutic modality. Waiting time, albeit increased, remains modest, at least in part because the decade has witnessed substantial surgical advances in reduced, split, and living donor LTxs. In spite of these technical developments, the decreased frequency of retransplantation, particularly for infants and young children during the past decade suggests concomitant improvements in surgical proficiency and / or immunosuppression.

TABLE 1: PROFILE OF PEDIATRIC LTx RECIPIENTS: 1990-92 vs 2000-02

CHARACTERISTIC	1990-92	2000-02	P-VALUE*
Age (years)	4.7 ± 0.1	5.2 ± 0.1	0.11
Gender (% Female)	50.9	53.6	0.13
Ethnicity (%)			<0.0001
White	63	53.4	
Black	17.6	18.1	
Hispanic	13.8	21.9	
Asian	1.7	3.8	
Height (cms)	97.6 ± 1.1	99.9 ± 1.0	0.15
Weight (kgs)	19.8 ± 0.5	22.9 ± 0.5	0.001
Diagnosis (%)			<0.0001
Acute Hepatic Necrosis	12.9	13.2	
Biliary Atresia	47.6	32.3	
Cholestatic Cirrhosis	11	18.7	
Metabolic Disease	14.9	11.4	
Neoplasm	2.2	4.4	
Non-Cholestatic Cirrhosis	8.2	9.3	
Other	3.1	10.8	
Donor Type (%)			<0.0001
Living	4.4	17.1	
Deceased	95.6	82.9	
Deceased Graft Type (%)			<0.0001
Whole	95.9	73.8	
Reduced / Split	4.1	26.2	
ABO Incompatible (%)	6.9	2.6	<0.0001
Previous Transplant (%)	17.6	12.4	<0.0001
Age <1	13.7	6.3	<0.0001
Age 1 - 5	18.1	13.6	0.034
Age 6-10	22.7	18.2	0.26
Age 11 - 17	18.6	14.8	0.17
Waiting Time (days)	90.6 ± 4.5	157.6 ± 7.3	<0.0001
Albumin at Transplant (mg/dL)	3.2 ± 0.0	3.0 ± 0.0	<0.0001
Bilirubin at Transplant (mg/dL)	13.9 ± 0.3	11.1 ± 0.3	<0.0001
Creatinine at Transplant (mg/dL)	0.6 ± 0.0	0.6 ± 0.0	0.43

*Chi-Squared or Wilcoxon

ISCHEMIA-REPERFUSION INJURY

Abstract# 877

IMPROVED PERFUSION IN HYPOTHERMIC MACHINE PRESERVATION OF THE LIVER.

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Introduction: Hypothermic machine perfusion (HMP) provides a better protection against ischemic kidney damage compared to cold-storage (CS). A switch from static CS to HMP in liver transplantation could prevent Ischemic-Type-Biliary-Lesions due to incomplete perfusion and allow the use of marginal and non-heart-beating donor livers. HMP could, furthermore, prolong preservation time and improve graft function. An important question concerning the application of HMP in liver preservation is the required perfusion pressure in the hepatic artery and portal vein.

Specific aim: To determine the optimal perfusion pressure during HMP preservation of the liver, enabling complete perfusion without inducing endothelial injury.

Methods: Rat livers were preserved using continuous perfusion with Belzer-UW. Group A was perfused with a mean arterial pressure of 12.5 mmHg at 360 beats per minute with a portal perfusion pressure of 2 mmHg, group B was perfused with 25 mmHg and 4 mmHg and group C at 50 mmHg and 8 mmHg respectively. UW was enriched with 13.5 µM acridine orange (AO), to stain viable hepatocytes, and 14.9 µM propidium iodide (PI) to stain dead cells. After 1 h preservation the percentage of liver perfusion was assessed with in-vivo fluorescent microscopy (that is: epi-illumination) of the liver surface using a 484/520 nm filter. Image analysis was performed to determine the percentage of perfusion. Cryosections (4 µm) were examined to identify the location of PI stained cells.

Results: Group A showed 70 +/- 4 % liver perfusion of zone 1 and 2. No PI positive staining was found for the endothelial cells. Group B and C showed complete perfusion for all three zones. Endothelial cell injury, as measured by PI, was found in group C but not in group B.

Conclusion: Hypothermic Machine Perfusion of the liver could improve liver preservation, however, a low grade perfusion is insufficient for complete liver perfusion and adequate preservation. Although arterial perfusion at 50 mmHg -perfectly normal for kidney perfusion preservation- and portal perfusion at 8 mmHg shows complete perfusion, a major drawback is the occurrence of arterial endothelial cell injury, and high-grade perfusion is thus not suitable for HMP preservation. Perfusion at 25 mmHg arterially and 4 mmHg portally is complete with no endothelial injury and is optimal for HMP preservation.

Abstract# 878

PRESERVING THE MUCOSAL BARRIER VIA NUTRIENT & ANTIOXIDANT TREATMENT DURING SMALL BOWEL STORAGE. Payam Salehi,[1] Christopher Samuel,[1] Thomas A. Churchill.[1] *[1]Surgical-Medical Research Institute, University of Alberta, Edmonton, AB, Canada.*

Introduction: The development of an effective preservation solution has been a major obstacle for the successful transplantation of small bowel (SB). The objective of this study was to improve SB preservation by combining known antioxidant agents with a proven amino acid-rich solution (AA solution) which is tailored to the specific metabolic requirements SB.

Methods: SB from Sprague-Dawley rats (n=6 in each Group) was flushed vascularly with modified UW solution and flushed luminally as: **Group 1-** none (control); **Group 2-** 1 h oxygenated perfusion then static storage with AA solution; **Group 3-** group 2 + trolox; **Group 4-** group 2 + superoxide dismutase/catalase. Energetics, oxidative stress and histology were assessed over 24 h at 4°C.

Results: Oxidative damage as assessed by levels of a by-product of lipid peroxidation, malondialdehyde (MDA), was significantly lower in all groups treated with AA solution than in untreated controls (Group 1). The addition of trolox in Group 3 resulted in a significant reduction in MDA levels compared to all other groups throughout 24 h cold storage. Tissue energetics correlated with reduced oxidative injury; ATP and total adenylates were superior in tissues treated with trolox (Group 3) versus AA solution alone (Group 2). Group 1 (clinical control) had the lowest levels of energetic parameters assessed compared to all AA-treated groups. Histologic integrity was markedly improved in Group 3 after 24 h cold storage. Group 3 exhibited only minor injury including moderate epithelial clefting of the villi; this was an improvement on treatment with AA solution (no additives, Group 2) which exhibited varying degrees of injury ranging from epithelial clefting to villus disintegration and crypt infarction. Control tissues (Group 1) exhibited severe injury, including villus degeneration, crypt infarction and transmucosal infarction.

Conclusion: Use of the novel amino acid-rich solution significantly reduces peroxidative damage; this effect is enhanced via supplementation with the water-soluble vitamin E analogue, Trolox. These antioxidant effects lead to a superior maintenance of mucosal integrity supported by sustained energy metabolism. This combined strategy may have implications for the successful preservation and transplantation of SB in the clinic.

Abstract# 879

CD39/ECTO-NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE-1 MAINTAINS VASCULAR INTEGRITY IN INTESTINAL ISCHEMIA-REPERFUSION INJURY. Olaf Guckelberger,[1] Xiofeng Sun,[2] Jean Sévigny,[2] Masato Imai,[2] Elzbieta Kaczmarek,[2] Keiichi Enjyoji,[2] Simon C. Robson.[2] *[1]Dept. of Surgery, Charité Campus Virchow-Klinikum, Berlin, Germany; [2]Dept. of Medicine, Beth Israel Deaconess Medical Center, Boston, MA.*

CD39/NTPDase-1 the dominant vascular ectonucleotidase has been shown to inhibit platelet aggregation as well as EC activation following nucleotide-mediated stimulation of P2-receptors *in vitro*. Increased catalysis of plasma nucleotides by NTPDases is associated with decreased vascular injury and prolonged graft survival in transplantation models. Here we evaluate effects of vascular NTPDase-1 expression and purinergic mediators on survival and vascular permeability in intestinal ischemia-reperfusion injury (IRI).

Methods: Wild-type and cd39-null mice were subjected to superior mesenteric arterial occlusion for 45 or 60 min. Matched control mice underwent sham surgery prior to tissue harvesting at 60 min. Treatment groups received soluble NTPDase (0.2U/g), adenosine (1µmol/kg/min)/amrinone (0.5µmol/kg/min), or saline continuously infused over 60 min commencing 5 min prior to reperfusion. Evans Blue (EB) (0.8µL/g, 0.5%) was injected and accumulation in jejunal specimens determined as a measure of vascular permeability.

Results: Mortality rates in vehicle treated cd39-null and wild-type mice were comparable. NTPDase supplementation protected only wild-type animals from death due to IRI (p=0.038 vs. vehicle), while adenosine/amrinone administration could not influence survival figures in either group. Post-ischemic EB tissue levels increased in cd39-null mice over wild-type mice (p=0.039), while baseline levels did not differ (p=0.29 NS). Wild-type mice had no increases in EB tissue levels following sham operation, while IRI led to increased EB accumulation (44.6 OD/g) compared to baseline levels (21.6 OD/g, p=0.002). In cd39-null mice, permeability increased both post-sham operation (39.7 OD/g, p=0.007) and IRI (51.7 OD/g, p=0.001), as compared to baseline levels (25.8 OD/g). Treatment with either soluble NTPDase or adenosine/amrinone maintained post-IRI tissue permeability at baseline levels.

Discussion: The cd39-null mice demonstrated higher susceptibility to intestinal injury when compared to wild-type animals. Soluble NTPDase as well as adenosine/amrinone effectively prevented the increases in post-ischemic vascular permeability. Therefore, we conclude NTPDase maintains vascular integrity in intestinal IRI and that exogenous NTPDase administration has protective effects post-ischemic insults.

Abstract# 880

CARBON MONOXIDE SIGNIFICANTLY REDUCES THE APOPTOTIC EVENTS ASSOCIATED WITH ISCHEMIA-REPERFUSION INJURY: OBSERVATIONS IN A RENAL PIG-TO-PRIMATE MODEL. E. Cozzi,[1,2,3] M. Seveso,[2] F. Calabrese,[3] G. De Benedictis,[3] N. Baldan,[1,2,3] M. Boldrin,[2] M. Castagnaro,[3] L. Ravarotto,[4] P. Carraro,[1] R. Busetto,[3] L. E. Otterbein,[5] M. Lavitrano,[6] D. Bernardini,[3] G. Thiene,[3] F. H. Bach,[7] E. Ancona.[1,2,3] *[1]Padua General Hospital, Padua, Italy; [2]CORIT (Consorzio per la Ricerca sul Trapianto d'Organi), Padua, Italy; [3]University of Padua, Padua, Italy; [4]Institute for Animal Health Prophylaxis, Legnaro, Italy; [5]University of Pittsburgh, Pittsburgh, PA; [6]University of Milano-Bicocca, Monza, Italy; [7]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

Background: Apoptosis is a hallmark of ischemia-reperfusion injury and high levels of apoptotic events at reperfusion have previously been correlated with poor initial graft function. This study has investigated whether carbon monoxide (CO) administration was able to confer protection against ischemia-reperfusion injury and, possibly, extend the survival of pig organs transplanted into primates.

Materials and methods: Kidneys from hDAF transgenic pigs exposed to CO were transplanted into 6 immunosuppressed cynomolgus monkeys in a life-supporting model. The anti-inflammatory effects of the CO regimen used was monitored by evaluating *in vitro* TNF-α production by PBMC from these pigs. A thorough histopathological assessment, including TUNEL evaluation of the apoptotic index (AI), was conducted on the kidney both in the 30-minute biopsy and at euthanasia.

Results: The transplanted primates survived from 2 to 37 days and were euthanized with rejection in 5 cases. CO treatment significantly reduced TNF-a production by PBMC isolated from donor pigs ($P<0.02$). Similarly, at the biopsy taken 30 minutes after reperfusion, the AI in the tubuli and interstitium was significantly lower in the grafts from CO treated animals than in controls ($P<0.05$ and $P=0.007$, respectively). No cases of overt DIC were observed.

Conclusions: This study is the first *in vivo* demonstration in a primate transplantation model that CO preconditioning is an effective treatment to attenuate ischemia-reperfusion injury. Further studies are needed to determine to what extent CO-based regimens can ultimately extend the survival of xeno- or allografts transplanted into primates.

Abstract# 881

NITRIC OXIDE (NO) CAN PROMOTE SURVIVAL OF TUBULAR EPITHELIAL CELLS (TEC) AND INCREASE SURVIVAL OF RENAL ALLOGRAFTS. C. Du,[1,4] J. Jiang,[3,4] Q. Guan,[2,4] Z. Yin,[2,4] R. Zhong,[1,2,3,4] A. M. Jevnikar.[1,2,3,4] *[1]LHRI, London, ON, Canada; [2]RRI, London, ON; [3]Med.Surgery, Micro & Imm., Univ. West. Ontario; [4]MOTS, LHSC, London, ON.*

NO is synthesized from the terminal guanidino nitrogen of L-arginine by a family of NO synthetases. iNOS is expressed by glomerular, mesangial and tubular epithelial cells (TEC) and induced by cytokines (i.e. IFN-g and TNF-a) present during renal allograft rejection. However NO has been shown to have disparate functions in cell survival, either inducing or inhibiting apoptosis which could alter graft survival. While recipient NO inhibition has been beneficial in murine cardiac allografts, the role of renal derived NO in the survival of renal allografts is not known. CS3.7 tubular epithelial cells (TEC) were used to test the effect of NO in vitro. Treatment with IFN-g and TNF-a upregulated both iNOS synthesis and release of NO. Addition of sodium nitroprosside (SNP), a NO-donor molecule for 24 h to TEC induced apoptosis in a dose-dependent manner with maximal apoptosis of 49% ($p<0.05$ from control) using 50 ug/ml. However at 100 ug/ml, TEC apoptosis was equivalent to basal levels of untreated TEC (20%). We analysed cell survival proteins in these TEC. 10-50 ug/ml of SNP decreased mRNA levels of anti-apoptosis proteins (c-FLIP, IAP-3 and Survivin). In contrast, SNP at 100 ug/ml did not alter c-FLIP or IAP-3 levels, but enhanced Survivin mRNA expression. Furthermore, TEC which survived SNP induced apoptosis were resistant to downregulation of c-FLIP, IAP-3 and Survivin on re-exposure to SNP (10-50 ug/ml), and remained resistant to further apoptosis.These data suggest that NO could induce survival proteins in TEC in vivo. To test this in vivo, wild type (WT) or iNOS null B6 (H-2b) kidneys were transplanted into nephrectomized untreated, BALB/c (H-2d) mice. WT grafts survived longer (48±10 d, n = 4) compared to those with iNOS null grafts (24±5d, n = 8, p=0.04). These data suggest that while NO can induce TEC apoptosis, at higher amounts NO can promote survival of TEC by upregulation of anti-apoptosis proteins. Furthermore we show that endogenous levels of NO within donor tissue may be important to graft function and survival. Maintaining renal derived NO as well as upregulation of survival proteins such as c-FLIP and survivin, might be of benefit in strategies for long-term survival of renal allografts.

Abstract# 882

A SINGLE SHORT-TERM DONOR TREATMENT FOR THE INDUCTION OF CO REDUCES GRAFT IMMUNOGENICITY AND IMPROVES ALLOGRAFT LONG-TERM FUNCTION. Paulo N. A. Martins,[1] Anke Jurisch,[1] Anja Reutzel-Selke,[1] Andreas Pascher,[1] Sven Jonas,[1] Johann Pratschke,[1] Peter Neuhaus,[1] Hans-Dieter Volk,[2] Stefan G. Tullius.[1] *[1]Dept. of Surgery, Charité, Virchow-Clinic, Berlin, Germany; [2]Dept. of Medical Immunology, Charité, Mitte, Berlin, Germany.*

Events leading to unspecific inflammatory damages occurring prior to organ transplantation reduce graft survival. It can be speculated that an increased immunogenicity accelerates specific immune responses. We tested the effects of CO induction in donor animals immediately prior to organ harvesting.

F-344 renal allografts were grafted into LEW recipients. Ischemia was prolonged to 6h to induce unspecific inflammatory damages. Recipients received a short term CyA treatment (1.5 mg/kg/d x 10 d) to overcome an initial acute rejection episode. Donor animals were either treated with Methylene Chloride (MC) at -4h prior to organ harvesting to induce CO or remained untreated. Effects of recipient CO induction were followed by a short (10 d) or long-term (180 d) MC administration (n=6/group).

COHb levels peaked by 2 h (COHb 5.5 % ±1.1%) and returned to norm values by 24 h. Proteinuria was significantly reduced by 6 mths in pretreated grafts (15±2mg/24h vs. control: 60±20mg/24h, p<0.01). Structural changes in control animals demonstrated characteristic signs of chronic graft deterioration by 6 mths while morphological changes in pretreated grafts were markedly reduced (glomerulosclerosis 82±9% vs. 20±5%, p<0.0001; arteriosclerosis 3.2±0.2 vs. 2.3±0.3, p<0.01; tubular atrophy 3.8±0.3 vs. 1.4±0.4, p<0.0001; fibrosis 3.5±0.4 vs. 0.7±0.4, p<0.0001; evaluation on a 0-4+ scale/4+ = strongest structural deterioration/>20 fields of view/section at 400x). Similarly, cellular infiltrates (CD4, CD8+ T cells, ED1+ monocytes/macrophages) were significantly reduced following donor pretreatment compared to controls (p<0.0001). Both, grafts of short-term and long-term MC-treated recipients demonstrated reduced functional and structural deterioration compared to controls. However, improvement of morphological changes was even more pronounced in grafts following a single donor pretreatment compared to long-term treatment of recipients (glomerulosclerosis: 20±5% vs. 37±13%, p=0.01).

CO induction in the donor shortly prior to organ harvesting was associated with a marked improvement of long-term graft function. Those observations may be based on a reduced immunogenicity.

Abstract# 883

BENEFICIAL EFFECT OF CARBON MONOXIDE-INHALATION AGAINST ISCHEMIA/REPERFUSION INJURY AFTER RAT LIVER TRANSPLANTATION: POSSIBLE MECHANISM OF iNOS/NO PATHWAY BLOCKADE. Takashi Kaizu, Eric L. Marderstein, Lee L. Sonis, Lifang Shao, Brian T. Bucher, Atsunori Nakao, Leo E. Otterbein, David A. Geller, Noriko Murase. *Surgery, University of Pittsburgh, Pittsburgh, PA.*

Background: Stress response genes, such as heme oxygenase (HO)-1 and inducible nitric oxide synthase (iNOS), have been reported to be induced after ischemia/reperfusion (I/R) injury; however, their definite roles remain undetermined. We have previously demonstrated that carbon monoxide (CO), byproduct of heme catalysis, protects kidney and small intestinal grafts against I/R injury. This study examined whether CO would protect the liver against cold I/R injury with a particular attention to iNOS. **Methods:** Orthotopic syngeneic LEW rat liver transplantation (OLT) was performed with 18 hrs preservation in cold UW solution. Recipients were exposed to air or a low dose CO (100 ppm) for 1 hr before and 24 hrs after OLT and sacrificed 3~48 hrs for serum AST and NO levels, histopathology, and hepatic iNOS protein expression. Efficacy of CO was further analyzed *in vitro* primary rat hepatocyte culture system after stimulation with cytokine mixture (CM) (TNF-α 500U/ml, IL-1 200U/ml, IFN-γ 100 U/ml). iNOS protein, NO production and hepatocyte viability after CM stimulation were analyzed. **Results:** After OLT, CO-inhalation effectively improved hepatic I/R injury. Serum AST levels and tissue necrosis was significantly reduced with CO, compared to air-treated controls. Hepatic iNOS protein expression was significantly decreased in CO-treated group versus air-treated controls at 3 and 48 hrs with lower serum NO levels (Table, mean±SE, n=3 animals per group, ∗P < 0.05). *In vitro* culture study confirmed the efficacy of CO. CM-induced iNOS protein expression was markedly inhibited, and NO production was significantly reduced in CO-treated hepatocytes (20.8 ± 5.7 vs. 37.8 ± 7.4 μM in Air, p < 0.05). Hepatocyte viability also was increased with CO (93.5 vs. 63.1% in Air, p < 0.05). **Conclusion:** The results demonstrate that exogenous CO treatment efficiently ameliorates hepatic I/R injury. The possible mechanism, by which CO protects the liver against cold I/R, may include the down-regulation of iNOS/NO pathway. Thus, low-dose CO inhalation treatment would be a novel therapeutic strategy to combat hepatic cold I/R injury.

	Necrotic area (%)	Serum AST (IU/L)		iNOS band intensity (/normal liver)	
Group	48h	6h	24h	48h	3h	48h
Air control	30.5±0.7	2434±646	13402±2452	8931±3233	10.6±1.5	31.2±7.5
CO (100 ppm)	10.7±0.2∗	1545±60	2930±636∗	2418±730	4.3±1.8∗	18.6±8.3

Abstract# 884

DECLINING NUMBER OF POTENTIAL LIVING DONOR CANDIDATES FOR ADULT LDLT. Dianne LaPointe Rudow,[1] Milan Kinkhabwala,[1] Silvia Hafliger,[1] Douglas Marratta,[1] Janet Lee,[1] Jean C. Emond,[1] Robert S. Brown, Jr..[1] *[1]Center for Liver Disease & Transplantation, Columbia University Medical Center, New York, NY.*

Introduction: After initial enthusiasm for adult LDLT in the late 1990's the number of LDLT performed has recently declined. This has likely been the result of increased scrutiny from professional societies, UNOS, The Health Department, and the public and increased caution in the transplant community. Whether the decrease in LDLT is due to fewer donors being evaluated or increased rejection of donors is unknown.

Aim: To analyze changing patterns in living donor (LD) evaluation and LDLT volume.

Methods: We retrospectively reviewed all LD who were evaluated from 1999 thru 2003 as part of adult LDLT. All LD had completed part of the evaluation. To be included they must have met with at least 1 member of the donor team and received education about LDLT. Our LD and recipient selection criteria have not changed throughout the study period. All non-urgent listed patients are considered for LDLT.

Results: 234 LD were evaluated. 73 LDLT (25% of adult OLT) were performed and 161 LD were declined (see below). The number of LD evaluations decreased significantly from a high of 74 in 2002 to 39 in 2003. The number of LDLT performed peaked in 2001 at 24 and dropped by 50% to 12 by 2003. Reasons for donor dropouts have not changed through out the study. Donor decision remains the main reason (43%), followed by donor medical issues (20%), and recipient issues (e.g. death, too sick etc.)(10%).

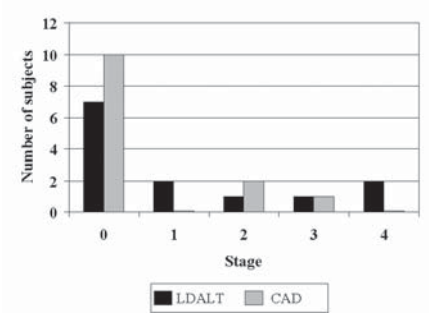
Conclusion: 30% of evaluated LD complete LDLT. Donor decision remains the most common reason for not undergoing LDLT. Thus, recent negative media attention and stricter quality improvement strategies have not changed the reasons for not performing LDLT but has led to fewer potential LD presenting for evaluation. It is premature to conclude that the decrease in volume is related to the stated changes in LDLT practices or other phenomenon. An alternate explanation would be exhaustion of a large reservoir of potential LDLT recipients on the UNOS wait list and thus a steady state based on new listings only.

	1999 (19)	2000 (16)	2001 (43)	2002 (56)	2003 (27)	Total
Evaluations	27	27	67	74	39	234
Transplants	8 (30%)	11 (40%)	24 (36%)	18 (24%)	12 (30%)	73 (31%)
Declines	19 (70%)	16 (60%)	43 (64%)	56 (76%)	27 (70%)	161 (69%)
recieved deceased donor	0	0	2%	14%	11%	7%
recipient issues	5%	12%	16%	9%	11%	11%
Medical	0	25%	19%	25%	22%	20%
Social	16%	0	14%	5%	4%	8%
Donor decision	53%	50%	37%	45%	41%	43%
Blood Type	16%	0	5%	4%	7%	6%
Coercion	5%	6.25%	2%	0	0	2%
Other	5%	6.25%	5%	0	4%	3%

Abstract# 885

THE SEVERITY OF RECURRENT HEPATITIS C (HCV) IN LIVING DONOR ADULT LIVER TRANSPLANT (LDALT) RECIPIENT IS THE SAME AS CADAVER (CAD) RECIPIENTS. Fredric D. Gordon,[1] Elizabeth A. Pomfret,[1] James J. Pomposelli,[1] Andrew Keaveny,[1] Mary Ann Simpson,[1] David Lewis,[1] Denise Morin,[1] Urmila Khettry,[2] Roger L. Jenkins.[1] *[1]Hepatobiliary and Liver Transplantation, Lahey Clinic Medical Center, Burlington, MA; [2]Pathology, Lahey Clinic Medical Center, Burlington, MA.*

Background: Recurrent HCV after liver transplantation (LT) is a well-described entity occurring in all patients transplanted with HCV. Anecdotal reports suggest that recurrent HCV in LDALT recipients has a more severe outcome than in CAD recipients. Comparative histologic data is lacking however. In this study we compare histologic outcomes in patients with HCV who received LDALT organs to case-matched CAD organ recipients. **Methods:** Subjects were matched with regard to year of LT and interval to biopsy (bx). All bx were event driven (abnormal liver enzymes) and reviewed by a single pathologist. Ludwig/Batts grade/stage were recorded for each pair. 66 patients with HCV underwent primary LT between 1/1/00 and 9/3/02. 19 received an LDALT and 47 received a CAD organ. 6 LDALT recipients were excluded (5 had no bx material, 1 required retransplantation) leaving 13 study subjects. Case-matches were found among the 47 CAD recipients. The mean interval from LT to bx was 11.5 months in both groups. **Results:** Histologic stage is summarized below. There was no statistical difference (Pearson's Chi square) in the stage or grade. Two patients in the LDALT group developed cirrhosis at 3 and 24 months. No CAD patient developed cirrhosis. One LDALT recipient died at 4 months from fibrosing cholestatic HCV. Two deaths occurred in the CAD group at 23 and 38 months from respiratory failure and recurrent HCV. Survival in LDALT vs. CAD at 6, 12 and 18 months was 92.3% vs. 100%. At 24m and 36m surivival in both groups was 92.3%. **Conclusions:** 1) The severity of recurrent HCV in LDALT recipients is no different than CAD recipients. 2) 3 year survival in both groups is excellent and comparable to non-HCV LT recipients. 3) Further studies with protocol liver bx are necessary to delineate the natural history of HCV after CAD and LDALT.



Abstract# 886

IMMUNOMONITORING OF LIVER RECIPIENTS TREATED WITH TOLERANCE-ENHANCING REGIMEN OF IMMUNOSUPPRESSION. Noriko Murase,[1] Diana Metes,[1] Adriana Zeevi,[1] Carol Bentlejewski,[1] David Guaspari,[1] Camila Macedo,[1] Angie Farren,[1] Pam McGregor,[1] Bijan Eghtesad,[1] Paulo Fontes,[1] Amadeo Marcos,[1] Jennifer Woodward,[1] Anthony J. Demetris,[1] John J. Fung,[1] Thomas E. Starzl.[1] *[1]Surgery, Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Liver allograft recipients were treated with therapeutic principles of pretreatment and minimum posttransplant immunosuppression consistent with graft survival and function. Thymoglubulin (THY, 3-6 mg/kg) or Campath (CAM, 30 mg) was infused before transplant and tacrolimus (TAC) monotherapy was used as postoperative treatment with other agents (e.g. steroid) for biopsy-proved rejection. Changes in immunological parameters during 6-12M follow-up were studied. **Methods**: Blood samples at 6 and 12M after transplantation were analyzed with four-color flow cytometry and in vitro cell proliferation assays. **Results:** Pretreatment with THY or CAM resulted in significant posttransplant lymphocyte depletion, which slowly recovered over the next 6-12M. CAM appeared to have stronger and prolonged impacts on depletion. Recovery of CD4 counts was slower than CD8, resulting in remarkably low CD4/CD8 ratios (<0.8) in nearly 40% of recipients at 6-12M with a high incidence in CAM group. Similarly, DC1 and DC2 populations significantly decreased. DC1 gradually recovered and became nearly normal by 12M (49% vs. 57% in normal volunteers). DC2 recovery was remarkably slow and was 5.5 ± 5.0% at 12M compared to 15.0 ± 9.0% in normals. CAM also effectively depleted B cells, and significantly low B cell counts persisted nearly 12M. At 1 year, the majority of patients (74%) were on TAC or cyclosporin monotherapy with doses spaced from QOD (19%), 3/W (9%), 2/W (30%), and 1/W (14%). Detailed in vitro studies (MLR, CML) were performed in 13 recipients with >1 year follow-up. In 10 of 13 on spaced monotherapy, 7 showed profound hyporesponsiveness to mitogens and to donor and 3rd party alloantigens in MLR. One developed donorspecific hyporeactivity in MLR and CML. Remaining 2 showed vigorous antidonor reactivity in MLR and CML with 13 and 43% donor killing. In 3 patients with daily treatment, 2 showed intact antidonor reactivity in MLR, and one had total hyporesponsiveness. **Conclusions**: Liver recipients treated with tolerance-enhancing regimen achieved stable engraftment. CAM appeared to have robust and prolonged impacts on immunological parameters. High frequency of profound in vitro hyporesponsiveness under minimum immunosuppression in liver recipients may be the result of pretreatment and complicated immunological status in this population.

Abstract# 887

REGIONAL VARIATIONS IN MELD EXCEPTIONS FOR NON-STANDARD DIAGNOSES. Hector Rodriguez-Luna,[1] Hugo E. Vargas,[1] Adyr Moss,[1] Kunam S. Reddy,[1] Richard B. Freeman,[2] Ann M. Harper,[3] Erick B. Edwards,[3] David C. Mulligan.[1] *[1]Transplant Medicine, Mayo Clinic Hospital, Phoenix, AZ; [2]Transplant Medicine, Tufts University School of Medicine/New England Medical Center, Boston, MA; [3]Research Department, United Network for Organ Sharing (UNOS), Richmond, VA.*

AIM: The introduction of MELD in 2002 was designed to eliminate subjective parameters from influencing priority on the waiting list. Recognizing that MELD may bias against certain diagnoses, a system is in place to review exceptional cases and adjust MELD score based on regional consensus. The OPTN recommended HCC, hepato-pulmonary syndrome, familial amyloid and metabolic liver diseases for standard upgrade consideration. Regional review boards approve or deny individual requests. The aim of this study is to compare the regional practices and to review the diagnoses that are used to justify MELD increases in non-HCC cases. **METHODS:** The UNOS database was queried to extract all adult cases where exceptions to MELD were requested from 2/27/02 until 8/27/03. For the purpose of this study, only non-HCC cases were included. Request narratives were reviewed by our group and a justification for exception request was assigned. The data was stratified by UNOS region and justification for exception. **RESULTS:** Data for 29510 pts was available. 3281 exceptions were requested in that period of time. Of those, 827 were for non-standard diagnoses 477 (58%) were granted, 39 (4.7%) withdrawn, and 302 (37.4%) denied. The percentage of petitions approved varied significantly among regions (28-75% p<0.0001). The most common diagnoses for these exceptions included ascites (164), biliary complications (78), porto-systemic encephalopathy (73), cholangitis (71) portal hypertension bleeding (62) regional agreement (31) and hepatic hydrothorax (26). The percentage of patients listed with non-HCC MELD exceptions varies significantly among regions (0.7-8.3 %, p<0.0001). Furthermore, the proportion of pts transplanted in this period of time had significant variability (2.1 to 31.9% p<0.0001) Pts who faced a re-LT were significantly more likely to be considered for exception (p< 0.0001). Gender, ABO group, or ethnicity had no influence on the choice to petition.**CONCLUSIONS:** Allocation of livers using the MELD score has resulted in fewer deaths on the waiting list and increased numbers of pts receiving organs. Widespread variations exist, however, between the different regions in the country where a significant percentage of pts are being transplanted using non-MELD criteria. These variations mandate a reform to standardized exception criteria that can be applied uniformly across the country to maintain equity for our patients.

Abstract# 888

HIGHER PELD SCORES AT LISTING ARE NOT ASSOCIATED WITH WORSE POST-TRANSPLANT OUTCOME IN PEDIATRIC LIVER TRANSPLANTATION (LT): A RETROSPECTIVE STUDY IN 100 RECIPIENTS. Raymond Reding,[1] Christophe Bourdeaux,[1] Tran Thanh Tri,[1] Jeremie Gras.[1] *[1]Pediatric Liver Transplantation Program, Universite Catholique de Louvain, Brussels, Belgium.*

Pediatric end-stage liver disease score (PELD), a severity-of-illness assessment, has been proposed as an objective tool to prioritize children awaiting LT, higher PELD scores being associated with increased pre-LT mortality. PELD is in use in the UNOS, whereas it is still debated in Eurotransplant (ET). We investigated whether PELD at listing may also impact on post-LT results. Patients and Methods: PELD was retrospectively analyzed in 100 pediatric recipients (median age:1.4y, range:0.4-13.9) of a primary LT, transplanted between 05/94 and 04/02. Hepatic malignancy and fulminant hepatitis were excluded from this study, the two main diagnoses being biliary atresia (n=64) and Byler's disease (n=12). PELD was calculated by computation of bilirubin (median:11mg/dl; range:0.3-51.9), albumin (median:3.8g/dl; range:1.7-5.1), INR (median:1.2; range:0.9-8.0), age, and growth retardation. Post-mortem liver grafts were allocated by ET using an allocation system taking into account waiting times as well as medical urgency. Post-LT results were studied according to PELD calculated at the time of listing on the post-mortem donor waiting list (n=51), or at the pre-LT assessment of children included the living donor program (n=49). Results: Overall 1y and 5y actuarial patient survival in this series was 97% and 96%, the corresponding figures for graft survival being 92% and 91%. Individual PELD (mean:13.3; SD:9.7; range:-5 to +46) showed an almost normal statistical distribution (Kurtosis: 1.112; standard error of Kurtosis: 0.478). One-year patient survivals were calculated within the following PELD categories (NS): 100% for children with PELD >+2SD (n=5);

91% with PELD +2/+1SD (n=11); 96% with PELD +1/0SD (n=24); 98% with PELD 0/-1SD (n=47); 100% with PELD <-1SD (n=13). Similarly, no statistical impact of PELD could be found for graft survival, retransplantation rate, as well as for acute rejection-free and chronic rejection-free survivals. Conclusion: PELD did not significantly impact on post-LT results, which suggests that giving priority to high PELD recipients may not result in worsening the post-LT outcome. PELD-based liver graft allocation in children de-emphasizes waiting time and directs organs to the sickest patients. In accordance to our data, we support such "sickest children first" allocation policy, which should contribute to reduce pre-LT mortality without worsening post-LT results and increasing organ waste.

Abstract# 889

TOLERANCE OF LIVER TRANSPLANT RECIPIENTS TO STRENUOUS PHYSICAL ACTIVITY IN HIGH ALTITUDE. J. Pirenne,[1] F. Van Gelder,[1] C. Verslype,[1] W. Peetermans,[1] F. Nevens.[1] *[1]UZ Gasthuisberg, University Hospital, Leuven, Belgium.*

Quality of life and performance are altered by liver failure and improved by Liver Transplantation (LTx) but no study compares physical capacity in LTx *versus* normal healthy subjects. How LTx tolerate strenuous physical activity (and extreme altitude) is unknown. **Methods**.6 LTx patients gave consent and participated to a trek up Kilimanjaro, Africa's highest point (5.895m). Inclusion citeria were:<50yo, LTx>1 yr, normal liver/cardio/pulmonary function, normal life-pattern, non-sport-professional. LTx were accompanied by 15 control subjects (similar profile, matched for age/body-mass-index/gender/VO2max). Daily data recording included: physical performance, Borg-scale, Lake Louise acute-mountain-sickness (AMS) score, cardiorespiratory parameters. Immunosuppression was steroid-free and tacrolimus-based (5-8ng/ml). Prevention against AMS and infection was given. **Results**.83.3 % Tx subjects summited *versus* 84.6 % controls (ns). No difference in Borg-scale was seen. Lake Louise score showed no increased vulnerability to AMS in LTx. O2 saturation (sat) decreased whereas arterial blood pressure and heart rate increased with increasing altitude in LTx and controls. The only difference was a higher arterial blood pressure at all time-points in LTx. One LTx with hepatitis C abandonned at 4,600m due to exhaustion, hypoglycemia, low O2 sat. A biopsy done immediately after the trek showed relapsing hepatitis that was clinically silent and compatible with normal activities at sea level but that impaired the liver adaptive response to exercice in altitude. No infection was seen among LTx subjects.

Conclusion: Selected LTx recipients, free of recurrent liver disease, tolerate extreme physical conditions (strenuous physical activities, extreme altitude) similarly to control subjects, suggesting that today's LTx technology has the potential to restore physical ability *ad integrum.*

Abstract# 890

COMBINED LUNG (HEART) AND LIVER TRANSPLANTATION: INDICATION AND OUTCOME. Gerrit Grannas,[1] Martin Strueber,[2] Rainer Lueck,[1] Thomas Becker,[1] Michael Neipp,[1] Juergen Klempnauer,[1] Bjoern Nashan.[1] *[1]Viszeral- und Transplantationschirurgie, Medizinische Hochschule Hannover, Hannover, Germany; [2]Herz-, Thorax- und Gefaesschirurgie, Medizinische Hochschule Hannover, Hannover, Germany.*

Background: Portopulmonary hypertension (PPHT) is often associated with pulmonary fibrosis based on cystic fibrosis or a1-antitrypsin deficency. The ultimative choice of treatment is a lung transplant (Tx). Since there is a significant co-incidence of liver cirrosis, a combined lung-liver transplantation (Lu-LTx) is the therapy of choice. The combination of endstage lung and liver disease leads to patients with poor nutritional condition related to severe intestinal malabsorption and chronic infections of the lung with often multiresistent organisms. The suitability of these patients with particullar poor outcome (1) is called in question, paricularly in the context of global organ shortage. **Patients and methods:** From 04/1999 to 12/2003 12 Patients (Pat.) (8 male, 4 female) with a therapy refractory PPHT underwent a combined liver and lung Tx; in 1 Pat. with secondary PPHT due to restrictive cardiomyopathy enbloc heart/ lung and liver Tx was performed. Median recipient age was 36 years (19 to 55 years). The underlying disease was in 2 Pat. an a1 antitrypsin deficency, in 4 Pat. a cystic fibrosis, in 1 Pat. a sarcoidosis with secondary liver cirrosis, in 5 Pat. an idiopathic pulmonary hypertension in combination with in 1 case each, a chronic HBV infection, a hemochromatosis, a cryptogenic cirrhosis, an autoimmune cirrhosis. In 10 cases a double lung Tx and in 2 cases a single lung Tx was performed. The immunosuppression was based on Cyclosporin A in combination with predisolon and Aza respectively MMF. **Outcome:** 3 Pat. died, 1 with an initial non function and toxic liver failure, the other 2 months post Tx due to a rupture of an splenic artery aneurysm respectively a brain edema after resuscitation after severe bleeding from pulmonary artery.10 Pat. are doing well (survival: 1yr 73%, 2ys 73%, 3ys 73%, 4ys 73%)*Rejection:* In the postoperative course we observed in 2 Pat. a rejection episode of the liver and in 1 Pat. a rejection of the lung. The rejections were successfully treated with steroids and in 1 Pat. additionally with an immunosuppressive switch to tacrolimus.*Complications:* one rectum perforation, one esophageal bleeding, two pneumonia, one CMV infection and one secondary wound healing. **Conclusion:** Combined lung/ liver or even heart/ liver and lung Tx in a selected population demonstrate a favourable outcome given the surgical, immunological and infectious risks

ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION

Abstract# 891

ACCOMMODATION IN ABO INCOMPATIBLE LIVING-DONOR KIDNEY TRANSPLANTATION. Atsushi Aikawa,[1] Tomomi Hadano,[1] Takehiro Ohara,[1] Kenji Arai,[1] Takeshi Kawamura,[1] Masaki Muramatsu,[1] Tosihiro Itabashi,[1] Ken Sakai,[1] Sonoo Mizuiri,[1] Akira Hasegawa,[1] Tetsuo Kanai,[2] Noriko Kawada.[2] *[1]Deapartment of Nephrology, Toho University School of Meidicine, Tokyo, Tokyo, Japan; [2]Blood Transfusion Center, Toho University, Omori Hospital, Tokyo, Tokyo, Japan.*

[Purpose] To investigate accommodation in ABO incompatible kidney transplantation, we studied anti-donor and compatible blood type antibodies in patients' sera and blood type antigen expression in renal allograft biopsy following transplantation. [Methods] Seventy seven except 2 patients had splenectomy and were pretreated with immunoadsorption and/or plasmapheresis and exchange. Immunosuppression consisted of cyclosporine or tacrolimus, steroid and azathioprine or mycophenolate mofetile. Forty five ABO incompatible recipients (13 A1 to O, 11 B to O, 9 A1 to B, 11 B to A1, and 1 A1B to O) more than 1 year post-transplantation were measured anti-donor and compatible blood type IgG and IgM antibodies. The titers were compared with those of control groups (6 O to O, 7 A1 to A1, and 7 B to B). Blood type antigens were stained with an indirect immunoperoxidase method in 35 renal allograft biopsy specimens. [Results] The titers of anti-A1 IgG and IgM antibodies were significantly lower in 13 recipients (A1 to O) than those in 6 recipients (O to O) (Log_2[IgG]: 3.2±1.7 vs. 6.2±2.6, $p<0.02$, Log_2[IgM]: 2.4±1.1 vs. 6.2±2.6, $p<0.001$). The titers of anti-B antibodies were significantly lower in 11 recipients (B to O) than those in 6 recipients (O to O) (Log_2[IgG]: 2.1±1.8 vs. 6.3±1.6, $p<0.00002$, Log_2[IgM]: 1.5±1.1 vs. 5.3±0.8, $p<0.0001$). These indicated donor specific antibody suppression in 24 recipients with blood type O. However donor specific suppression was observed only in IgM of 11 recipients (B to A1) and 9 recipients (A1 to B), compared with A1 to A1 and B to B respectively (Log_2[IgM]: 2.3±1.0 vs. 5.6±1.7, $p<0.0007$, Log_2[IgM]: 3.3±1.6 vs. 5.7±1.0, $p<0.001$). Donor blood type antigens were strongly expressed on vascular endothelium even 7 year-post transplantation and the intensity was stable under all conditions. Only one patient (A1 to O) had delayed hyperacute rejection and maintained the high titers (x512) of anti-donor antibodies even after ABO compatible retransplantation. Three patients with chronic allograft nephropathy remained donor specific antibody suppression. [Conclusion] Donor blood type antibody suppression appears to be a key to protect from hyperacute or humoral rejection and result in accommodation in ABO incompatible kidney transplantation.

Abstract# 892

ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION WITHOUT SPLENECTOMY USING ANTIGEN-SPECIFIC IMMUNOADSORPTION AND RITUXIMAB. Gunnar Tydén,[1] Gunilla Kumlien,[2] Ingela Fehrman.[3] *[1]Department of Transplantation Surgery, Huddinge University Hospital, Stockholm, Sweden; [2]Department of Transfusion Medicine, Huddinge University Hospital, Stockholm, Sweden; [3]Department of Renal Medicine, Huddinge University Hospital, Stockholm, Sweden.*

Purpose: Historically, ABO-incompatible kidney transplantations have only been undertaken following splenectomy and unspecific plasmapheresis and with quadruple drug immunosuppression plus B-cell specific drugs. We have now evaluated a protocol for ABO-incompatible kidney transplantation without splenectomy, using antigen-specific immunoadsorption, rituximab and a conventional triple-drug immunosuppressive protocol.

Methods: The protocol called for a 10-day pretransplant conditioning period, starting with one dosage of rituximab, followed by full-dose tacrolimus, mycophenolate mofetil and conventional prednisolone tapering. Antigen-specific immunoadsorption was performed on pretransplant days -6, -5, -2 and -1. Following the last session, 0.5 g/kg of intravenous immunoglobulin was administered. Postoperatively three more apheresis sessions were given every third day. Furthermore, if there was a significant increase in the antibody titers, extra sessions were considered.

Results: Eight patients have been transplanted with this protocol. The donor/recipient blood groups have been two each of A2/O, B/O, B/A and A1/O. The ABO-antibodies were readily removed by the antigen-specific immunoadsorption before transplantation and were kept at a low level post transplantation by further adsorptions. There were no side effects and all the patients are discharged with normal renal transplant function.

Abstract# 893

THREE-YEAR OUTCOME OF ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION UNDER PRETRANSPLANT ONE-WEEK IMMUNOSUPPRESSION WITH TACROLIMUS, MYCOPHENOLATE MOFETIL, AND STEROID. Kazunari Tanabe,[1] Tadahiko Tokumoto,[1] Hideki Ishida,[1] Nobuo Ishikawa,[1] Naoshi Miyamoto,[1] Tsunenori Kondo,[1] Hiroaki Shimmura,[1] Kiyoshi Setoguchi,[1] Hiroshi Toma.[1] *[1]Department of Urology, Tokyo Women's Medical University, Shinkuku, Tokyo, Japan.*

Introduction: The supply of cadaveric and living kidneys is not sufficient to satisfy the increasing number of patients requiring renal transplantation. Expansion of the donor pool by overcoming ABO-incompatibility would help to solve this problem. Since 2001, we have been employing one-week pretransplant immunosuppression with tacrolimus (FK) / mycophenolate mofetil (MMF) /methylprednisolone (MP) and have obtained excellent short-term results without any serious complications. Mid-term results of ABO-incompatible renal transplantation under one-week pretransplant immunosuppression with low-dose FK/MMF/MP maintenance immunosuppression are reviewed.

Patients and Methods: Thirty-two adult patients underwent ABO-incompatible LKT at our institute between January 2001 and September 2003. There were 16 males and 16 females, with a mean age of 33 years. Plasmapheresis was carried out to remove anti-AB antibodies prior to the kidney transplantation. Since January 2001, we administered FK (0.1 mg/kg/d) / MMF (1-2 g/d) / MP (125 mg/d) concomitantly with plasmapheresis starting from 7 days before transplantation. Splenectomy was done at the time of kidney transplantation in all patients. In the maintenance phase (6 months after transplantation), all patients were maintained on low-dose immunosuppression with FK/MMF/MP. The average dose of FK, MMF, and MP was 0.05mg/kg, 1000mg/day, and 5mg/day, respectively. The target trough level of FK was 5ng/ml in the maintenance phase.

Results: Patient and graft survival was 100% and 95% at three years, respectively. Only one graft was lost due to humoral rejection during the observation time. The incidence of acute rejection was 25%. Also, the incidence of steroid-resistant acute rejection was 7%. There was no serious infectious complication during the observation period. Protocol biopsies did not show any late-onset rejection.

Conclusion: Mid-term results of ABO-incompatible renal transplantation under one-week pretransplant immunosuppression with low-dose FK/MMF/MP maintenance immunosuppression showed excellent patient and graft outcome.

Abstract# 894

EXCELLENT LONG-TERM OUTCOME OF ABO-INCOMPATIBLE RENAL TRANSPLANTATION. A SINGLE-CENTER EXPERIENCE. Hiroshi Toma,[1] Kazunari Tanabe,[1] Tadahiko Tokumoto,[1] Hideki Ishida,[1] Nobuo Ishikawa,[1] Naoshi Miyamoto,[1] Hiroaki Shimmura,[1] Ichiro Nakajima,[2] Shouhei Fuchinoue,[2] Satoshi Teraoka.[2] *[1]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan; [2]Kidney Surgery, Tokyo Women's Medical University, Tokyo, Japan.*

INTRODUCTION: Despite great efforts to promote the donation of cadaveric organs, a serious shortage of cadaveric organs exists in Japan. ABO-incompatible renal transplantation has been performed for expansion of the donor pool. We will review our results of 141 cases of ABO-incompatible renal transplantation since 1989.

PATIENTS AND METHODS: One hundred and forty-one patients underwent ABO-incompatible living kidney transplantation at our institute between January 1989, and December 2001. To remove anti-ABO antibodies, recipients received plasmapheresis before transplantation. Methylprednisolone, cyclosporine (CyA) or tacrolimus (TAC), and azathioprine or mycophenolate mofetil (MMF) were used as basic imunosuppressants. Antilymphocyte globulin and deoxyspergualin were used in most of the cases which were performed in the pre MMF era (1989-1999). Splenectomy was done at the time of transplantation in all patients except one. Seven hundred and seventy-seven concurrent ABO-compatible kidney transplant recipients were employed as a control.

RESULTS: The patient survival of ABO-incompatible recipients was 94% at 5 years after transplantation, and 88% and 84% at 10 and 13 years post-transplantation, respectively. The patient survival was not significantly different from that of the 777 ABO-compatible kidney transplant recipients (5 years, 97%; 10 years, 92%; 13 years, 90%). The graft survival of ABO-incompatible recipients was 76% at 5 years, and 56% at 10 and 13 years. The graft survival of ABO-compatible recipients was 85% at 5 years, 67% at 10 years, and 58% at 13 years. Although there was a significant difference in graft survival between ABO-incompatible and ABO-compatible renal transplants (log-rank test, P=0.007), 13-year graft survival was almost the same between the two groups. Acute rejection episodes were significantly frequent in the ABO-incompatible grafts (85 of 141, 60%) compared with the ABO-compatible grafts (377 of 777, 49%; P=0.010). However, since 1998, when TAC, MMF, and CyA-Neoral were introduced, graft survival has been markedly improved. Namely, five-year graft survival is more than 90% which is not significantly different from that of ABO-compatible cases.

CONCLUSION: Long-term results of ABO-incompatible renal transplantation are excellent. ABO-incompatibility dose not seem to be a difficult immunological barrier to overcome in renal transplantation.

Abstract# 895

OUTCOMES OF ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION RECIPIENTS WITH POSITIVE PRA. Hiroaki Shimmura,[1] Kazunari Tanabe,[1] Tadahiko Tokumoto,[1] Hideki Ishida,[1] Nobuo Ishikawa,[1] Naoshi Miyamoto,[1] Kiyoshi Setoguchi,[1] Hiroshi Toma.[1] *[1]Department of Urology, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.*

Introduction: Due to the continuing shortage of cadaveric donors in Japan, ABO-incompatible living kidney transplantation (LKT) is being carried out. It is well known that highly sensitized patients with positive panel reactive antibody (PRA) often present with acute rejection. Until now, it is not clear whether there is an association between the results of ABO-incompatible LKT and the existence of anti-HLA antibody. Therefore, we examined the impact of positive PRA on the results of ABO-incompatible LKT.

Materials and Methods: One-hundred and seventy-seven recipients underwent ABO-incompatible LKT at our institution between January 1989, and March 2003. Of these patients, 45 who had been examined for PRA before transplantation were included in this study. There were 33 males and 12 females with a mean age of 37.0 years. Plasmapheresis was carried out to remove the anti-ABO antibodies prior to transplantation. In the induction phase, methylprednisolone, azathioprine or mycophenolate mofetil and cyclosporine or tacrolimus were used for immunosuppression. Splenectomy was done at the time of kidney transplantation in all patients. PRA was measured using FlowPRA (One Lambda, CA, USA) by flow cytometer.

Results: Twelve of the 45 patients had positive PRA before transplantation (class I; 8, class II; 1, class I and class II; 3). The incidence of acute rejection was 36.4% in the patients with negative PRA. The patients with positive PRA showed slightly higher incidence of acute rejection (50.0%). However, there was no statically difference between two groups in the incidence of acute rejection (p=0.4090). Graft survival also similar between two groups (97.0% vs 80.2 at 5 years, negative PRA vs positive PRA, respectively, p=0.1000 logrank test).

Conclusions: The results in ABO-incompatible patients with positive PRA was similar to that in patients with negative PRA. Plasmapheresis and splenectomy may also help eliminate anti-HLA antibody in ABO-incompaatible LKT.

Abstract# 896

EFFECT OF ANTI-CD20 ANTIBODY ON SPLENIC AND PERIPHERAL BLOOD B LYMPHOCYTES IN POSITIVE CROSSMATCH KIDNEY TRANSPLANT RECIPIENTS RECEIVING PLASMAPHERESIS AND INTRAVENOUS IMMUNOGLOBULIN. James M. Gloor,[1] Joseph P. Grande,[2] William R. Macon,[2] Mark D. Stegall.[1] *[1]Transplant Center, Mayo Clinic, Rochester, MN; [2]Pathology, Mayo Clinic, Rochester, MN.*

Introduction: Successful positive crossmatch (+XM) kidney transplantation is possible using a protocol combining the B lymphocyte depleting anti-CD20 antibody rituximab and plasmapheresis followed by intravenous immunoglobulin (PP/IVIG). Whether PP/IVIG administered soon after rituximab interferes with B lymphocyte depletion is undetermined. The purpose of this investigation was to study the effect of rituximab followed by PP/IVIG on peripheral blood and splenic B-lymphocytes in +XM kidney transplant patients.

Methods: 9 positive crossmatch (+XM) and 10 ABO incompatible (ABOI) kidney transplant recipients were studied. Both groups underwent a series of pretransplant plasmaphereses followed by 100 mg/kg IVIG as pretransplant conditioning, and both underwent splenectomy at transplant. +XM recipients received rituximab 375 mg/m2 1 day prior to beginning PP/IVIG, while ABOI patients did not receive rituximab. Both groups had peripheral blood T and B lymphocyte analysis on postoperative day 3. Peripheral blood B-lymphocytes were identified by expression of CD19. 8 ABOI and 4 +XM patients also had analysis of splenic B-lymphocytes (CD20 and CD79 positive staining using standard immunohistochemical techniques). A semiquantitative 0-3+ scale was utilized to measure extent of CD20 and CD79 expression in splenic follicles and interstitium. Peripheral blood and splenic B lymphocyte counts and distribution in the rituximab treated and untreated groups were compared using Student's t-test.

Results: +XM patients underwent a mean of 3.7 (range 2-5) PP/IVIG treatments following rituximab administration. Peripheral blood B lymphocytes were significantly decreased in the rituximab treated compared to the nontreated group (2.3±2.9/µl vs. 33.0±31.6/µl, p=0.01). B lymphocytes were significantly reduced in splenic follicles in treated vs nontreated patients (1.50±0.58 vs. 2.75±0.46 on a scale of 0-3+ expression of CD20 and CD79, p=0.002). CD20 and CD79 positive lymphocytes were densely distributed in splenic interstitium in the nontreated group, and were absent in the treated group.

Conclusion: Administration of rituximab 375 mg/m2 results in significant B cell depletion in spleen and peripheral blood even when closely followed by plasmapheresis and IVIG.

Abstract# 897

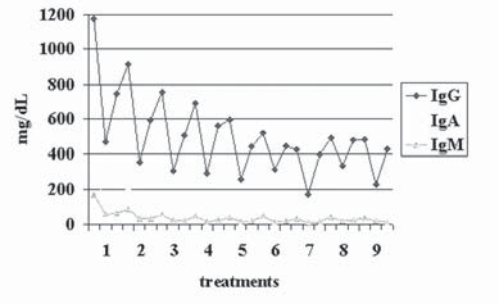
IMMUNOGLOBULIN G-A-M REMOVAL DURING PLASMAPHERESIS TREATMENT FOR POSITIVE CROSSMATCH AND ABO INCOMPATIBLE KIDNEY TRANSPLANTATION. Lloyd Ratner,[1] Mark Chaballa,[3] Beth Colombe,[2] Nancy Edger-Hall,[4] Liise Kayler,[1] Donald Dafoe.[1] *[1]Surgery; [2]Medicine, Thomas Jefferson University; [3]Pharmacy; [4]Blood Donor Center, Thomas Jefferson University Hospital, Philadelphia, PA.*

Background: Plasmapheresis (PP) with immune globulin (IVIg) has been used to abrogate a positive crossmatch (+ XM) or ABO incompatibility (ABOI) prior to live donor renal transplantation (LDRT). The affects of PP/IVIg have not been fully evaluated.

Purpose: To evaluate the kinetics of immunoglobulin (Ig) G-A-M removal & reconstitution during PP/IVIg therapy in ABOI & +XM recipients.

Methods: We analyzed IgG-A-M levels in 4 pts. receiving PP/IVIg therapy for an ABOI (n=2) or + XM (n=2) LDRT. Pts. received 5-8 one-volume PP treatments prior to a LDRT & an additional 2-16 treatments post-Tx. Colloid was reconstituted with albumin unless profound coagulopathy ensued. IVIg (CytoGam®) 100mg/kg was administered after each PP. IgG-A-M levels were drawn before and after each PP & immediately after the IVIg infusion. All pts. received immunosuppression with tacrolimus, mycophenolate mofetil, daclizumab & steroids

Results: Mean Ig levels at baseline were 1176 mg/dL for IgG, 310 mg/dL for IgA and 171 mg/dL for IgM. Following each PP the Ig levels decreased by a mean of 56% (33-64%), 60% (41-67%) and 64% (51-79%) for IgG, A and M, respectively. Despite treatment with IVIg, Ig levels continued to decline. After PP#9, mean Ig levels decreased to 231 mg/dL, 48 mg/dL and 19 mg/dL, and post IVIg levels were 432 mg/dL, 53 mg/dL and 15 mg/dL for IgG-A-M, respectively. All pts. are doing well with a mean f/u of 140 days.



Conclusions: 1) Ig G-A-M levels fall precipitously immediately post PP. 2) IVIg results in an increase in the IgG but not IgA or IgM. 3) Ig G-A-M rises in the interval between PP treatments (despite immunosuppression). 4) Overall, Ig levels continue to decline relative to the number of PP treatments. 5) Extensive PP yields very low levels of Ig despite reconstitution with low dose IVIg. Consideration should be given to increasing the dose of IVIg if infectious complications occur.

Abstract# 898

REJECTION EPISODES IN PATIENTS FOLLOWING POSITIVE CROSSMATCH AND ABO INCOMPATIBLE LIVE DONOR RENAL TRANSPLANTATION UNDER THE PLASMAPHERESIS/ IVIg PROTOCOL. Liise K. Kayler,[1] Donald C. Dafoe,[1] Beth Colombe,[2] Deborah LaCava,[2] James A. Rothschild,[1] George C. Francos,[2] James F. Burke, Jr.,[2] John J. Friedewald,[2] John L. Farber,[3] Lloyd E. Ratner.[1] *[1]Surgery, Thomas Jefferson University Hospital, Philadelphia, PA; [2]Medicine, Thomas Jefferson University Hospital, Philadelphia, PA; [3]Pathology, Thomas Jefferson University Hospital, Philadelphia, PA.*

Background: Live kidney donors are excluded, if the recipient has donor specific antibody (DSA) to HLA antigens (+XM) or ABO blood type antigens (ABOI). Plasmapheresis combined with immune globulin (PP/IVIg) to remove DSA allows successful live donor Tx. Antibody-mediated (AMR) and acute cellular rejection (ACR) remain, nevertheless, significant risks. Rejection episodes in these recipients are poorly characterized, and a need exists to better define prognostic indicators and optimal therapy.

Purpose: To characterize the features of rejection episodes following +XM or ABOI Tx.

Methods: A retrospective review was performed of consecutive pts that received PP/IVIg to abrogate +XM or ABOI. Extent of PP/IVIg was determined by the pretransplant DSA titer. Immunosuppression consisted of tacrolimus, mycophenolate mofetil, daclizumab, and steroids. Biopsies were obtained on POD#7 and at the onset of graft dysfunction. Characterization of rejection episodes was by histopathology, immunoflourescence, and DSA titer. Antirejection therapy and renal function were assessed.

Results: 12 pts were Txd under the PP/IVIg protocol (+XM=9, ABOI=2, +XM/ABOI=1). No hyperacute rejections occurred. Patient and graft survival of 100% has been achieved with a creatinine of 1.8 ± 1.1 mg/dl after a mean follow-up of 181 days (range 13-430). No rejection occurred in 5. Rejection occurred in the remaining 7 pts. In 4 +XM pts, DSA was detected at the time of graft dysfunction despite no histologic features of AMR (7 biopsies) and negative C4d staining. In 1 of these patients, DSA preceded the histologic and immunofluorescent features of AMR. AMR occurred with and without an ACR component (Banff scores 0-IIA). In 2 pts with Borderline changes and DSA, graft function improved after PP/IVIg, despite no histologic or immunoflourescent evidence of AMR. One pt with Banff IIA ACR and DSA treated with antithymocyte antibody but not PP/IVIg had recurrent rejections and poor graft function.

Conclusions: In +XM and ABOI recipients with graft dysfunction: 1) DSA may represent AMR in the absence of C4d or histologic features of AMR; 2) DSA can precede C4d or light microscopic features of AMR; 3) AMR occurs independently of ACR; 4) A poor outcome results if DSA is present and not treated despite a negative biopsy, including C4d.

Abstract# 899

SUCCESSFUL PRIMARY DECEASED DONOR TRANSPLANTATION IN THE PRESENCE OF DONOR-SPECIFIC HLA CLASS I ANTIBODY WITHOUT IVIG OR PLASMAPHERESIS THERAPY. Christopher F. Bryan, Scott B. McDonald, Alan M. Luger, Franz T. Winklhofer, A. Michael Borkon, Charles F. Shield, Bradley A. Warady, Mark I. Aeder. *Midwest Transplant Network, Westwood, KS.*

Introduction/Methods: Renal transplantation in the presence of low levels of HLA IgG antibody may not influence short- or long-term graft survival. Between 12/15/00 and 3/15/03, 6 of 338 (1.8%) primary recipients of deceased donor organs were transplanted when their flow cytometric T cell IgG donor-specific crossmatch was positive but the AHG T cell crossmatch was negative (Flow T^+/AHG^-). Immunosuppression of the recipients was center-directed, *but no pre- or peri-operative IVIg or plasmapheresis were administered.* Five of the 6 recipients were female.We now report on the outcomes with a mean follow-up of 13 months (12-24 months).

Results/Conclusions: All organs are currently functioning (see table). High definition antigen beads for flow cytometry allowed the identification of at least one donor-specific HLA class I antibody in each of the six cases. Among the six patients transplanted, there was one episode of acute rejection in the SPK recipient on day 14 and two in the heart recipient on days 300 and 349. Each rejection was successfully treated.We have evaluated the HLA antibody(ies) around 1 year post-transplantation for 3 patients and see evidence that the antibody specificity(ies) present before transplantation are undergoing epitope spreading as well as epitope collapse. Furthermore, we can see in the heart recipient that the strength of the donor antibody is decreasing.

Patient	Crossmatch AHG	Crossmatch Flow	Donor-specific HLA Antibodies	Organs	Creatinine @ 1 yr	Function
#1	Neg	Pos	A2	Kidney	1.0	Yes
#2	Neg	Pos	A1, B8	Kidney	1.5	Yes
#3	Neg	Pos	B60	Kidney	1.0	Yes
#4	Neg	Pos	A3	Kidney	1.2	Yes
#5	Neg	Pos	A2	SPK	1.9	Yes
#6	Neg	Pos	A2, A24	Heart	—	Yes

We have found that short- and long-term graft survival in primary transplants is not influenced by low levels of donor-specific HLA class I antibody that were defined as being Flow T^+ but AHG crossmatch negative. Those findings indicate that there may be a level of diagnostic sensitivity at which HLA antibodies can be safely transplanted without the need to use IVIg and/or plasmapheresis. We speculate that the low levels of HLA antibody may induce graft accommodation rather than portend rejection.

Abstract# 900

CAMPATH 1H (ALEMTUZUMAB) IN RENAL TRANSPLANTATION: 5-YEAR COMPARATIVE FOLLOW UP.

Christopher J. Watson,[1] John Firth,[2] John Bradley,[2] Kenneth G. Smith,[2] Neville V. Jamieson,[1] Peter J. Friend,[3] Geoff Hale,[4] Herman Waldmann,[4] Andrew J. Bradley,[1] Sir Roy Y. Calne.[1] *[1]University Dept of Surgery, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Department of Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom; [3]Nuffield Dept of Surgery, University of Oxford, Oxford, United Kingdom; [4]Department of Immunology, University of Oxford, Oxford, United Kingdom.*

Alemtuzumab is a powerful lymphocyte depleting monoclonal antibody that is licensed for the treatment of lymphoreticular malignancy and is under evaluation in transplantation. This paper describes the 5-year follow up of a study which comprised alemtuzumab followed by low dose ciclosporin monotherapy. These patients have been compared to contemporary controls. The only selection criteria for alemtuzumab was the giving of informed consent.

Method

Cadaveric renal transplant recipients (n = 33) were given two doses of 20mg alemtuzumab followed 48 hours later by low dose ciclosporin monotherapy. A cohort of patients (n = 66) transplanted in the same time period in the same centre and receiving conventional ciclosporin-based triple therapy (n = 61) or sirolimus, ciclosporin and prednisolone (n = 5) were selected for comparison. Patients receiving living donor or multi-organ transplants were excluded.

Results

Follow up was complete in the alemtuzumab group and while 2 of the control group were lost to follow up at varying intervals post transplant; both had normal graft function at the time. Table 1 illustrates the outcomes.

		Patient and graft survival		
		Alemtuzumab	Controls	Significance
Graft survival	1 year	94%	83%	
	5 years	79%	75%	p = 0.57
Patient survival	1 year	97%	88%	p = 0.13
	5 years	88%	83%	p = 0.28

One patient in each group died from PTLD. The other deaths were due to ischaemic heart disease (n = 2) and calciphylaxis (n = 1) in the alemtuzumab group, or to ischaemic heart disease (n = 6), cerebrovascular disease (n=1), sepsis (n = 2), cerebral tumour (n = 1) and unknown (n=1) in the control patients. Notable events in alemtuzumab treated patients include one de novo autoimmune haemolytic anaemia and one ileocaecal TB infection.

In spite of profound initial lymphocyte depletion in the alemtuzumab group, the total lymphocyte counts in both groups were similar by 6 months. Renal function remained similar in each group throughout. The incidence of acute rejection was also similar in both groups, but time of the first acute rejection episode was much later in alemtuzumab treated patients (medians 170 days vs 16 days).

Conclusion

Alemtuzumab combined with low dose ciclosporin is a safe and effective regimen that is well tolerated and avoids steroids. Patients need to be followed up closely for late acute rejection.

Abstract# 901

CAMPATH-1H IN PATIENTS WITH DELAYED GRAFT FUNCTION: REDUCED REJECTION AND IMPROVED GRAFT SURVIVAL.

Stuart J. Knechtle,[1] John D. Pirsch,[1] Barbara J. Voss,[1] Glen E. Leverson,[1] Bryan N. Becker,[2] Luis A. Fernandez,[1] L. Thomas Chin,[1] Yolanda T. Becker,[1] Jon S. Odorico,[1] Anthony M. D'Alessandro,[1] Hans W. Sollinger.[1] *[1]Surgery, University of Wisconsin, Madison, WI; [2]Medicine, University of Wisconsin, Madison, WI.*

Purpose: The purpose of this study was to evaluate the impact of Campath-1H (Alemtuzumab, ILEX Oncology) therapy on outcomes of renal transplants which experience delayed graft function (DGF).

Methods: Outcomes of 23 renal transplants with DGF treated with two doses of 30 mg of Campath-1H (day of transplant and day 1) were compared to outcomes of 195 renal transplants with DGF who received anti-CD25 antibody (n=127), Thymoglobulin (n=41), or other (n=27; anti-thymocyte globulin, ATG, n=18; OKT3, n=4, no antibody, n=5). All patients received immunosuppression with a calcineurin-inhibitor, mycophenolate mophetil, and steroids, and were transplanted between 12/97 and 5/03, with minimum 6 month followup.

Results: Demographic features of the groups were not different. There was no difference in the incidence of DGF according to antibody therapy, with an overall incidence of 15%. Between 95 and 100% of the grafts experiencing DGF were from deceased donors in all treatment groups. At three months, the incidence of rejection was 12.5% in the Campath-1H group, 35% in the anti-CD25 group, 36% in the Thymoglobulin group, and 61% in the "other" group. Log-rank analysis of the incidence of rejection between the groups over time, using chi-square test, reveals a significant advantage of Campath-1H (p=0.0078). Graft survival was also significantly better in the Campath-1H group with no graft loss to date (p=0.012). There was no difference in patient survival, incidence of infection, or incidence of malignancy between the groups.

Conclusions: Campath-1H provides a significant benefit compared to other antibody therapies used in transplantation when used in patients who receive renal transplants that experience DGF. DGF is associated with a high incidence of rejection under conventional immunosuppressive regimens, and the profound immune cell depletion associated with Campath-1H therapy reduces the risk of rejection to such a degree that graft survival is also improved.

Abstract# 902

THE USE OF CAMPATH-1H AS INDUCTION THERAPY IN RENAL TRANSPLANTATION: PRELIMINARY RESULTS.

Gaetano Ciancio,[1] George W. Burke,[1] Jeff J. Gaynor,[1] Adela D. Mattiazzi,[1] Anne Rosen,[1] Ramir Roohipour,[1] David Roth,[2] Warren Kupin,[2] Delvis Jorge,[1] Joshua Miller. *[1]Surgery. Division of Transplantation, University of Miami School of Medicine, Miami, FL; [2]Medicine, University of Miami School of Medicine, Miami, FL.*

Introduction and Objective: In attempt to reduce long-term nephrotoxic calcineurin inhibitor dosage and totally eliminate maintenance corticosteroids, Campath-1H was used as induction therapy in first cadaver and non-HLA identical living related donor renal transplantation.

Methods. Forty one de novo renal allograft recipients were treated with Campath-1H (0.3 mg/kg) on day 0 and day 4 proceeded by a methylprednisolone bolus. Twenty five were in another randomized trial and 16 were non-randomized and chose to be treated with Campath-1H. Maintenance 12 hour Tacrolimus trough levels of 5-7 ng/ml were operational from the outset as well as reduced mycophenolate mofetil (MMF) dosage of 500 mg twice daily, no corticosteroids were given after the first week postoperatively. There was at least 1 DR antigen donor/recipient compatibility. Primary outcome measures included delayed graft function, episode of rejection, complications, incidence of opportunistic infections, graft and patient survival after median follow-up of 8 months range 1-18) months.

Results. Patient and graft survival is 100% and 100% respectively. Biopsy-proven rejection was diagnosed in 4 (10%) patients. One patient experienced an acute humoral rejection that was reversed with plasmapheresis and intravenous immunoglobulin. Two patients developed infections that required hospitalization. The geometric mean/S.E. serum creatinine concentrations at 1, 6, and 12 months are 1.53/1.07, 1.39/1.08, and 1.45/1.12 mg/dl respectively. Four patients had delayed graft function and only one patient required dialysis. There were minimal side-effects related with Campath-1H infusion. Thirty six (88%) patients have totally avoided maintenance corticosteroids.

Conclusions. The combination of Campath-1H with low dose tacrolimus and MMF with avoidance of maintenance steroids appear to be safe and effective for kidney transplant recipients. Campath-1H as induction therapy appears to allow for the safe avoidance of maintenance steroids in renal transplantation. Longer term outcomes will be updated.

Abstract# 903

MONOCYTES ARE DOMINANT INFLAMMATORY CELLS FOR ACUTE RENAL REJECTION AFTER COMBINED TREATMENT WITH PREOPERATIVE Campath-H AND POSTOPERATIVE IMMUNOSUPPRESSION.

Ping L. Zhang,[1] Sayeed K. Malek,[1] Jeffery W. Prichard,[1] Fan Lin,[1] Taher M. Yahya,[1] Michael S. Schwartzman,[1] Ruth P. Latsha,[1] Evan R. Norfolk,[1] Robert E. Brown,[1] James E. Hartle,[1] Santosh Potdar.[1] *[1]Department of Laboratory Medicine and Divisions of Transplantation & Nephrology, Geisinger Medical Center, Danville, PA.*

Background: T lymphocytes are conventionally known to mediate acute rejection in renal transplants, but the role of monocytes in acute rejection has not been well understood. Since Campath-H, a humanized antibody against CD52, can more profoundly deplete T lymphocytes than monocytes, a study in renal recipients with Campath-H treatment may uncover effects of monocytes contributing to acute rejection.

Methods: Total 27 renal recipients received induction therapy with 30 mg IV Campath-H preoperatively. The patients subsequently were treated with maintenance therapy of tacrolimus or mycophenolate mofetil. In addition, all patients also received 20 mg of predinisone after operation, which were weaned to off at a rate of 2.5 mg/week. All patients were followed with weekly labs. Renal transplants were biopsied at 2 w, 3 m and 6 m or as needed. Special stains in renal biopsies were done using Dako Autostainer.

Results: Lab data showed that Campath-H resulted in a dramatic depletion in both lymphocytes and monocytes. Over 7 months, only one clinical plus biopsy proven acute rejection (Banff criteria Ib) was identified in a recipient 2 weeks after receiving a living non-related transplant. Inflammatory cells during rejection were composed of 95% CD68 positive monocytes and 5% CD3 positive T lymphocytes. Two additional acute rejections (Ia) were seen in protocol renal biopsies, in absence of elevated creatinine, 3 or 5 months after the transplantation. Eighty % of inflammatory cells were CD68 positive monocytes, but only 20% of inflammatory cells were CD3 positive. Negative CD34 and CD1a stains for inflammatory cells suggest no involvement of

CD34 derived dendritic cells in the acute rejection. Interestingly, all renal transplant biopsies (22/22) showed positive staining for CD3 in renal tubules but were CD3 negative in glomeruli. Renal tubules were markedly positive for CD68 (11/12), while focal mesangial cells were positive for CD68.

Conclusions: Our data indicate that the current treatment regimen resulted in a low rate of acute rejection, which was predominantly mediated via monocytes. Findings of positive staining in renal tubules for CD3 and CD68 suggest that renal tubules share similar antigens with T lymphocytes and monocytes, which may explain why acute rejection mainly targets at renal tubules rather than glomeruli.

Abstract# 904

CELLULAR PHENOTYPES AFFECTED BY INDUCTION THERAPY WITH CAMPATH-1H VS THYMOGLOBULIN VS ZENAPAX IN KIDNEY ALLOGRAFT RECIPIENTS. Manuel R. Carreno,[1] Gaetano Ciancio,[1] George W. Burke,[1] Anne Rosen,[1] Camillo Ricordi,[1,2,3] Andreas Tzakis,[1] Joshua Miller,[1,2,4] Violet Esquenazi.[1,2,4] *[1]University of Miami School of Medicine, Department of Surgery-Transplantation; [2]Department of Microbiology and Immunology; [3]Diabetes Research Institute; [4]The VA Medical Center, Miami, FL.*

Introduction: Several independent studies have shown the safety and efficacy of antibody induction in renal transplantation using several different maintenance immunosuppressive protocols. This describes an 18 month follow-up in a randomized study, comparing the cytoablative effect of Campath-1H® (**C**), Thymoglobulin® (**T**) and Zenapax® (**Z**) on peripheral blood and iliac crest bone marrow of kidney allograft recipients.

Methods: In **C** half maintenance dosages of tacrolimus (tacro) and mycophenolate (MMF) but no corticosteroid were given. In **T** and **Z** higher maintenance doses of tacro, MMF as well as steroids were given. Depletion of discrete mononuclear cell phenotypes in peripheral blood and iliac crest bone marrow samples taken sequentially post-transplant at 10, 60, and 200 days were compared. The absolute number of mononuclear cells in peripheral blood or iliac crest bone marrow aspirates per mm^3 and the CD3, 19, 56/16 (NK cells), 25, 14 (Monocytes) and 34 (Stem cells) phenotypes in flow cytometry were calculated.

Results: In peripheral blood, when compared to normal values, in the **C** group, there was depletion of virtually all lymphocytes, T cells by ~100%, B cells by ~98%, NK cells by ~ 95%, monocytes by ~ 83% and Stem cells by ~40%, with an earlier recovery (in descending order) of stem cells, B cells, monocytes, and NK cells, and later (at >6 months) with T cells. In contrast, **T** depleted T cells (~99%) and NK cells (~98%), but B cells, and monocytes were only moderately affected (~20% and ~45% respectively). **Z** depleted all CD25 positive cells with early modest depletion of T cells, B cells and NK cells by ~50%, ~72% and ~62% respectively, and with no effect seen on monocytes. In iliac crest marrow, at 10 days **C** also strongly depleted all lymphocyte, $CD3^+$, $CD19^+$, $CD56/16^+$, and $CD14^+$ counts by ~99%, ~98%, ~96%, and ~68% respectively. However, virtually normal numbers of $CD34^+$ cells were present. At 200 days post-transplant, an increased number of B cells (double the normal values) were seen. The early depletion of affected subsets by **T** in this compartment were 50% that of **C** with an earlier return to normal ranges in all phenotypes, while with **Z** there was only a minimal effect.

Conclusion: **C** is the more potent lymphoablative induction agent (followed by **T**) allowing in most cases for early and total avoidance of corticosteroids and lower doses of maintenance immunosuppressive agents.

Abstract# 905

IMMUNOPHENOTYPIC ANALYSIS OF CELLULAR INFILTRATE OF RENAL ALLOGRAFT BIOPSIES IN PATIENTS WITH ACUTE REJECTION AFTER INDUCTION WITH CAMPATH 1H. Lorenzo G. Gallon,[1] Marina Noris,[2] Elena Gagliardini,[2] W. James Chon,[1] Joseph R. Leventhal,[3] Dixon B. Kaufman,[3] Giuseppe Remuzzi.[2] *[1]Medicine, Northwestern University, Chicago, IL; [2]Medicine, Mario Negri, Bergamo, Italy; [3]Surgery, Northwestern University, Chicago.*

Background: Campath 1H is a humanized anti-CD52 monoclonal antibody that has emerged as a safe and effective lymphocyte depleting antibody for induction therapy in renal transplant. Peripheral T cell depletion after a single dose of 30mg of Campath 1H usually last for 2-5 months. Recent reports by Kirk et al (Transplantation, 76:120-129) have suggested that rejection of renal allografts in pts receiving Campath 1H induction may be mediated by an atypical population of monocytes, and not through " classical " T cell dependent pathways of allorecognition. We have hypothesized that renal allograft rejection in pts recieving Campath 1H induction will be qualitatively and quantitatively comparable to rejection observed in pts receiving other form of antibody induction. **Methods:** 9 biopsies from pts treated with a single dose (30mg) of Campath 1H at the time of transplant who subsequently developed acute cellular rejection (ACR), were studied with the following cell markers: CD3 (T cell), CD68 (Macrophage), and CD20 (B cell). 4 biopsies from pts treated with IL2 receptor antagonist as induction and with ACR were used as controls. Both groups received the same maintenance immunosuppression (Tacrolimus + Mycophenolate Mofetil without the use of chronic steroid). **Results:** See table.

Immunophenotypic analysis and quantification of cellular infiltrate in kidney biopsies*

Patients	% of peripheral T cells at the time of ACR	CD3 + T cells in Cortical Interstitium	CD3 + T cells in Medullary Interstitium	CD20 + B cells in Cortical Interstitium	CD20 + B cells in Medullary Interstitium	CD68 + Macrophages in Cortical Interstitium	CD68 + Macrophages in Medullary Interstitium
Controls (n=4)	20± 0.5	168± 62.7	96.6± 80.4	21± 15.2	21.1± 27.8	82.6± 41.4	48.8± 45.4
Campath treated pts (n=9)	10.9± 11	82± 56.6	61.0± 45.7	16.5± 20.3	7.7± 8.2	109.2± 25.1	103± 49.6

* Data are expressed as mean SD. Quantification of cell infiltrate is expressed in # cells/hpf counted in 10 different fields.

Conclusion: Despite peripheral T cell depletion induced by Campath 1 H, pts that develop ACR do have T cells infiltrating the graft and their number correlates with the % of peripheral lymphocytes (r=0.74). Monocytes are equally present during ACR in Campath 1H treated and in controls.

Abstract# 906

CAMPATH 1-H PRECONDITIONING AND TACROLIMUS MONOTHERAPY WITH SUBSEQUENT WEANING IN RENAL TRANSPLANT RECIPIENTS. Ron Shapiro,[1] Henkie P. Tan,[1] Amit Basu,[1] Akhtar Khan,[1] Edward A. Gray,[1] Parmjeet S. Randhawa,[1] Noriko Murase,[1] Adriana Zeevi,[1] Anthony J. Demetris,[1] Jennifer Woodward,[1] Mark L. Jordan,[1] Kristine M. Ruppert,[1] Amadeo Marcos,[1] John J. Fung,[1] Thomas E. Starzl.[1] *[1]Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

After elucidating the mechanisms of alloengraftment, we proposed a strategy of tolerogenic immunosuppression that facilitates these mechanisms. Two principles were applied: recipient pretreatment and the minimalistic use of post-transplant immunosuppression. In an earlier series, kidney recipients were preconditioned with Thymoglobulin and given post-transplant monotherapy with tacrolimus. Between November 2002 and September 2003, we substituted 30 mg Campath 1-H for Thymoglobulin in 96 adult kidney recipients (mean 50.3 ± 16.4 years). 13 (13.5 %) were undergoing retransplantation, and 20 (20.8 %) had a PRA >20%. Mean HLA mismatches were 3.7 ± 1.6. There were 59 (61.5 %) cadaver donors (mean cold ischemia 20.8 ± 7.2 hours) and 37 (38.5 %) live donors. Mean donor age was 41.4 ± 15.9 years (range 1 - 72). Post-transplant immunosuppression was with tacrolimus monotherapy (target levels 10 mg/ml), with dose spacing to every other day or longer at various times after about four months. With a mean follow up of 4.8 ± 2.3 months, the six-month patient and graft survival rates are 100%, with a mean serum creatinine of 1.71 ± 0.99 mg/dl. Only one patient had acute rejection (steroid responsive) prior to weaning. Weaning was started in 20 patients. One developed a readily reversed rejection. The other 19 are on every other day tacrolimus. The incidence of CMV disease, diabetes, and PTLD was 0%. One patient had a BK virus infection. Although the follow-ups in these cases are still short, we conclude that the use of Campath 1-H for pretreatment has significantly improved the efficacy of our tolerogenic regimen of immunosuppression.

Abstract# 907

INTRAOPERATIVE CAMPATH-1H INDUCTION WITH TACROLIMUS OR MYCOPHENOLATE MOFETIL(MMF) BASED MAINTENANCE IMMUNOSUPPRESSION - IN ADULT CADAVERIC KIDNEY TRANSPLANTATION. Santosh Potdar,[1] Sayeed Khan Malek,[1] Patricia Seneko,[1] Ping L. Zhang,[2] Fan Ling,[2] Jeffery W. Prichard,[2] Micheal Schwartzman,[3] Evan Norfolk,[3] Taher Yahya,[3] James E. Hartle.[3] *[1]Transplant Surgery, Geisinger Medical Center, Danville, PA; [2]Laboratory Medicine, Geisinger Medical Center, Danville, PA; [3]Nephrology, Geisinger Medical Center, Danville, PA.*

Body-Campath -1H has been shown to cause profound depletion of T lymphocytes,.A high incidence of early,monocyte predominant,acute rejection has been reported when Campath was used without maitenence immunosuppression in renal transplantation.Recent studies also suggest that steroids can produce apoptosis of monocytes.We postulate that intra operative Campath induction coupled with minimum immunosuppression and short term steroid therapy would result in low post-transplant rejection rates.

Methods-All cadaveric renal transplants after April 2003 were performed with the following protocol:Perioperatively Solumedrol(1gm), followed by Campath-1H (30mg) was given before reperfusion of kidney.Subsequent immunosuppression was either Tacrolimus (aiming for trough level of 10ng/ml) or MMF 1gram bid(.MMF was used in expended donors, non heart beating donors and if cold ischemia time was greater than 24hrs).All patients in addition received 20 mg of prednisone from day one post-transplant,which was then weaned off at a rate of 2.5mg/week. All patients were placed on CMV prophylaxis with oral Valcyte 450 mg, dose adjusted according to creatinine clearance.All patients were followed with weekly labs.Renal biopsies were performed as clinically indicated, as well within 2 weeks, 3 months and 6 months post-transplant.

Results-20 cadaveric renal transplants were performed with a follow-up of 40-240 days.Patients and graft survival has been 100%.Mean creatinine is 1.36mg/dl. Absolute lymphopenia (<1000) was noted in all 20 patients.The median time to development of lymphopenia was 1 day post operative. The range of nadir lymphocyte count was 0 to 140.To date no patient has returned to a absolute lymphocyte count greater than 1000 (longeast follow up 240 days.)No episode of CMV antigenemia has been detected.Only 1 episode of 1A rejection has occured (detected by protocol biopsy). The rejection was predominantly monocytic (70%). At present time 11 patients are on Tacrolimus monotherapy and 9 patients are on MMF monotherapy.

Conclusion- Campath induction followed by rapid steroid taper and monotherapy maintenance immunosuppression has been found by our center to be associated with excellent short term renal function and only 5% incidence of acute rejection. Long term follow -up is still required to confirm our initial results.

Abstract# 908

CAMPASIA: A PILOT RANDOMISED CONTROLLED TRIAL OF THE EFFECTIVENESS OF CAMPATH-1H (MABCAMPATH®) AS AN INDUCTION AGENT FOR PREVENTION OF GRAFT REJECTION AND PRESERVATION OF RENAL FUNCTION IN PATIENTS RECEIVING KIDNEY TRANSPLANTS. Anantharaman Vathsala,[1] Enrique T. Ona,[2] Si-Yen Tan,[3] Shirley Suresh,[4] Yiong-Huak Chan,[4] Huei-Xin Lou,[1] Concesca B. Cabanayan Casasola,[2] Jorgen Seldrup,[4] Roy Calne.[5] *[1]Renal Medicine, Singapore General Hospital, Singapore; [2]National Kidney Transplant Institute, Quezon City, Philippines; [3]Nephrology, University Hospital, Kuala Lumpur, Malaysia; [4]Clinical Trials and Epidemiology Research Unit, Singapore; [5]Surgery, National University of Singapore, Singapore.*

CAMPATH-1H, a humanised anti-CD52 monoclonal antibody, is a powerful lytic agent for both T- and B- lymphocytes. A pilot, multicentre randomised controlled trial was initiated in October 2001, to evaluate the safety and efficacy of low-dose Cyclosporine (CsA) monotherapy following lymphocyte depletion with CAMPATH, in preventing rejection and maintaining renal function in renal transplant recipients (RTX). RTX were randomised to receive either CAMPATH or standard CsA immunosuppression. In the Campath group, CAMPATH (iv 20 mg) was administered within 6 hours after anastamosis and 24 hours later; CsA was started at 72 hours after the first CAMPATH dose to achieve trough levels of 90-110 ng/mL; steroids were only administered at surgery and pre-CAMPATH infusion. In the standard group, CsA doses were adjusted to achieve trough levels of 180-225 ng/mL with Azathioprine and steroids.

The 6 month analysis after recruitment of 30 RTX (50% males; 36.7% Chinese, 46.7% Filipino, 16.6% others; age 39.9±10.8 yrs) is shown (Table). 10 of 14 cadaveric and 10 of 16 live-donor RTX were randomised to CAMPATH. Lymphocyte depletion was profound after 2 doses of CAMPATH (% lymphocytes: 20.6±9.3 Pre vs 0.5±0.4 Post, P<0.001) and remained depleted till 5 months post TX. Likewise, lymphocyte subsets (CD3, CD4, CD8, CD44, CD52) were depleted to 5-6 months post TX.

Efficacy and Safety Parameters at 6 Months Post Renal Transplant by Intent to Treat Analysis

	Standard	Campath
Acute Rejection (AR)	22.2%	27.8%
Steroid Resistant AR	11.1%	16.7%
Recurrent AR ∗	0%	5.6%
Serum Creatinine µmol/L	131 ± 33	126 ± 26
Patient Survival	100%	94.6%
Treatment Failure	11.1%	27.8%
CMV Infections (Treated)	14.3%	0%
Malignancy / PTLD	0%	0%
Serious Adverse Events	22.2%	33.3%
Trough CsA Level, ng/mL ∗	179 ± 39	116 ± 32
RTX on Maintenance Steroids ∗	100%	22.2%
Prednisolone Dose (mg/day) ∗	9.4 ± 3.0	1.2 ± 3.3

§ Death with function is graft loss. ∗ P < 0.001 Standard vs Campath.

These results suggest that CAMPATH is an effective induction agent that permits low dose steroid-free immunosuppression in RTX. Followup to 3 years, as per protocol, will determine the long term safety and efficacy of this regimen.

Abstract# 909

IMMATURE DENDRITIC CELLS PLUS SUBDOSING SIROLIMUS ACHIEVE LONG-TERM ALLOGRAFT SURVIVE. A MECHANISTIC INSIGHT. Ran Tao,[1] Lina Lu,[1] Shi-He Wang,[1] Richard Demarco,[2] Michael T. Lotze,[2] John J. Fung,[1] Shiguang Qian.[1] *[1]Thomas E. Starzl Transplantation Institute, Surgery, University of Pittsburgh, Pittsburgh, PA; [2]Molecular Medicine Institute, University of Pittsburgh, Pittsburgh, PA.*

Administration of immature dendritic cells (iDC) that fail to deliver costimulation prolongs allograft survival via promoting apoptotic death of activated T cells, but does not achieve graft long-term survival. We attempted in this study to boost iDC efficacy through the adjuvant subdose immunosupression. DC propagated from B10 ($H2^b$) BM were transfected with NF-κB binding site specific oligodeoxyribonucleotide (ODN) resulting in completely blocked NF-κB activity (gel shift assay), inhibition of CD80, CD86 and D40 expression. The *in vitro* allostimulatory activity of ODN DC was markedly impaired in MLR and CTL assays. To examine *in vivo* allostimulatory activity, 2 x 10^6 ODN DC were i.v. injected into C3H ($H2^k$) recipients 7 days before B10 cardiac transplant. ODN DC significantly prolonged allograft survival (MST 25d, vs.10d in non-treated control, 6d in mature (m) DC groups, both $p<0.05$). In contrast to cyclosporine that failed to enhance the effect of ODN DC, combination of ODN DC with subdosing sirolimus (6mg/kg/d for 3 days post-transplant) prolonged MST to 43.5d (sirolimus alone MST 25.7d, $p<0.05$), while combined with sirolimus treatment at 6mg/kg/d for 6 days resulted in long-term survival in all allografts (>100d) (n=6 in all groups). CTL activity of either graft infiltrating cells or splenic T cells (d7 post transplant) in the sirolimus combined treatment group was significantly lower than the control or single treatment group. More TUNEL positive cells were identified in T cell areas of mesenteric lymph nodes in the combined treatment group than the control groups ($p<0.05$), suggesting that sirolimus promotes activated T cell apoptosis. We utilized the Cellomics® system to analyze the influence of sirolimus on activation of transcription factors in T cells stimulated with DC. ODN DC inhibited nuclear translocation of Stat1, Stat3, ERK and ATF-2, but not NF-κB and P38, compared with mDC. The selective inhibition of Stat1, ERK, ATF-2 signal transduction can be enhanced by sirolimus, but not cyclosporin. Stat1 has been shown to be importance for cell-mediated immunity. ERK and p38, subgroups of MAP kinases, regulate cellular proliferation, apoptosis, survival and differentiation. ATF-2 involves in a feedback mechanism that regulates MAPK signaling. These transcription factors may be the useful targets in the development of new immunosuppressive agents. Sirolimus, but not cyclosporine, can enhance iDC tolerogenicity.

Abstract# 910

RAPAMYCIN INHIBITS EPSTEIN BARR VIRUS + B CELL LYMPHOMA PROLIFERATION THROUGH MODULATION OF CELL CYCLE PROTEIN EXPRESSION. Maria Vaysberg,[1] Cynthia E. Balatoni,[1] Ronald R. Nepomuceno,[1] Sheri M. Krams,[1] Olivia M. Martinez.[1] *[1]Department of Surgery, Program in Immunology, Stanford University, Stanford, CA.*

Post transplant lymphoproliferative disorder (PTLD) is a serious complication of solid organ and bone marrow transplantation that is closely associated with Epstein Barr Virus (EBV) infection. In healthy individuals the expansion of EBV-infected B cells is controlled by EBV-specific CD8+ T lymphocytes. However, impaired T cell immunity as a result of immunosuppression places transplant recipients at increased risk for EBV^+ B cell lymphomas. Our lab has previously shown that rapamycin (RAPA), unlike other immunosuppressive therapies, directly inhibits *in vitro* proliferation of EBV-infected B cell lines (SLCL) derived from patients with PTLD by arresting cells in the G_1 phase of the cell cycle. Moreover, we demonstrated that RAPA significantly inhibits B cell lymphoma growth *in vivo* in a xenogeneic SCID mouse model of PTLD. To determine the mechanism by which RAPA induces cell cycle arrest in EBV-infected B cells we analyzed G_1-associated cell cycle proteins in SLCL cultured in the absence or presence of RAPA (10 ng/ml). Western blot and densitometric analysis revealed that RAPA

significantly decreases cyclin D3 protein levels (range=71-78%) in 3/3 SLCL and cyclin D2 protein levels (range=29-63%) in 2/3 SLCL while cyclin E levels remain unchanged. The activation and association of cyclins with cyclin dependent kinases (cdk) is essential for the G_1/S transition and is regulated by cdk inhibitors. Accordingly, RAPA decreased the protein levels of cdk4 (range=17-56%) and significantly increased the expression of the cdk inhibitor p27 (range=65-88%). In contrast, expression of another cdk inhibitor p21 was markedly inhibited by RAPA (range=58-74%) in 2/3 SLCL. p21 is a complex regulatory protein that can inhibit cdk4 and cdk6, but is also necessary for proper cyclin D/cdk interaction. These results suggest that p27 is the key inhibitor in the G_1 cell cycle arrest in EBV-infected B cells, while p21 promotes the cyclin D/cdk interactions which drive cellular proliferation. Thus, RAPA modulates multiple proteins associated with progression through the G_1 phase of the cell cycle resulting in inhibition of cell proliferation. These findings provide insight into the growth pathways of the EBV^+ B cell lymphomas and demonstrate the potential for RAPA treatment to prevent PTLD and other EBV^+ lymphomas.

Abstract# 911

RAPAMYCIN BLOCKS TUMOR PROGRESSION INDUCED BY CYCLOSPORINE IN A MOUSE TUMOR-TRANSPLANT MODEL. Joachim Andrassy,[1] Gudrun E. Koehl,[2] Markus Guba,[2] Alexander Kroemer,[2] Marcus N. Scherer,[2] Christian Graeb,[2] Karl-Walter Jauch,[2] Edward K. Geissler.[2] *[1]Surgery, Division of Transplantation, University of Wisconsin, Madison, WI; [2]Surgery, University of Regensburg, Regensburg, Germany.*

Introduction: Rapamycin (RAPA) has recently been shown to have antiangiogenic effects related to its remarkable anticancer activity. Conversely, cyclosporine (CsA) use is correlated with tumor progression. Here, we tested if the procancer and proangiogenic properties of CsA could be blocked by simultaneous usage of RAPA. **Methods:** *Tumor-transplant model*: Syngeneic B16 melanoma cells were s.c.-injected into C57BL/6 mice on day 0 (d0). On d7, treatment was initiated with 1.5 mg/kg/d RAPA or 40 mg/kg/d CsA, either as mono- or combination therapy (n=4-6/group). Also on d7, allogeneic, heterotopic, C3H mouse heart Tx (HTx) was done in specified experimental groups. *In vitro angiogenesis model*: Angiogenesis was tested in the presence of CsA (100 ng/ml), RAPA (5 ng/ml), or the combination, using an aortic-ring assay. Wistar rat aortae sections (1-2mm) were cultured on matrigel-coated wells; vascular sprouting area was measured as a "% of control" after 4d. **Results:** Untreated C57BL/6 mice with B16 tumors (±HTx) had to be euthanized within 2-3 weeks due to cancer complications. All HTx in untreated mice were rejected (±tumor) by d11, whereas RAPA and CsA prolonged HTx survival to 75% and 50% on d28 (experimental endpoint), respectively. In tumor-HTx mice, RAPA improved animal survival to 83% at d28 (no controls survived) by reducing tumor growth (d10: control-tumor vol.=542±74 mm^3 vs. RAPA=192±25 mm^3); all surviving mice had a beating HTx at d28 and tumors remained small (574±110 mm^3). In CsA-treated tumor-bearing mice, tumor growth was accelerated (d10 vol.=1391±185 mm^3), and mice were sacrificed due to tumor effects before HTx rejection. Importantly, when both CsA and RAPA were used, tumor growth was not promoted by CsA, and in fact, RAPA exerted its full anticancer effect, with beating allografts on d28. In vitro, CsA promoted (572%), while RAPA inhibited (31%) aortic-ring sprouting, vs. controls (100%), and the CsA effect was abrogated in the presence of RAPA (27%). **Conclusions:** (1) RAPA in a tumor-HTx situation protects a HTx and simultaneously inhibits tumor growth; (2) CsA, in contrast, promotes tumor growth, but this effect is blocked by concurrent use of RAPA, and (3) consistent with these data, the in vitro proangiogenic effects of CsA are blocked by RAPA. Therefore, when a transplant patient has cancer, concurrent use of RAPA may negate tumor progression attributed to CsA-based immunosuppression.

Abstract# 912

THE EFFECT OF RAPAMYCIN (RAPA) AND MYCOPHENOLIC ACID (MMF) ON VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) FUNCTION IN VITRO. Stefan G. Kiessling,[1,2] Christopher Geehan,[1,2] David M. Briscoe.[1,2] *[1]Division of Nephrology, Children's Hospital Boston, Boston, MA; [2]Harvard Medical School, Boston, MA.*

We have found that VEGF is expressed in association with chronic rejection; and that blockade of VEGF attenuates the development of graft vascular disease (GVD) in a murine cardiac transplant model. Rapa, an immunosuppressive agent has recently been reported to inhibit VEGF mRNA and has anti-angiogenic effects. We propose that this function of Rapa is important following transplantation, but little is known about the effect of other immunosuppressants (I/S) on VEGF expression and function. Here, we first evaluated the effect of Rapa (1-10 ng/ml), CsA (0.1-1 mcg/ml) or MMF (5-10 mcg/ml) on VEGF transcription in human endothelial cells. Total RNA was extracted at 16 hours and Real time PCR was performed for VEGF mRNA expression. As expected, Rapa treated cells showed a consistent decrease in VEGF mRNA transcription, whereas treatment with CsA, in general, resulted in an increase in VEGF transcription. In contrast, MMF had minimal or no detectable effect on VEGF mRNA expression. To next evaluate the effect of I/S on VEGF function, EC were stimulated with VEGF (5-10 ng/ml)) in the absence or presence of Rapa (1-10ng/ml), CsA (0.1-1 mcg/ml) or MMF (5-10 mcg/ml). VEGF alone enhanced endothelial cell proliferation and both Rapa and MMF inhibited VEGF-induced EC proliferation. In contrast, CsA consistently increased VEGF-induced EC proliferation in a dose dependent manner. The inhibitory effect of Rapa on EC proliferation was less pronounced in the presence of higher concentrations of VEGF (20 ng/ml) whereas MMF inhibited proliferation at all concentrations. This is suggestive that MMF might be more potent than Rapa to inhibit post VEGF Receptor signaling. To further evaluate this effect of MMF, we next evaluated its effect on VEGF-induced expression of EC adhesion molecules. By RNase protection, we found that MMF markedly reduced the expression of EC ICAM-1 and VCAM-1 mRNA, and that the addition of VEGF to MMF treated cells lead to a partial restoration of adhesion molecule transcription. Together, these findings suggest that both MMF and Rapa have anti-VEGF properties, but appear to exert their major effects through different mechanisms. We suggest that the use of rapamycin and MMF in combination as VEGF antagonists in the absence of CsA will be therapeutic to attenuate the development of chronic rejection.

Abstract# 913

DEOXYSPERGUALIN (DSG) DISRUPTS THE MYD88 INNATE IMMUNE PATHWAY AND PROMOTES DENDRITIC CELL APOPTOSIS: A RATIONAL BASIS FOR USE OF DSG IN TOLERANCE. Jianguo Wu,[1] Jin He,[1] Clement Asiedeu,[1] Frank T. Thomas,[1] Anne Hutchings,[1] Stephanie Le Bas-Bernardet,[1] Judith M. Thomas.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL.*

Innate immunity has a pivotal role in adaptive immune responses. Heat shock proteins (HSPs) activate innate toll receptor signal pathways, leading to inflammatory cytokine production, myeloid dendritic cell (mDC) activation and maturation. Preventing innate immune responses facilitates tolerance induction. Concomitant with promoting allograft tolerance, brief therapy with DSG curbs early proinflammatory cytokines and mDC maturation, and diminishes ischemia-reperfusion injury, suggesting an inhibitory effect on innate immunity. Here we show that DSG blocks innate immune signaling pathways, involving HSP70, MyD88, TRAF2, and NF-κB2 in mDC. Rhesus mDC were prepared from monocytes by culture in GMCSF and IL-4. TNFα was added to activate mDC with or without DSG. Protein expression was detected by western blotting and normalized to tubulin expression. DSG exerted a dose-dependent inhibition on nuclear translocation of RelB with significant reduction at 5mg/ml. In contrast, no suppression was observed on RelA or c-rel, suggesting a selective action of DSG on NFκB2, a transcription factor critical for mDC. Consistent with this finding, expression and nuclear translocation was 50% inhibited for the NF-κB2 partner p52 and p100, but not inhibited for the NF-κB1 partner p50 or p105. DSG also blocked expression and nuclear translocation of HSP70 in response to TNFα, and reduced MyD88 expression by 60%, independent of TNFα activation. Expression of TNF family members was differentially targeted by DSG; anti-apoptotic TRAF2 was inhibited; TRAF6 was unaffected, and the proapoptotic TNF-death receptor 4 (DR4) was increased. Addition of the DR4 ligand, TRAIL, to DSG-treated, TNFα-activated mDC induced 88% apoptosis, confirming a proapoptotic effect of DSG on mDC. Immunoprecipitation studies showed MyD88 and TRAF2 to be complexed in mDC, consistent with the linked inhibitory effect of DSG on MyD88 and TRAF2 expression. These data provide new insights into the molecular action of DSG in downregulating the innate immune pathway in primate mDC. DSG disrupts mDC maturation by inhibiting NFκB2 and down-regulating expression of MyD88 and anti-apoptotic TRAF2, while up-regulating expression of pro-apoptotic DR4. Since apoptotic DC are tolerogenic, the inhibitory action of DSG on innate immune signal pathways in mDC offers a plausible explanation for the synergistic effect of DSG in tolerance induction and a rational basis for its application.

Abstract# 914

FTY720 INDUCES PROLONGED ALLOGRAFT SURVIVAL IN THE PRESENCE OF MEMORY CD4 T CELLS. Anna Valujskikh,[1] Alla Gomer,[1] Peter S. Heeger.[1] [1]*Immunology, The Cleveland Clinic Foundation, Cleveland, OH.*

It has been previously reported that memory alloreactive T cells prevent the graft prolonging effects of costimulatory blockade. One of the strategies to overcome this problem and to control memory T cells is to inhibit their infiltration into the graft. FTY720 prolongs survival of solid organ allografts through retention of lymphocytes in secondary lymphoid organs without affecting their priming. The effect of FTY720 on the trafficking and functions of alloreactive memory T cells has not been previously addressed and was the focus of these studies.

We first tested how FTY720 affects migration of memory T cells, using the ability to rapidly produce IFNg after in vitro restimulation as a marker for memory and not naïve T cells. 4 weeks after placement of C3H skin grafts onto B6 recipients, the animals were either fed with FTY720 (3mg/kg/day) or with water as a control. 4 days later, the total numbers of donor reactive IFNg producing cells in the LNs and spleen were determined by a short term recall ELISPOT assay. The FTY720 treatment resulted in a dramatic increase in the absolute number of C3H-specific T cells in the lymph nodes (7,324±923 vs 867±302 in control group) and also facilitated the retention of C3H-specific T cells in the spleen (24,922±498 vs 15,248±883). We next tested if the addition of FTY720 to the costimulatory blockade restored the ability to prolong skin graft survival in primed recipients. B6 recipients were primed to C3H alloantigens through the rejection of C3H skin allografts. 6 weeks later, all recipients were given C3H donor specific transfusion plus anti CD154 mAb MR1 (DST/MR1) treatment followed by placement of second C3H skin allografts. One group of recipients was fed with FTY720 (3mg/kg/day on days –2 through 4), and the control group was given water. The control sensitized recipients (fed with water) rejected the skin grafts with MST of 10.2±0.2 d despite DST/MR1. Combined treatment with FTY720 and DST/MR1 resulted in modest but statistically significant prolongation of skin graft survival (MST of 13.4±0.6 d). Notably, this delay of graft rejection was comparable to the duration of treatment with FTY720 (4 days posttransplant). These findings demonstrate that FTY720 results in relocation of alloreactive memory T cells (along with naïve T cells) to the secondary lymphoid organs and, for the duration of therapy, prevents the memory cells from infiltrating the graft and mediating rejection. As human T cell repertoires contain memory alloreactive T cells, this finding may have important therapeutic implications in transplant recipients.

Abstract# 915

THE SPHINGOSINE-1-PHOSPHATE RECEPTOR AGONIST FTY720 REGULATES DENDRITIC CELL TRAFFICKING BY MODULATION OF ADHESION MOLECULE EXPRESSION. Yuk Yuen Lan,[1] An De Creus,[1] P. Toby Coates,[1] Angus W. Thomson.[1] [1]*Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Introduction: The immunosuppressive agent FTY720 prolongs allograft survival by promoting lymphocyte sequestration in secondary lymphoid tissues. Its active metabolite FTY720-phosphate (FTY720P) is a structural homologue of the blood-borne bioactive lipid mediator sphingosine-1-phosphate (S1P) that regulates cell migration, proliferation, and survival. G-protein-coupled S1P receptors have been identified as the molecular targets of S1P and its synthetic analogue FTY720P. We hypothesized that the S1P receptor agonist FTY720 might regulate dendritic cell (DC) trafficking and function via the S1P receptor pathway.

Methods: Immature murine (C57BL/10) bone marrow (BM)-derived myeloid DC (BMDC) were generated by 7-day culture in rmGM-CSF and IL-4. Blood and splenic DC were isolated from mice mobilized with the DC poietin fms-like tyrosine kinase 3 ligand (Amgen; 10 µg/day for 10 days). Immunobead-purified CD11c$^+$ DC (>90% purity) were then exposed to FTY720P (1 µM, Novartis, Basel, Switzerland) for various times. S1P receptor and adhesion molecule expression were determined by RT-PCR and by flow cytometry respectively. *In vitro* chemotaxis assay was used to measure DC migratory response to S1P.

Results: Using RT-PCR, we demonstrate for the first time, the differential expression of all 5 S1P receptors subtypes ($S1P_{1\text{-}5}$) by murine blood and splenic DC. $S1P_5$ was not expressed by BMDC. DC (blood, splenic and BMDC) matured *in vitro* following 24 h exposure to LPS (1 µg/ml) showed increased S1P receptor expression ($S1P_{1\text{-}5}$) compared with immature DC. This correlated with migration of splenic DC to S1P in an *in vitro* chemotaxis assay. *In vivo* treatment of mice with an immunosuppressive dose of FTY720 (0.35 mg/kg) i.p. for 24 h reduced CD11c$^+$ DC in the spleen but not in the blood. Flow cytometric analysis revealed that treatment of BMDC with FTY720P (4 h, 24 h) downregulated expression of CD11b and CD31/PECAM, surface adhesion molecules required for transmigration from blood into tissue. Further, CD54/ICAM-1 and CD44, which are costimulatory molecules important during DC-T cell interaction, were also downregulated after FTY720P treatment.

Conclusion: In contrast to its well-recognized capacity to deplete circulating lymphocytes, the immunomodulator FTY720 reduced CD11c$^+$ DC in the spleen, but not in the circulation. Our data suggest that FTY720 may regulate DC trafficking via modulation of surface intercellular adhesion molecule expression, a mechanism that may contribute to its immunosuppressive effects.

Abstract# 916

NIBR713: A NEW, MORE SELECTIVE FTY720 ANALOGUE WITH SIMILAR EFFICACY BUT LONGER DURATION OF ACTION IN ANIMAL TRANSPLANT MODELS. Klaus Hinterding,[1] Carsten Spanka,[1] Charles Pally,[1] Barbara Nuesslein-Hildesheim,[1] Volker Brinkmann,[1] Marc Bigaud,[1] Klaus Menninger,[1] Gisbert Weckbecker,[1] Christian Beerli,[1] Danilo Guerini,[1] Karl Welzenbach,[1] Gabriele Weitz,[1] Christian Bruns.[1] [1]*Transplantation Research, Novartis Institutes for Biomedical Research, Basel, Switzerland.*

Introduction: FTY720 has a unique immunomodulatory mechanism that targets migration of lymphocytes via sphingosine-1-phosphate receptors (S1P-R) and is highly effective in animal models of organ transplantation and autoimmunity. NIBR713 is a novel FTY720 analogue which displays increased selectivity on S1P-receptors by lacking S1P-3 activity. Reduction in S1P-3 activity may potentially result in an improved side-effect profile for this drug class.

Method: NIBR713 was selected because of its potent S1P-1-, but lack of S1P-3 activity in [GTP-γ^{35}S] binding assays. NIBR713 was compared with FTY720 to determine its effects on peripheral lymphocyte depletion in rats and monkeys, and its efficacy in the DA-to-LEW heterotopic heart allotransplantation model in combination with everolimus.

Results: NIBR713 depleted peripheral blood lymphocytes in both rats and cynomolgous monkeys with similar potency and efficacy but longer duration of action than FTY720. In rats, NIBR713 had an ED_{50} of 0.2 mg/kg (FTY720: 0.1 mg/kg) and a median plasma half-life of 6d (FTY720: 1.5d). Remarkably, in cynomolgous monkeys one oral dose of NIBR713 at 1 mg/kg reversibly depleted blood lymphocytes for >35d, while the effect of FTY720 at the same dose lasted for 5-7d. The median plasma half-life of NIBR713 in monkeys was 16d versus 4d for FTY720. Blood level data on NIBR713 and its phosphate demonstrated an almost complete *in vivo* phosphorylation and a close correlation between phosphate concentrations and the degree of peripheral lymphocyte depletion (PK/PD correlation). In transplantation, the combination of NIBR713 with everolimus was equipotent and equi-efficacious compared to FTY720 in preventing rat heart allograft rejection: NIBR713 at 0.1 mg/kg/d in combination with everolimus at 0.3 mg/kg/d led to graft prolongation of >28 days in all animals treated.

Histological and early toxicological examinations indicated a good tolerability of NIBR713.

Conclusion: NIBR713 is a novel S1P receptor agonist lacking S1P-3 activity that demonstrated equivalent efficacy in a rat heart allotransplantation model and a prolonged duration of action compared with FTY720. This study demonstrates for the first time, that the lack of S1P-3 activity does not compromise the immunomodulatory potency of FTY720 analogues.

Abstract# 917

THE NOVEL IMMUNOMODULATOR FTY720 DESENSITIZES AND DISRUPTS ENDOGENEOUS S1P RECEPTOR SIGNALING PATHWAYS IN VITRO. Danilo Guerini,[1] Rao Movva,[1] Thi-Thanh-Thao Tran,[1] Christoph Hangartner.[1] [1]*Transplantation & Immunology, Novartis Institutes for Biomedical Research, CH-4002 Basel, Switzerland.*

Introduction: FTY720 is a novel immunomodulator whose mode of action is different to that of other current drugs in transplantation. FTY720 was efficacious in allograft protection in Phase II trials of kidney transplantation in humans. FTY720 prevents the egress of lymphocytes from secondary lymphoid organs. A hypothesis is that FTY720 is phosphorylated in vivo, becoming a potent agonist at four sphingosine-1-phosphate-receptors (called S1P1, S1P3, S1P4, S1P5), which belong to the family of GPCR receptors. Some members of this protein family are removed upon activation from the surface of the cells and transported into intracellular compartments (internalization). This novel study was designed to evaluate receptor internalization by FTY720 and some close analogues.

Methods: We generated stable cell lines (using CHO and HeLa cells) expressing myc-tagged receptors S1P1, S1P3, S1P4 and we developed a method to quantify the agonist-mediated internalization process.

Results: Phosphorylated FTY720 caused a complete (>95%) and long lasting (>3h) disappearance of the S1P1 from the surface of the cells, in contrast to equimolar sphingosine-1-phosphate (S1P), which had a partial (40-50%) and reversible effect (reversed in 1-2 h). S1P, however, promoted the complete (>90%) internalization of S1P3 while after treatment with phosphorylated FTY720; nearly half (40 to 60%) of S1P3 was still present on the surface of the cell. S1P and phosphorylated FTY720 caused only moderate (35-45%) and transient internalization of the S1P4. Since a derivative, which cannot be phosphorylated, did not promote internalization, our data support the idea that internalization requires phosphorylated FTY720. Internalization mediated by phosphorylated FTY720 at S1P1 was invariably followed by the loss of responsiveness toward agonists that lasted up to 3 hours. In contrast, the complete internalization of S1P3 mediated by sphingosine-1-phosphate was transient and full surface expression was restored after around 1 h.

Conclusions: These results show for the first time that phosphorylated FTY720 promotes the post-transcriptional reorganization of S1P receptors. The long lasting

receptor internalization of the S1P1 (the most widely expressed receptor of this family) by phosphorylated FTY720 is expected to dramatically affect signaling by the endogenous agonist S1P. We propose that the agonist dependent internalization of S1P receptors is an important molecular component of the action of FTY720 in vivo.

Liver Transplantation: Hepatitis C

Abstract# 918

LIVER TRANSPLANTATION FOR HCV CIRRHOSIS: RESULTS ARE NOT GETTING WORSE IN RECENT YEARS. Luca S. Belli,[1] Dimitrios N. Samonakis,[2] Alberto B. Alberti,[1] George V. Papatheodiridis,[2] Marcello Vangeli,[1] David W. Patch,[2] Aldo Airoldi,[1] Alberto Quaglia,[3] Keith Rolles,[2] Luciano de Carlis,[1] Andrew K. Burroughs,[2] Giovambattista Pinzello.[1] *[1]Department of Gastroenterology - Liver Transplantation Unit, Ospedale Niguarda, Milan, Italy; [2]Department of Medicine- Liver Transplantation Unit, Royal Free Hospital, London, United Kingdom; [3]Histopathology Department, Royal Free Hospital, London, United Kingdom.*

Background: A worse outcome in HCV positive recipients with a faster progression of recurrent disease to overt cirrhosis has been reported in recent years and increased donor age and stronger immunosuppression have been indicated as major contributors to these worse results (M Berenguer, Hepatology 2002;36:202-210). We have reviewed the experience of two European Centers with a particular attention at histologic disease progression over the last 13 years

Methods: a retrospective analysis conducted on 404 HCV positive recipients transplanted between January 1990 and December 2002 (216 pts in Milan and 188 in London). Protocol liver biopsies available at regular intervals for all patients. Different immunosuppressive protocols used in the two Centers and over time. 30 patients transplanted after 1992 in Milan were treated with combined antiviral therapy (interferon + ribavirin).

Results: Overall survival progressively improved over the last 13 years table1(p=0.02). As for the disease progression study, the histological outcome was assessed for a total of 327 patients with at least 6 months of follow up (168 pts in Milan and 159 in London). A total of 960 protocol liver biopsies were reviewed corresponding to a median of 3 biopsies per patient (range 1-10). The cumulative probability of developing a severe fibrosis (≥ 4 Ishak) over the last 13 years are reported in tab.2. Finally, severe fibrosis progression was observed in 12 of 39 (30%) and 52 of 288 (18%) pts receiving a graft respectively from donors older and younger than 60 yrs (p=0.06).

Conclusions: In our experience patients and graft survival in HCV patients improved over the last 13 years despite the increasing number of older donors. The recent adoption of lighter immunosuppressive protocols and the selective use of antivirals may justify these favorable results.

Cumulative probability of survival in 404 HCV recipients: effect of year of LT

	3 months	1 year	3 years	5 years
1990-93	83%	70%	61%	60%
1994-96	81%	69%	63%	56%
1997-99	85%	81%	71%	69%
2000-02	89%	86%	84%	

tab.1

Cumulative probability of developing severe fibrosis (S=4-6, Ishak) in 327 HCV recipients with a f.up > 6 mos: effect of year of LT.

	1 year	3 years	5 years
1990-93	5%	12%	21%
1994-96	7%	16%	24%
1997-99	3%	13%	21%
2000-02	9%	20%	

tab.2

Abstract# 919

RECONSTITUTION OF HEPATITIS C VIRUS-SPECIFIC T CELL-MEDIATED IMMUNITY FOLLOWING LIVER TRANSPLANTATION. Scott Weston,[1] Rachel Leistikow,[1] Rajendar Reddy,[2] Maria Torres,[3] Michael Davey,[1] Anne M. Wertheimer,[1] Hugo Rosen.[1] *[1]Medicine/ Hepatology, Portland VAMC, Portland, OR; [2]Medicine/ Hepatology, U. Penn, Philadephia, PA; [3]Medicine, U. Miami, MIami, FL.*

Background: Hepatitis C virus (HCV)-related liver failure is the leading indication for liver transplantation worldwide. Post-transplantation, virological recurrence is the rule, but the spectrum of histologic injury is wide, ranging from the development of allograft cirrhosis within a few years to minimal hepatitis despite long-term follow-up. The immunological correlates of the variable natural history are poorly understood. *Methods:* we studied the kinetics of the cellular immune responses, viral replication, and allograft histology in 20 patients who had undergone liver transplantation for HCV-related liver failure. We prospectively tracked T-lymphocyte responses in 3 groups of HCV-seropositive patients who underwent liver transplantation: patients who received pre-emptive antiviral therapy (or placebo) starting within the first month after transplantation (patients 1 –10), patients who received antiviral therapy for severe histologic recurrence more than 3 months after transplantation (patients 11- 17), and patients with long-term follow-up who have demonstrated minimal evidence of histologic recurrence and have not required antiviral therapy (patients 18 - 20) *Results*: Using direct ex vivo methodologies, i.e., interferon-gamma ELISPOT and major histocompatibility complex class I-peptide tetrameric complexes, we found that patients who experienced viral eradication following antiviral therapy demonstrated restoration of HCV-specific T cell responses whereas patients with progressive HCV recurrence that failed to respond to therapy showed declining frequencies of these viral-specific effector cells. The HCV-specific CD8+ T cells that peripherally reconstituted after liver transplantation were clonotypically identical to CD8+ T cells present within the recipient's explanted liver (identical Vbeta chain and CDR3 sequence). Moreover, the subset of patients who spontaneously demonstrated minimal histologic recurrence had more vigorous CD4+ T cell responses targeted predominantly against the HCV nonstructural (NS3, NS4, and NS5) proteins at 2 and 3 months after transplantation. *Conclusions:* These results may help identify patients more likely to develop severe HCV recurrence and therefore benefit from current antiviral therapy, as well as provide a rationale for the future use of novel immunotherapeutic approaches.

Abstract# 920

SUSTAINED RESPONSE TO HCV TREATMENT FOLLOWING TRANSPLANTATION LEADS TO LOWER HCV ACTIVITY AND FIBROSIS AND GREATER GRAFT SURVIVAL. Stephen C. Rayhill,[1] Patricia Kirby, Michael Voigt, Douglas Labrecque, Daniel Katz, Rachael Miller, Alan Stolpen, Frank Mitros, You Min Wu, Warren Schmidt. *[1]University of Iowa.*

The rapid recurrence of severe hepatitis C viral (HCV) hepatitis after liver transplantation remains a major problem. To avert recurrent HCV in our transplant population, our patients are aggressively treated with interferon alpha and ribavirin following transplantation. Herein we analyze the effect of sustained response to anti-viral treatment (vs. no sustained response). Methods: Using our longitudinal database, survival for all recipients of liver transplants for cirrhosis due to HCV was analyzed (1991 onward). Fifty-three of the 112 patients in the database were treated with anti-HCV therapy. All liver transplant biopsies were analyzed (in a blinded fashion) and activity and fibrosis were graded using the Batts-Ludwig scale. Severe activity was defined as moderate or worse activity and severe fibrosis was defined as bridging fibrosis or cirrhosis. Graft survival, graft survival until the onset of severe activity, and graft survival until the onset of severe fibrosis were determined using Kaplan Meier estimates. The log rank test (LR) and Wilcoxan test (WC) were used to compare survival and Cox multivariate analysis was used to determine relative risks (RR). Results: Of the 53 treated patients, 13 (25%) had a sustained response, while 40 (75%) did not.

Kaplan Meier Survival After HCV Treatment

	Graft Survival			Graft Survival without Severe HCV Activity			Graft Survival without Severe Fibrosis		
	1 year	3 years	p	1 year	3 years	p	1 year	3 years	p
No Response	78%	70%	0.03 LR	55%	33%	0.02 LR	66%	50%	0.06 LR
Sustained Response	100%	100%	0.04 WC	84%	84%	0.02 WC	100%	100%	0.03 WC

Patients who did not achieve a sustained response to anti-HCV therapy were at 3.9 times greater risk for death, graft loss, or severe HCV activity and at 3.8 times greater risk for death, graft loss, or severe fibrosis. Conclusions: A sustained response to HCV antiviral therapy results in significantly less severe HCV activity and fibrosis after transplantation and improved graft survival. Thus, every effort must be made to treat liver transplant recipients at risk for severe HCV recurrence.

Abstract# 921

INFLUENCE OF CLASS II HUMAN LEUKOCYTE ANTIGENS ON RECURRENT HEPATITIS C AND GRAFT SURVIVAL IN LIVER TRANSPLANT RECIPIENTS. Richard T. Stravitz, Pamela Kimball, Velimir A. Luketic, Richard K. Sterling, Arun J. Sanyal, Robert A. Fisher, Adrian H. Cotterell, Daniel Maluf, Marc P. Posner, Scott A. Mills, Melissa Contos, Mitchell L. Shiffman. *Liver Transplant Program, Virginia Commonwealth University, Richmond, VA.*

Although recurrent HCV infection occurs universally after liver transplantation (LT), disease progression is variable. We hypothesized that recipient class II HLA antigens, which regulate the immune response to HCV, contribute to this variability. OBJECTIVES: To determine whether specific HLA class II antigens and donor-recipient mismatch (MM) are associated with disease progression and graft survival (GS) in LT recipients with recurrent HCV. METHODS: HLA genotypes were determined by PCR in 409 LT recipients and their donors, including 147 HCV RNA (+) recipients with ≥ 1 liver biopsy obtained ≥ 1 yr after LT. Recurrent HCV was defined as necroinflammatory activity of ≥ 3 points (Knodell) at 1 yr. Fibrosis rate (FR) was defined as fibrosis score/ yr post-LT. Acute rejection was assessed histologically. Patients with chronic rejection and vascular/biliary complications were excluded. RESULTS: Actuarial 10 yr GS was 45.6% in HCV-infected LT recipients compared to 61.7% transplanted for other liver diseases (P=.001). In recipients with HCV, the prevalence of HLA DR3 was higher (18.9 vs 9.8%; P=.0002), and HLA DR2 (10.9 vs 17.9%; P=.007) and DR5 (5.9 vs 11.3%; P=.007) lower, than in patients transplanted for other diseases; therefore, these genotypes were chosen for further study

HLA	GS@10Y (%)	Regraft Distribution (%)	Recurrence (%)	FR	Acute Rejection (%)
DR2	54	25	71	0.69(0.58)	42
DR3	48**	46*	81*	0.84(0.87)	37
DR5	58	8*	36**	0.11(0.14)**	25
Other DR	62	21	68	0.71(0.95)	29

(SD). *P≤0.05, **P≤0.02 vs other DR

Patients with HLA DR3 had reduced GS, and higher regrafting percent, HCV recurrence, and FR than other genotypes. In contrast, patients with HLA DR5 had lower regrafting percent, HCV recurrence and FR than other genotypes. HLA genotype had no effect on acute rejection rates. There were no significant differences between recipients with 0, 1, or 2 DR MM in HCV recurrence or in FR, although GS in 0 MM recipients was significantly better than 1 or 2 MM (75 vs 46%; P<.05). CONCLUSIONS: Recurrent HCV in LT recipients expressing the DR3 genotype follows a more aggressive clinical course, while DR5 confers relative protection, compared to other HLA DR genotypes. These observations occur independently of rejection, and suggest that host genetic factors play a significant role in the severity of recurrent HCV after LT.

Abstract# 922

HCV CORE PROTEIN IMPAIRS T CELL ACTIVATION AND ENHANCES RESPONSE TO CYCLOSPORINE. Pam M. Kimball,[1] Scott Verbeke,[1] Mitchell Shiffman.[1] *[1]Transplant Surgery, Medical College of Virginia Hospital, Richmond, VA.*

Hepatitis C (HCV) recurrance following liver transplantation (LT) is universal. The speed and severity of recurrance may be linked to the level of immunosuppression. Since HCV infection reduces cellular immunity, we speculated that soluble HCV proteins might potentiate the effect of cyclosporine (CsA) upon T cell responses. This study examined the impact of HCV Core protein upon T cell proliferation, expression of early activation markers and proliferative suppression by CsA. T cells were activated with anti-CD3 for 2-6 days. Cultivation with 1, 2, 4 and 8 ug/ml Core reduced the stimulation index from 163 ±41 to 152 ±11(p=ns), 60 ±20 (p<.001), 49 ± 10 (p<.001) and 13 ±3 (p<.001), respectively, without evidence of toxicity. Actual cell counts (X 10^4) confirmed the lack of clonal expansion in untreated vs treated cultures after 2 days (72 ±11 vs 11 ± 3, p<.05), 4 days (397 ±50 vs 28 ±5, p<.05) and 6 days (250 ±100 vs 29 ±3, p<.05). Expression of activation markers was reduced in Core treated cells. Treatment with 4 ug/ml Core for 2, 4 and 6 days reduced CD3+CD25+ from 37 ±3% to 12 ±3% (p<.05), 12 ±1% (p<.05) and 18 ±4% (p<.05), respectively. Similarly, CD3+DR+ was reduced from 13 ±6% to 6 ±2% (p<.05), 7 ±2% (p<.05) and 6 ± 2% (p<.05), respectively. Thermal inactivation of Core abolished proliferative suppression (p=ns). Core protein was subsequently titrated into cultures containing various concentrations of CsA. The impact of combining Core with CsA upon proliferation was analyzed by isobologram analysis, a mathematical method which indicates whether interactions are synergistic, additive or inhibitory. Combining Core with CsA resulted in an additive effect upon proliferative suppression. For example, the proliferative suppression induced by the combination (70 ± 10%) was equivalent (p=ns) to the suppression produced by individual exposure to CsA (30 ±5%) or Core (43 ±7%). Linear regression of the data confirmed an additive interaction between Core and CsA with an r^2 value of 0.98 (p=ns). The data show that HCV Core protein, at physiologically attainable levels, specifically inhibits T cell activation at an early stage which blocks T cell clonal expansion. In addition, Core protein mitigates proliferative suppression by CsA. These results suggest that release of Core during periods of viremia may potentiate clinical immunosuppression with CsA. Although preliminary, we suggest that reduction in pharmacologic immunosuppression may be feasible and beneficial in HCV+ recipients following LT.

Abstract# 923

MULTICENTER RANDOMIZED HEPATITIS C (HCV) THREE TRIAL POST LIVER TRANSPLANTATION (OLT): A PRELIMINARY REPORT. Carlos G. Fasola,[1] Goran B. Klintmalm,[1] C. Hepatitis Three Study Group. *[1]Transplantation, Baylor University Medical Center, Dallas, TX.*

Purpose: To assess the effect of mycophenolate mofetil (MMF) and steroid (Pred)-free immunosuppression (IS) identifying the most effective IS that will minimize incidences of HCV recurrence (HCVR), acute rejection (ACR) and adverse events (AE) post OLT. **Methods:** Trial designed as open label, prospective, randomized multicenter study involving adult HCV-OLT recipients (pt) allocated into 3 IS regimens. Arm 1: tacrolimus (TAC) + Pred; arm 2: TAC + Pred + MMF and, arm 3: TAC + MMF + 2 dose-daclizumab, but no steroids. HCV-OLT pt (n = 312) from 17 different institutions are allocated at a 1:1: 2 ratio, respectively. Laboratory data and graft histology (LBx) are evaluated when clinically indicated and by protocol at 90, 365 and 730 days (d). HCVR is staged according to Batts and Ludwig. ACR is graded according to Banff schema. Primary endpoints are clinically significant: HCVR (fibrosis stage [Sg] ≥ 2 at 90, 365 d. or Sg ≥ 3 at anytime) and ACR (Banff grade [Gr] ≥ 2 + RAI ≥ 4). **Results:** To maintain the validity of the trial, report based on Data Safety Monitoring Board assessment from 11/2003. Out of 228 pt enrolled, 182 pt have a median follow up of 89 d. Table summarizes average pt characteristics.

Recipient Characteristics			
Age (years):	51.1± 7.1	HLA MM:	4.9±1.0
Weight (kg):	85.1±18.2	MELD score:	21.8±7.3
Male gender:	69.8%	HCV Ab (+):	98.9%
Race:	70.9% caucasian	HBV Ab (+):	37.9%[1]
Cad. vs. LRD:	87.4% vs. 12.6%	CMV Ab (+):	59.9%
Whole graft:	84.1%	EBV Ab (+):	68.1%
Cad. CIT (h.):[2]	7.3±3.0	LRD CIT (h.):	2.6±1.3

HBV sAg (+) and cAb (+) donors were excluded[1];CIT: cold ischemia time[2]

Day 90 protocol LBx were done in 82% pt. Current information on 98 pt: HCVR (Sg ≥ 2) found on 8 pt (8.2%), median of 94 d. post OLT and placed on antiviral therapy (endpoint). ACR found in 10 pt (10.2% - Gr: 2 [6]; 3 [4]), median = 13 d. post OLT. Five pt died due to: lung failure + cancer; CVA; idiopathic lung disease; HA thrombosis; sepsis. Study withdraw = 3 pt. No major AE reported in any arms. **Conclusions:** This 90-d. preliminary report suggests the safety of the IS used (Pred, TAC, MMF, daclizumab and a steroid-free regimen) in the trial, since no major AE has been reported in any group. HCVR and ACR incidences appear encouraging at this point. High protocol LBx rate obtained will provide invaluable information on natural course of HCV post OLT on pt treated similarly and followed up with same standards in multiple centers. Enrollment should be completed by 03/2004. Results, based on 90 and 365 d, will be presented at the ATC 2004 meeting.

Abstract# 924

THE IMPACT OF CALCINEURIN INHIBITORS (CNIs) ON RECURRENT HEPATITIS C INFECTION. Gary A. Levy,[1] Leslie B. Lilly,[1] David R. Grant,[1] Nigel T. Girgrah,[1] Atul Humar,[1] Paul D. Greig,[1] Mark S. Cattral.[1] *[1]Multi Organ Transplant Program, Toronto General Hospital, Toronto, ON, Canada.*

The long term outcome following transplantation for HCV has deteriorated questioning whether use of more potent immunosuppressant agents contributes to lower patient and graft survival. This study was designed to evaluate outcome differences in patients receiving Neoral C2 as compared to tacrolimus C0 following liver transplantation for HCV. 128 patients underwent liver transplantation for HCV at our center; 48 received Neoral and 80 Tacrolimus. All received Solumedrol/prednisone. C2 levels of Neoral were adjusted to 1000 ng/mL for the first 6 months whereas C0 levels of Tacrolimus were kept between 10-15 ng/mL for the first 6 months; subsequently C2 levels were lowered to 800 ng/mL months 6-12 and then 600 ng/mL thereafter whereas tacrolimus levels were reduced to 5-10 ng/mL after month 6. Rates and severity of rejection were similar for patients receiving Neoral or Tacrolimus (Neoral 37% vs Tacrolimus 34% p=NS). Patient and graft survival was higher in patients receiving Neoral compared to those receiving Tacrolimus (90% vs 78% p<0.05). The incidence and severity of recurrent HCV was much higher in patients receiving Tacrolimus with many patients progressing to severe fibrosis or cirrhosis by year 3 post transplant. Genotype differences could not explain the results seen. HCV viral titers increased more rapidly in patients treated with Tacrolimus than Neoral and by 3 years mean viral titers were 3.2 million copies in patients receiving Tacrolimus versus 1.05 million copies in patients treated with Neoral (p<0.05). Levels of AST, ALT, ALP and bilirubin were more markedly disturbed in patients receiving Tacrolimus compared to patients who received Neoral. Use of rebetron only provided a sustained virologic response in patients on Neoral as previously reported by another group of investigators, although the sustained virologic response was only seen in 20% of treated patients. The incidence of diabetes mellitus was significantly higher in patients treated with Tacrolimus. Addition of Cellcept (MMF) resulted in more severe recurrent HCV infection in both Neoral and Tacrolimus treated groups. These data strongly suggest that use of Tacrolimus in comparison to Neoral leads to a poorer outcome following transplantation for HCV. Multivariate analysis of this single center data as well as analysis of the Lis2t study will hopefully provide further insight into factors contributing to these results which will allow us to design better immunosuppressive protocols for this group of patients.

Abstract# 925

PRIMARY HEPATOCYTES DERIVED FROM THE DONOR LIVER EXHIBIT A SIMILAR PATTERN OF HEPATITIS C QUASISPECIES SELECTIVITY AS THE LIVER ALLOGRAFT. Tae W. Chong,[1] Robert L. Smith,[1] Michael G. Hughes,[1] Christine K. Rudy,[1] Robert G. Sawyer,[1] Timothy L. Pruett.[1] *[1]Surgery, University of Virginia, Charlottesville, VA.*

Introduction: Allograft infection after liver transplantation for chronic Hepatitis C (HCV) is nearly universal, and the presumed mechanism of infection is related to HCV envelope protein interaction with putative liver receptors. This represents a unique opportunity to study an early event in HCV infection, quasispecies (QS) selection by the allograft. We have developed a primary hepatocyte culture system derived from donor livers to study QS selectivity *in vitro* and to correlate it with allograft association *in vivo*.

Methods: In two patients undergoing liver transplantation secondary to HCV infection, the following samples were obtained: blood prior to the removal of the explant (inoculum), biopsy from the donor liver before transplantation (hepatocytes), and allograft after two hours of perfusion (*in vivo* selection). Hepatocytes were obtained using a two-step collagenase perfusion and cultured at $3.0x10^5$ cells/ml. The cells were inoculated with serum obtained from the patient's blood and harvested after 5 days. RNA was extracted from the samples and reverse transcribed. The cDNA was amplified using a semi-one-sided PCR for the HVR1 region. Single strand conformational polymorphisms (SSCP) were analysed by denaturing the PCR product and separating them by electrophoresis.

Results: The HVR1 sequences were amplified and demonstrated similarity in band patterns for both patients when analyzed by SSCP. In patient A, there were 3 bands in the serum and 6 bands in the 2 hour post perfusion biopsy. The post perfusion sample demonstrated differential selectivity with 3 new bands represented that were not seen in the inoculum. *In vitro*, there were 3 bands in the primary hepatocytes with 2/6 bands from the 2 hour post perfusion represented in the *in vitro* sample. A single band persisted in all three samples. There was a higher degree of correlation between the *in vitro* and *in vivo* samples for patient 2. The *in vitro* sample had 6 bands which all demonstrated a similar pattern with the 2 hour sample (9 bands).

Conclusion: There is a differential association of the viral quasispecies for the allograft, and primary hepatocytes derived from the same liver demonstrate some similarity to the *in vivo* sample in this selectivity. The differences may represent phenotypic differences in cultured hepatocytes or viral adaptation to the host without immunologic pressure. This represents a unique model to study microenvironment and quasispecies selection.

Abstract# 926

INTRAHEPATIC TISSUE HEPATITIS C PCR TESTING TO HELP DETERMINE SUSTAINED VIROLOGICAL RESPONSE IN LIVER TRANSPLANT RECIPIENTS FOLLOWING SUCCESSFUL COMPLETION OF HCV ANTIVIRAL THERAPY. Guy W. Neff,[1] Christopher B. O'Brien,[1] Robert Cirocco,[2] Kamran Safdar,[1] Marzia Montalbano,[1] Jose A. Gascon,[1] Eugene Schiff.[1] *[1]Medicine, University of Miami, Miami, FL; [2]Surgery, University of Miami, Miami, FL.*

Introduction

Hepatitis C virus (HCV) is the most common indication for liver transplantation in patients suffering from end stage liver disease. Recurrence post liver transplantation is common and frequently results in progressive liver disease requiring treatment with a pegylated interferon and ribavirin. However, relapse upon withdrawal of combination therapy is very common. Therefore, optimal duration and the stopping point of therapy in this patient population remain unclear.

Aim

We wanted to determine if the presence or absence of HCV RNA liver tissue would be a better predictor of success following 48 weeks of therapy with treatment of pegylated alpha-2b interferon and ribavirin of therapy in patients treated for recurrent HCV post liver transplantation than the present detection of HCV RNA in serum.

Methods

All recipients received combination pegylated alpha-2b interferon (1.5mcg/kg) and ribavirin (200-600mg/d) therapy for at least 48 weeks of therapy. The diagnosis of serum HCV-R was determined histopathologic findings with inflammation along with viral recurrence using COBAS AMPLICOR™ Hepatitis C virus Test, version 2.0 (HCV RNA Qualitative PCR) and COBAS AMPLICOR™ HCV MONITOR TEST-version 2.0 (HCV RNA quantitative PCR) assays. Tissue HCV PCR confirmation was done using Light Cycler (Roche) Real time PCR for the HCV quantitation. SVR was determined as serological testing nondetectable at 6 months.

Results

Ten transplant recipients were included: 3 (30%) females and 7 (70%) males, mean age (53 yrs), mean time from OLT was 29.2 months. Four (40%0 were Caucasians and 6 (60%) were Hispanic. All 10 were nondetectable HCV RNA in their blood at the end of therapy. However, 7/10 (70%) remained HCV PCR detectable in their liver tissue and 3/10 (30%) were nondetectable. SVR without relapse only occurred in the 3/10 that were liver tissue HCV PCR negative at the end of therapy.

Conclusion

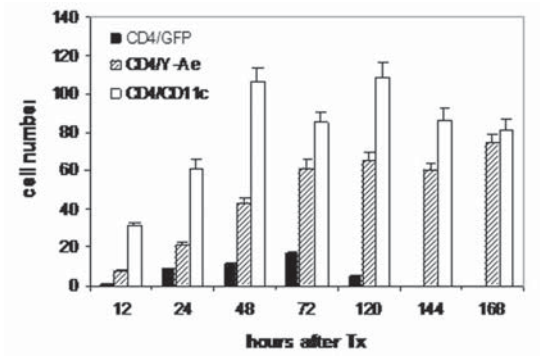
Direct detection of HCV PCR in liver tissues appears to be a more accurate predictor of SVR following PEG interferon and ribavirin therapy for recurrent HCV post liver transplantation. This information will require long term follow up and further confirmation.

MECHANISMS OF ACUTE GRAFT INJURY

Abstract# 927

DIRECT AND INDIRECT ALLORECOGNITION: VISUALIZATION OF DENDRITIC CELL INTERACTIONS IN GRAFT REJECTION. Jordi C. Ochando,[1] Jaime Llodra,[1] Gwendalyn J. Randolph,[1] Alexander Y. Rudensky,[3] Jonathan S. Bromberg,[1,2] Nancy R. Krieger.[1,2] *[1]Carl C. Icahn Center for Gene Therapy and Molecular Medicine, Mount Sinai School of Medicine, New York, NY; [2]Recanatti/ Miller Transplantation Institute, Mount Sinai School of Medicine; [3]Department of Immunology, University of Washington, Seattle, WA.*

Interactions between T cells and both donor and recipient dendritic cells (DC), characterize alloresponses in organ transplantation, where recipient T cells respond either directly to donor MHC or indirectly to processed donor MHC allopeptides in context of recipient MHC molecules. The present study evaluates the nature of the DC-CD4 T cell interactions in the lymph node (LN) during AR after murine cardiac transplantation. CX3CR1$^+$/GFP$^+$ BALB/c (I-A^d) donor hearts were transplanted into C57BL/6 (I-A^b) mice; AR occurred by 7 days. LNs were harvested after 12, 24, 48, 72, 120, 144 and 168 hours, and fluorescent immunohistological staining was performed for CDllc, CD4, and Y-Ae mAb (which recognizes the complex of I-Eα 56-73 donor allopeptide in the context of recipient I-A^b, thus evaluating indirect presentation). Confocal microscopy enabled visualization of donor DC (CD11c$^+$GFP$^+$cells), as well as indirect (CD4$^+$Y-Ae$^+$) and direct (CD4$^+$donor DC) interactions. The number of donor and recipient DCs, CD4$^+$, Y-Ae$^+$ cells, and their interactions per 0.6 mm2 of LN tissue over time were enumerated.



Our data indicate an early increase of DC-CD4$^+$ interactions in the LN following AR. Donor GFP$^+$ DC-CD4$^+$ direct interactions are present as early as 12 hours following engraftment, peak at day 3, but disappear by the 6th day. Conversely, recipient DC-CD4$^+$ indirect interactions increase to a maximum between 48-120 hours, but persists for at least 7 days. Y-Ae-CD4$^+$ interactions, which evaluate indirect presentation of the specific allopeptide pEα:I-A^b, similarly persist and even increase at day 7. In conclusion, both direct and indirect interactions between CD4 T cells and donor and recipient DCs occur shortly after engraftment in AR episodes. However, only donor MHC peptides are still presented in context of recipient DC to CD4 T cells at post-transplant day 7, indicating persistent indirect allorecognition.

Abstract# 928

ACUTE REJECTION OF HEART ALLOGRAFTS WITH A SINGLE MHC II DISPARITY IS MEDIATED BY BLOCKING CD25+ REGULATORY CELLS BUT NOT BY DONOR-SPECIFIC DENDRITIC CELL PRIMING. Soren Schenk,[1] Danielle D. Kish,[2] Anton V. Gorbachev,[2] Kiyotaka Fukamachi,[1] Peter S. Heeger,[2] Robert L. Fairchild.[2] *[1]Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH; [2]Immunology, The Cleveland Clinic Foundation, Cleveland, OH.*

Heart allografts expressing single MHC II disparities are not acutely rejected. We hypothesized that $CD25^+$ regulatory cells prevent the expansion of alloreactive T cells. The goal of this study was to test if increased donor-specific priming or direct blockage can impede early regulation. We first confirmed that C57BL/6 (B6) (H-2b) recipients accept MHC II mismatched B6.H-2^{bm12} (bm12) heart grafts (MST >100d, n=10). Control B6 acutely rejected complete mismatched A/J (H-2a) grafts by d8, and priming with A/J dendritic cells (DC) 3 days before transplant, reduced graft survival to d5. In contrast, bm12 DC priming of B6 (n=10) only resulted in a 50% loss of bm12 grafts by d60 (*P* NS vs non-primed B6). Although bm12 grafts of primed B6 had severe cellular infiltration at d7 (ISHLT grade 3A-3B) compared to non-primed B6 (1A-1B), the number of alloreactive T cells increased less than 2-fold (from 74 cells/ 6 x 10^6 in non-primed to 110/ 6 x 10^6 in primed B6), smaller than observed in complete mismatched grafts (950/ 6 x 10^6). By d21, donor-specific T cells decreased 5-fold in primed vs non-primed B6 (21/ 6 x1 0^6 vs 60/ 6 x 10^6, $P<0.05$), suggesting down-regulatory mechanisms augmented by DC priming. Accordingly, multiple bm12 DC priming of B6 led to indefinite graft survival. We next blocked $CD25^+$ cells with PC61 mAb (0.25 mg on d -1 to d9), which resulted in a striking 80% loss of bm12 grafts by d25 (n=6, MST 21d, $P<0.01$ vs control B6). Recall assays on d21 revealed a 15-fold increase of alloreactive T cells compared to untreated B6 recipients (750/ 6 x 10^6 vs 50/ 6 x 10^6, $P<0.01$). PC61-blocked CD25+ cells were present 1 day after the treatment; but not at 9 days after treatment, when all grafts were already severely infiltrated. We finally transplanted bm12 hearts into T cell deficient B6 $Rag^{-/-}$ recipients, which were then reconstituted with 30 x 10^6 B6 splenocytes that did or did not contain $CD25^+$ cells. Reconstitution with $CD25^+$ depleted cells resulted in acute rejection of bm12 grafts (n=6, MST 12d, $P<0.001$ vs control B6), whereas $CD25^+$ containing B6 cells did not. Thus, donor-specific priming cannot mediate rejection of grafts with a single MHC II disparity, possibly due to the presence of strong regulation. This study is the first to show that temporary blockage of $CD25^+$ cells can lead to increased priming of alloreactive cells and to acute rejection of MHC II mismatched grafts.

Abstract# 929

SYNERGISTIC INFECTIOUS TOLERANCE BY ADOPTIVE TRANSFER OF TOLEROGENIC DENDRITIC CELLS AND T REGULATORY T CELLS. Wei-Ping Min,[1,2,3] Dameng Lian,[1] Mu Li,[1] Thomas E. Ichim,[1] Bertha Garcia,[1,2] Robert Zhong.[1,2,3] *[1]The Multi-Organ Transplant Program, London Health Sciences Centre, London, ON, Canada; [2]Transplantation, Lawson Health Research Institute, London, ON, Canada; [3]Departments of Surgery, Microbiology and Immunology, and Pathology, University of Western Ontario, London, ON, Canada.*

We have previously demonstrated that targeting both dendritic cells and T cells simultaneously achieved reliable tolerance in a mouse transplant model, which depended on a regulatory feedback loop between tolerogenic dendritic cells (Tol-DC) and T regulatory cells (Treg). The present study was undertaken to determine if there is synergy in the induction of tolerance by simultaneous adoptive transfer of Tol-DC and Treg. **METHODS:** BALB/c mice receiving C57BL/6 cardiac allografts were treated with LF 15-0195, a novel analogue of 15-deoxysperqualine at dose of 2 mg/kg for 20 days and long-term survivors (>100 days) were defined as tolerant recipients. $CD11c^+$ dendritic cells, $CD4^+CD25^+$ and $CD8^+CD28^-$ Treg cells were isolated from spleens of tolerant recipients. Tol-DC alone (5x10^6/mouse), Treg alone (1x10^6/mouse) or a combination of Tol-DC and Treg were intravenously injected into naïve BALB/c recipients prior to and/or after allogeneic (C57/BL6->Balb/c) transplantation. The recipients received sublethal radiation and no additional immunosuppressants were used. **RESULTS:** The $CD4^+CD25^+$ and $CD8^+CD28^-$ T cells were significantly increased in the tolerant recipients as compared with rejecting recipients and non-transplanted mice. Both ex vivo isolated $CD4^+CD25^+$ and $CD8^+CD28^-$ T cells potently suppressed ongoing MLR in vitro, suggesting they are Treg. The Tol-DC isolated from tolerant recipients demonstrated immature phenotypes, impaired antigen presenting function, and inhibited allostimulatory capacity. Adoptive transfer of Tol-DC significantly prolonged allograft survival to a median survival time (MST) of 38 days, while recipients without transfer survived to MST of 13 days. Transfer of a subtherapeutic dose of Treg did not prolong the allograft survival. In contrast, adoptive transfer of a combination of Tol-DC and the subtherapeutic dose of either $CD4^+CD25^+$ or $CD8^+CD28^-$ Treg significantly prolonged survival to MST of 85 days ($P<0.05$) and MST of 105 days ($P<0.01$), respectively. **CONCLUSIONS:** This study provides the first direct evidence that Tol-DC synergize with Treg in the induction of "infectious tolerance". These findings shed light on mechanisms of tolerance induction through the regulatory feedback loop between Tol-DC and Treg.

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Abstract# 930

NKT CELLS PARTICIPATE IN REJECTION OF ISLET ALLOGRAFTS IN THE LIVER OF MICE. Atsushi Toyofuku,[1,5] Yohichi Yasunami,[1] Kentaroh Nabeyama,[1] Masahiko Nakano,[1,5] Masayuki Satoh,[1] Nobuhide Matsuoka,[1] Junko Ono,[2] Toshinori Nakayama,[3] Masaru Taniguchi,[4] Masao Tanaka,[5] Seiyo Ikeda.[1] *[1]SurgI, Fukuoka Univ, Fukuoka, Japan; [2]Lab Med, Fukuoka Univ, Fukuoka, Japan; [3]Mol Immunol, Chiba Univ, Chiba, Japan; [4]Allergy and Immunology, RIKEN Research Center, Yokohama, Japan; [5]SurgI, Kyushu Univ, Fukuoka, Japan.*

Natural killer T (NKT) cells have been recently identified as a novel lymphoid lineage distinct from T , B and NK cells and their role in transplant immunology remains undetermined. Previously, we have shown that NKT cells are essential for accptance of rat islet xenografts in mice (JCI 105:1761, 2000). The aim of the present study was to determine a role of NKT cells in islet allograft rejection. For those purposes, CD1d-deficient (CD1d KO) as well as Va14NKT cell-deficient (NKT KO) mice were used for the experiments.

When BALB/c islets (500) were grafted into the liver of STZ diabetic wild-type C57BL/6 mice, islet allografts were rejected at 9.2±1.4 (n=8) days after transplantation (tx). Morphologically, islets infiltrated with mononuclear cells were seen in the liver. FACS analysis revealed an up-regulation of intracytoplasmic IFN-γ expression as well as CD25 expression of NKT cells in the liver at the time of rejection. When islet allografts were grafted into CD1d KO mice without treatment, the MST was prolonged to 26.0±9.3 (n=5) days. When diabetic wild-type or CD1d KO mice were treated with 0.2 mg/kg rapamycin (ip, day 0-6), the MST of islet allografts was 16.2±6.9 (n=9) or >64.5±24.1 (n=6) days, respectively. When the dosage of rapamycin was increased to 1.0 mg/kg, the MST in wild-type or CD1d KO mice was >41.3±22.2 (n=8) or >86.7±10.9 (n=11) days, respectively. One out of 8 wild-type and 10/11 CD1d KO mice receiving islet allografts and treated with 1.0 mg/kg rapamycin were normoglycemic at 90 days after tx. Morphologically, intact islets with well-granulated β cells were seen in the liver of normoglycemic mice. The similar findings were obtained when NKT KO mice were used as recipients. The survival rate of islet allografts in CD1d KO mice treated with rapamycin (1mg/kg) and receiving the intraportal transfer of hepatic MNC (5x10^6) from wild-type (n=3) or CD1d KO (n=3) mice at the time of islet tx was 0 or 100%, respectively, at 60 days after transplantation.

These findings clearly demonstrate that NKT cells play a significant role in acute rejection of islet allografts in the liver of mice and indicate that NKT cells might be considered as a target for prevention of rejection. Further studies are in progress to determine whether it is also the case with the use of calcineurin inhibitors instead of rapamycin.

Abstract# 931

IN VIVO REGULATION OF TH1 RESPONSES AND MURINE CARDIAC ALLOGRAFT REJECTION BY MUTIDRUG RESISTANCE P-GLYCOPROTEIN. Atsushi Izawa,[1] Natasha Y. Frank,[2] Shona S. Pendse,[1] Armen Margaryan,[1] Masayuki Sho,[1] Mohamed H. Sayegh,[1] Markus H. Frank.[1] *[1]Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA; [2]Division of Genetics, Chidren's Hospital, Boston, MA.*

We have previously demonstrated a critical role of multidrug resistance (MDR1) P-glycoprotein (P-gp) in alloimmunity: P-gp blockade inhibits human alloimmune T cell activation in vitro via both T cell- and antigen presenting cell (APC)-dependent mechanisms. A possible in vivo immunoregulatory role of P-gp has not been investigated to date. We used a vascularized murine heterotopic cardiac transplant model to examine the effects of P-gp blockade on allograft survival and alloimmune T cell responses. RT-PCR detection of mdr1a mRNA and flow cytometric detection of a P-gp antagonist-sensitive calcein-AM efflux capacity demonstrated functional expression of mdr1a P-gp in murine splenocytes. P-gp blockade in BALB/c recipients of C57BL/6 allografts via i.p. administration of the specific P-gp antagonist PSC833 prolonged mean allograft survival from 8.5+/-0.5 to 11.7+/-0.5 days vs. controls ($p<0.01$), and inhibited recipient IFN-γ but not IL-5 production, while enhancing the IgG1/IgG2a ratio of donor-specific alloantibody ($p<0.05$). The results suggested that delayed rejection in P-gp-blocked animals occurred predominantly via a Th2-dependent humoral response. Concurrent blockade of P-gp and CD86, which regulates Th2 responses, markedly prolonged allograft survival (40.1+/-3.9 days) vs. either P-gp-blockade or CD86 blockade alone or vs. concurrent P-gp and CD80 blockade ($p<0.01$). Mice exhibited suppressed Th1-dependent IFN-γ production and blocked Th2-dependent humoral immunity, demonstrating in vivo synergy of P-gp inhibition and CD86-directed costimulatory blockade. When hearts from MHC class II knockout (KO), but not wildtype mice, were transplanted into mdr1a/b KO mice, graft survival was also significantly prolonged compared to wildtype recipients ($p<0.05$), suggesting that P-gp functions predominantly in indirect allorecognition. Our findings demonstrate that P-gp is a critical regulator of Th1 responses in vivo and show that the molecule functions in indirect allorecognition. Thus, our results raise the possibility that P-gp-targeted approaches may open new therapeutic approaches in clinical allotransplantation, and in particular in chronic allograft rejection.

Abstract# 932

IMPAIRED REJECTION IN THE ABSENCE OF LIGHT: A COMPARISON OF CD8 AND CD4 T CELLS. Zhong Guo,[1] Ying Dong,[1] Jun Wang,[1] Marvin Newton-West,[1] Yang-Xin Fu,[2] Kenneth A. Newell.[1] *[1]Surgery, Emory University, Atlanta, GA; [2]Pathology, University of Chicago, Chicago, IL.*

We have previously shown that CD4 T cells lacking the costimulatory molecule LIGHT ineffectively reject allografts. We now examine the role of LIGHT in rejection mediated by CD8 T cells and contrast its effect on CD8 and CD4 T cell function. **Methods.** Two approaches were used to examine the effect of LIGHT on T cell-mediated rejection. 1) $RAG^{-/-}$ allograft recipients reconstituted with wild-type (WT) CD8 or CD4 T cells were treated with fusion proteins that block LIGHT (LTβR-Ig or HVEM-Ig). 2) CD4 or CD8 WT or $LIGHT^{-/-}$ T cells were transferred into $RAG^{-/-}$ allograft recipients. The effect of LIGHT on T cell activation after transplantation was assessed by IFNγ Elispot and MLR. **Results.** Blocking LIGHT with LTβR-Ig or HVEM-Ig prolonged skin allograft survival in mice reconstituted with WT CD8 T cells (20 and 16 days respectively vs. 11 days for controls, $p<0.001$) but had no effect on survival in CD4-reconstituted mice. Relative to $RAG^{-/-}$ mice reconstituted with WT T cells, skin allograft survival was prolonged in $RAG^{-/-}$ mice reconstituted with either $LIGHT^{-/-}$ CD8 (52 vs. 16 days, $p = 0.0003$) or $LIGHT^{-/-}$ CD4 T cells (40 vs. 13 days, $p < 0.0001$). Intestinal allograft rejection at day 28 was also less severe in mice reconstituted with $LIGHT^{-/-}$ CD8 T cells (absent/mild rejection) compared to WT CD8 T cells (all with severe rejection). The number of T cells recovered from $RAG^{-/-}$ mice reconstituted with WT or $LIGHT^{-/-}$, CD8 or CD4 T cells was similar suggesting that differences in graft survival were not due to differences in homeostatic proliferation. $LIGHT^{-/-}$ CD4 T cells isolated prior to rejection demonstrated significantly impaired priming in vivo and proliferation to donor antigen in vitro relative to WT CD4 T cells. Following allograft rejection, $LIGHT^{-/-}$ and WT CD4 T cells demonstrated equivalent priming and proliferation to donor antigen. Surprisingly, even in non-rejecting recipients there were large numbers of primed $LIGHT^{-/-}$ CD8 T cells. These $LIGHT^{-/-}$ CD8 cells also proliferated well to donor antigen in vitro. **Conclusions.** Although LIGHT contributes to rejection mediated by both CD4 and CD8 T cells, the fusion protein data suggest that LIGHT has a greater effect on CD8 T cells mediating rejection. Following transplantation LIGHT appears to be important for the initial activation of CD4 T cells. In contrast, $LIGHT^{-/-}$ CD8 T cells appear to undergo initial activation but remain relatively ineffective in mediating allograft rejection suggesting that LIGHT acts on CD8 T cells down-stream of initial activation.

Abstract# 933

DISTINCT REQUIRMENTS FOR HOST CD80/CD86 COSTIMULATORY MOLECULES IN CARDIAC VERSUS ISLET REJECTION. Zachary Johnson,[1] Joshua Beilke,[1] Biagio Pietra,[2] Brian Kelly,[2] Ronald G. Gill.[1] *[1]Barbara Davis Center for Childhood Diabetes, Univ. Colo. Hlth. Sci. Center, Denver, CO; [2]Dept. Pediatrics, Univ. Colo. Hlth. Sci. Center, Denver, CO.*

A major role for the B7 family members CD80 and CD86 in providing costimulation to T cells is well established. Interestingly, previous studies show that host, but not donor CD80/CD86 expression is required for cardiac allograft rejection. However, the role for host costimulation by these molecules in the rejection of cellular islet allografts and xenografts is unclear. The purpose of this study was to determine whether islet allografts and/or rat islet xenografts required recipient CD80/CD86 molecules for acute rejection

Methods: Streptozotocin-induced diabetic C57Bl/6 (B6, $H\text{-}2^b$) or CD80/CD86 double-deficient B6 mice were grafted with allogeneic BALB/c ($H\text{-}2^d$) islet allografts or with WF rat ($RT1^u$)) islet xenografts. Non-diabetic animals were grafted with BALB/c cardiac allografts. Islet survival was determined by monitoring blood glucose levels while cardiac allograft survival was assessed by graft palpation. Graft rejection was determined by return to hyperglycemia (islets) or cessation of heartbeat (hearts). Rejection was confirmed be histological examination of the transplant.

Results: Consistent with previous studies, BALB/c cardiac allografts were acutely rejected in wild-type B6 mice (5/5 grafts rejecting in <12 days) but survived >100 days in CD80/CD86 deficient mice (6/6). In stark contrast, both islet allografts (10/10) and rat islet xenografts (6/6) demonstrated acute rejection in both control B6 and in CD80/CD86 deficient hosts ($p<.01$ relative to cardiac allografts). Parallel studies using CD80/CD86 deficient islet donors failed to demonstrate a requirement for donor B7 expression in islet allograft rejection. Thus, neither host nor donor CD80/CD86 expression was required for islet allograft rejection.

Conclusion: Varied studies imply that the inherent pathways for rejecting primarily vascularized versus cellular allografts or xenografts may be distinct. The present study illustrates this concept by showing a marked difference in the role of host-derived CD80/CD86 costimulatory molecules for cardiac allograft versus islet allograft/xenograft rejection in vivo. While such costimulation is rate-limiting for cardiac allograft rejection, these same molecules are not necessary for acute rejection of either islet allograft or xenografts.

Abstract# 934

EARLY ALLOGRAFT REJECTION IS MEDIATED BY CD3+CD4+CD45RA-CD62L- MEMORY CELLS FOLLOWING ALEMTUZUMAB DEPLETION. Steven C. Hoffmann,[1] Jonathan P. Pearl,[1] Douglas A. Hale,[1] Roslyn B. Mannon,[1] S. J. Swanson,[2] Allan D. Kirk.[1] *[1]Transplantation Branch, NIDDK, Bethesda, MD; [2]Organ Transplant Service, Walter Reed Army Medical Center, Washington, DC.*

Alemtuzumab facilitates marked depletion of T-cells and monocytes following organ transplantation and allows for reduced maintenance immunosuppression. Peripheral cellular phenotyping of renal transplant recipients showed that naïve T-cells and T-cells with potential regulatory function (CD4+CD25+) were not prevalent following aggressive depletion with Alemtuzumab. Rather, post-depletion T-cells were of a single depletion-resistant effector memory phenotype (CD3+CD4+CD45RA-CD62L-; M1) that expanded in the first month and were uniquely prevalent at the time of rejection. These cells were resistant to steroids, deoxyspergualin and sirolimus, in vitro. In this study, we examined patients undergoing profound T-cell depletion with Alemtuzumab following renal transplantation, evaluating the phenotype and transcriptional characteristics of their residual PBMC and cellular infiltrates present on renal biopsies. Patient PBMC were isolated by ficoll and characterized by flow cytometry. RNA was extracted from sorted naïve and M1 memory cells, following 2-hour stimulation with PMA/ionomycin, and from renal protocol biopsies for quantitative real-time PCR. Recipients typically experienced a reversible acute rejection within the first month after transplantation and these rejection biopsies were characterized by a significant elevation of costimulatory molecule transcripts (CD80, CD86 and CD154), pro-inflammatory cytokines (IL10, IFNg, TNFa, TNFb) and chemokines and growth factor transcripts (MIP1a, RANTES, MIG, IP-10 and GM-CSF). Stimulation of M1 memory cells from patient PBMC, post-depletion, also generated a significantly elevated, effector memory transcriptional profile (RANTES, MIP1a, IFNg, MIG, IP-10, GM-CSF, and TNFa), remarkably similar to transcripts observed in renal biopsies. In addition, CCR7 and CD62L, critical regulators of immune cell trafficking, were significantly upregulated in renal biopsies but were strongly down-regulated in circulating, activated M1 memory. These data demonstrate that a limited population of functional memory cells result from aggressive depletion with Alemtuzumab and quickly hone to allograft and mediate rejection following transplantation. Therefore, strategies to account for and restrict the activity of resurgent T-cell populations following depletion must be considered in designing anti-rejection or tolerance-inducing therapies.

Abstract# 935

PRO-INFLAMMATORY EFFECTS OF NON-COMPLEMENT ACTIVATING ANTIBODIES ON ENDOTHELIAL CELLS AND MACROPHAGES. Barbara A. Wasowska,[1] Zhiping Qian,[1] Morteza Loghmani,[1] Salma Rahimi,[1] Karen Fox-Talbot,[1] William M. Baldwin III.[1] *[1]Pathology, Johns Hopkins School of Medicine, Baltimore, MD.*

Alloantibodies are a clinically significant component of the immune response to organ transplants. In our experimental model, B10.A ($H\text{-}2^a$) cardiac transplants survive significantly longer in C57BL/6 ($H\text{-}2^b$) immunoglobulin knock out (IgKO) recipients than in their wild type counterparts (WT). Passive transfer of a single 50-200 µg dose of complement activating IgG2b (15-1-P) alloreactive monoclonal antibodies (Allo-mAbs) to IgKO recipients reconstituted acute rejection of cardiac allografts. Although passive transfer of a subthreshold dose of 25 µg of IgG2b or a single 100-200 µg dose of non-complement activating IgG1 (AF3-12.1.3.) Allo-mAbs did not restore acute rejection to IgKO recipients, a combination of these Allo-mAbs did cause acute graft rejection. Histologically, rejection was accompanied by extensive aggregates of platelets that stained intensively for von Willebrand factor. These platelet aggregates occluded the arteries, capillaries and veins of the rejected allografts. Flow cytometry and ELISA assays demonstrated that the IgG1 Allo-mAbs (AF3-12.1.3.) used in our studies did not activate complement on their own and did not augment complement activation by IgG2b Allo-mAbs. However, IgG1 Allo-mAbs specific for mouse SVEC4-10 endothelial cells stimulated them to produce monocyte chemotactic protein 1 (MCP-1), the mouse homolog of human Gro-α that has 80% homology with human IL-8 (KC) and RANTES. Since MCP-1, KC and RANTES are all chemoattractant for macrophages, we cultured antibody-sensitized endothelial cells with macrophages. Using a protein macroarray assay and then Real-Time PCR and ELISA, we found that in addition to MCP-1, KC and RANTES, IgG1 stimulated high levels of MIP-2, IL-1-α and IL-6 and moderate levels of TNF-α in cultures of endothelial cells with macrophages. Exogenous TNF-α was demonstrated to augment the effects of IgG1 on endothelial cells. Our findings indicate that non-complement-activating Allo-mAbs can augment injury to allografts by complement-activating Allo-mAbs. Non-complement activating Allo-mAbs stimulate endothelial cells to produce chemokines (MCP-1, MIP-2, KC, RANTES) which in turn activate macrophages. Subsequently macrophage-secreted cytokines (IL-1-α, IL-6, TNF-α) augment antibody-induced activation of endothelial cells. The interaction of antibody-activated complement with complement receptors on macrophages and T cells is under investigation.

Abstract# 936

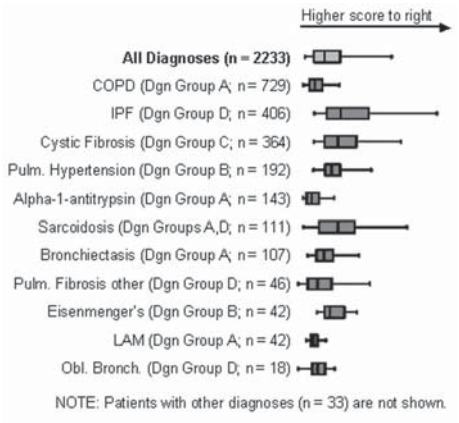
PROPOSED LUNG ALLOCATION SYSTEM BASED ON MEDICAL URGENCY AND TRANSPLANT BENEFIT. Robert M. Merion,[1,2] Tempie E. Hulbert-Shearon,[1,2] Rami T. Bustami,[2] Edward R. Garrity,[3] Thomas M. Egan,[4] Friedrich K. Port,[2] Robert A. Wolfe,[1,2] Susan Murray.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI; [3]Department of Medicine 110-6271, Loyola University Medical Center, Maywood, IL; [4]University of North Carolina Hospitals, Chapel Hill, NC.*

Background: Patients (pts) awaiting lung transplantation (LTX) are prioritized largely by waitlist (WL) time. Donor shortages suggest the need for maximizing LTX benefit among WL pts. A practical allocation system would prioritize pts at high risk of death on the WL while avoiding futile transplants.

Methods: Measures of LTX benefit were developed to rank candidates using WL and post-LTX survival models based on 5,109 pts, age ≥12, on the OPTN LTX WL and 2,700 recipients of a first LTX (age ≥ 12) between 1999-2001, respectively. The models identified factors based on clinical meaningfulness and objectivity that were statistically significant across all diagnoses plus those that varied significantly among 4 major diagnosis groups: A (mainly COPD), B (mainly PPH), C (CF) and D (mainly IPF).

Results: Higher WL mortality rates were significantly associated with decreasing FVC and BMI; increasing age, O_2 requirement (groups A, C, D), and PA systolic pressure (groups A, C, D); ventilator use, diabetes, higher NYHA class, and 6 min walk < 150 ft. Higher post-LTX mortality rates were significantly associated with decreasing FVC (groups B, D), increasing age, increasing creatinine, PCW ≥ 20 mm/Hg (group D), ventilator use, and higher NYHA class. Allocation scores combined utility and medical urgency measures for 2,233 WL pts active on 1/1/2003 by calculating the expected life lived with LTX in the following year minus twice the expected life lived on the WL (without LTX) during that year, with organs offered first to pts with highest scores. Equity was demonstrated by the large overlap in allocation scores among WL pts by diagnosis (Figure), and also by gender, race, and age (not shown).

Conclusion: The proposed lung allocation system would allocate organs to those likely to benefit the most from LTX.



Abstract# 937

USE OF DONOR HEARTS WITH LEFT VENTRICULAR EJECTION FRACTION ≤50% FOR TRANSPLANTATION. Fotios A. Mitropoulos,[UCLA] Hillel Laks,[UCLA] Reza Kermani,[UCLA] Daniel Marelli,[2] Abbas Ardehali,[UCLA] Mark Plunkett,[UCLA] Fardad Esmailian,[UCLA] Jonah Odim,[UCLA] Jamie Moriguchi,[UCLA] Linda Hamilton,[UCLA] Jignesh Patel,[UCLA] Jon Kobashigawa.[UCLA] *[1]Cardiothoracic Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Cardiac Surgery, University of Kansas Medical Center, Kansas City, KS; [3]Cardiac Transplantation and Heart Failure, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Purpose: The use of donor hearts with depressed left ventricular (LV) ejection fraction (EF) for transplantation is a controversial topic. In this single-institution retrospective study, we report our experience.

Methods: All patients who were transplanted with donor heart EF≤50% were identified. Statistical analysis was carried out using SPSS and Kaplan-Meier actuarial survival analysis.

Results: Seventy-two patients were identified with a mean follow up of 42.4±42.3 months. Mean donor age was 27.8 ± 14.4 (range, 1-61). Males made up 67.6% of the donors. Donor/recipient height ratio was 1.01± 0.08, and weight ratio was 1.14 ± 0.52. Only the highest EF was analyzed and the mean value was 46.7% ± 5.3% (range, 22-50). 10% of the donors died of trauma and 21.1% of intracranial bleeding. High dose inotropes (>10µg/kg/min dopamine) were required for 5.6% and LV hypertrophy was present in 1.4%. Mean recipient age was 48.5 ± 19.4 years, 70.4% were male, 44.5% were UNOS Status I, 45.8% Status II and 9.7% alternate. Actuarial survival at 30-day, 1-year, 3-years and 10-years was 91.4%, 83%, 75.9%, and 63.1% respectively. Actuarial TCAD-free survival 97.2% at 1-year and 91% at 3-years. At five years and ten years, dropped to 74.9% and 34% respectively. 30-day rejection was 15.2% and overall rejection was 29.6%, all within 3 years. Mean EF at latest follow up was 56.3%±7.7%. The mean difference between EF at follow up and harvest was an increase of 9.26% ± 9.11. There was no statistical significance between the group of 50% and the one less than 50% in the incidence of TCAD (p=0.625), rejection within 3 years (p=0.349) and long-term survival (p=0.152)

Conclusion: Actuarial survival estimates for donor hearts with EF≤50% is comparable to our overall institution reported survival of 84% at 1 year. Donor hearts with EF≤50%, with appropriate management and careful patient selection may present a viable option for the nationwide shortage of donor hearts.

Abstract# 938

NATIONAL EXPERIENCE WITH LIVE DONOR RENAL TRANSPLANTATION OF A_2/A_2B INTO B AND O PATIENTS. Christopher F. Bryan,[1] Wida S. Cherikh,[2] Jude Maghirang,[2] Mark I. Aeder,[1] James Gloor,[3] Paul W. Nelson,[1] Douglas J. Norman,[4] Charles F. Shield,[1] John B. Sorensen,[5] Mark D. Stegall,[3] Francis H. Wright, Jr..[6] *[1]Midwest Transplant Network, Westwood, KS; [2]United Network for Organ Sharing, Richmond, VA; [3]Mayo Clinic, Rochester, MN; [4]Oregon Health Sciences University, Portland, OR; [5]LDS Hospital, Salt Lake City, UT; [6]Methodist Specialty and Transplant Hospital, San Antonio, TX.*

Introduction: The live donor pool may be further maximized by considering the use of blood group A_2 donors for B or O candidates, or blood group A_2B donors for B candidates. The purpose of this study is to examine the national experience with live donor renal transplantation of B and O candidates with A_2/A_2B donor kidneys. Methods: We examined 79 ABO-verified A_2/A_2B into B and O live donor transplants reported to the United Network for Organ Sharing (UNOS) between 1990 and 2002. Each patient's transplant center was asked to provide anti-A titer data and whether the patient's anti-A titer was reduced by plasmapheresis (PP) and/or IVIg before transplantation. Kaplan-Meier graft survival of the 79 recipients was estimated and compared with that of the B/O recipients from B/O donors during the same period using the log-rank test.

		Graft Survival (years)				
Group	**n**	**1**	**2**	**3**	**4**	**p-value**
A2/A2B → B/O	79	88%	85%	78%	72%	0.04
B/O → B/O	28,239	94%	90%	87%	82%	
A2/A2B → B/O	44	88%	85%	82%	72%	0.26

Results: The data in Table 1 show that 1 to 4 year graft survival in the group of 79 B or O recipients of A_2/A_2B live donor kidneys was moderately lower (p=0.04) than those who received a B or O kidney. The 1 to 4 year graft survival of the 44 A_2/A_2B to B/O patients for whom the center returned the questionnaire was not significantly different from the B/O to B/O group (p=0.26).

		Graft Failure in 1st month	
Anti-A IgG Titer	**n**	**Yes (%)**	**No (%)**
Low (≤4)	20	1 (5%)	19 (95%)
High (≥8)	4	2 (50%)	2 (50%)

The data in Table 2 show the anti-A IgG titer data (all by non-AHG method) for the 24 patients who did not have anti-A titer reduction therapy before transplantation. The 1 month graft failure rate was greater (50%) in patients whose anti-A titer was ≥8 (50%) compared to those with low titers (5%) (Fisher's exact test p=0.06). Twelve patients, 10 of whom had high anti-A titers before transplantation, were treated with either PP alone (n=10) or PP and IVIg. All of the 12 patients have functioning kidneys. Conclusions: The long-term survival of kidneys from A_2 or A_2B live donors into B or O patients function as well as those from B or O donors, if the anti-A IgG titer is low or if it was lowered before transplantation. Consideration should be given to using A_2 or A_2B donors for B or O patients to increase access of live donor transplantation.

Abstract# 939

ELIMINATING POINTS FOR HLA-B SIMILARITY INCREASED KIDNEY ALLOCATION TO MINORITY, PEDIATRIC, SENSITIZED, AND ZERO MM CANDIDATES. Friedrich K. Port,[1] Valarie B. Ashby,[1,2] Alan B. Leichtman,[1,2] Maureen A. McBride,[3] James J. Wynn,[4] Winfred W. Williams,[5] John P. Roberts,[6] Sarah H. Rush,[1] Robert A. Wolfe.[1,2] *[1]SRTR/URREA, Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI; [3]OPTN/UNOS, Richmond, VA; [4]Department of Surgery, Medical College of Georgia, Augusta, GA; [5]Multicultural Affairs Office, Massachusetts General Hospital, Boston, MA; [6]Liver & Kidney Transplant Service, University of California, San Francisco, San Francisco, CA.*

In the United States, deceased-donor kidney transplants are allocated first to candidates with zero HLA-A,B,DR mismatches (MM) with the donor and then according to a

system that assigns points for HLA similarity, waiting time, pediatric age group, panel reactive antibody (PRA) level, and previous living donation. The current OPTN algorithm provides two points for a zero and one point for a one HLA-DR MM. Points are no longer assigned for HLA-A or HLA-B similarity. Prior to May 7, 2003, points assigned were 7, 5 and 2, for zero, one and two HLA-B,DR MM between candidates and deceased donors. We report the outcomes from the first four months of this new allocation policy. **Methods:** The study population consisted of 8,254 patients who received a solitary deceased donor transplant from 9/7/02 to 9/6/03, of which 2,758 (33%) patients received a transplant after the policy changed. The table below shows the fraction of recipients in each group during the pre- and post-policy time periods. A chi-square test was performed to test whether the differences were significant.

Results:

Group	% of Transplants Pre-Policy	% of Transplants Post-Policy	% Increase from Pre- to Post-Policy	p-value
Non-White	36.0	38.6	7.2	0.022
Age < 17	3.9	4.9	26.7	0.027
PRA 80+	4.4	5.5	25.3	0.030
Zero Mismatch	14.1	15.0	6.7	0.248
Zero DR Mismatch	30.1	27.2	-9.5	0.007

Conclusions: Eliminating points for HLA-B similarity and assigning a modest number of points for HLA-DR similarity increased the allocation of deceased-donor kidneys significantly to minority, pediatric, and sensitized candidates and non-significantly to zero MM candidates. As a consequence of the previous allocation system, candidates who are unlikely to receive zero HLA-DR deceased-donors transplants are currently accumulated at the top of the kidney transplant waiting lists. As the new allocation system comes to steady state, it is expected that the percentage of zero DR MM transplants will increase.

Abstract# 940

THE EFFECT OF CREG MATCHING ON GRAFT SURVIVAL AND HLA SENSITIZATION IN RENAL RECIPIENTS. Deborah O. Crowe,[1] William A. Nylander,[2] Harold Helderman,[3] Anthony J. Langone,[3] Tarik Kizilisik,[2] David Shaffer.[2] *[1]Transplant Immunology, DCI Laboratory, Nashville, TN; [2]Kidney and Pancreas Transplantation, Vanderbilt University Medical Center, Nashville, TN; [3]Nephrology Division, Vanderbilt University Medical Center, Nashville, TN.*

The UNOS algorithm for renal allocation has removed all HLA points for Class I HLA matching. There was some concern in our transplant program that this may increase the level of sensitization to HLA and make re-transplantation much more difficult. Review of UNOS data also suggested that long term graft survival may be adversely affected by increased mismatching of Class I HLA. The use of cross-reactive groups (CREG) for Class I matching was suggested as an option to decrease sensitization to broad public epitopes without discriminating against recipients with rare HLA antigens. Our local sharing region has a UNOS variance that incorporates CREG matching points into the algorithm. To help justify the use of this variance in allocation, we have examined graft survival retrospectively when stratified by HLA matching and CREG matching. We have also looked the level of HLA antibody (Panel Reactive Antibody - PRA) in patients activated for re-transplant and compared this with the degree of HLA mismatch of the first transplant.

Graft survival was examined in transplants performed from January,1990 through December,2000. The results show very little differences in one year graft survival, except for a slight decrease in graft survival for the 5-6 HLA mismatched grafts. Graft survival rates for 0ABDR, 0CREG-0DR, 0DR, and 5-6 ABDR mismatches were 93.5%, 88.9%, 84.5%, and 81.9%, respectively at one year. Five year graft survival, however, showed increased survival in the grafts that were better matched Graft survival rates for 0ABDR, 0CREG-0DR, 0DR, and 5-6 ABDR mismatches were 70.9%, 70.4%, 54.5%, and 59.6%, respectively at five years. This suggests that Class I matching may be important in long term graft survival. Broad Class I matching using the public epitopes identified as the CREG groups appeared to improve graft survival at five years, especially when there were also no mismatches at the DR locus.

72% of patients re-listed for transplantion were sensitized to HLA, compared to 21% of patients waiting for first transplant. Fifty re-transplant patients were evaluated for sensitization to HLA following the loss of the first transplant. Only 2 of 10 patients were sensitized to Class I HLA antigens when the first transplant was a 0 CREG mismatch. 78% of the patients were sensitized if there was a CREG mismatch. All six patients with 3 or more CREG mismatches were sensitized when re-listed. Donor-specific antibody was detected in all but three of the sensitized patients.

Abstract# 941

ASSIGNMENT OF ACCEPTABLE MISMATCHED HLA ANTIGENS ACCURATELY PREDICTS RESULTS OF CYTOTOXICITY AND FLOW CROSSMATCHES. Karen A. Nelson,[1] Danny Youngs,[1] William H. Marks,[2] Connie Davis,[3] Ruth McDonald,[4] Robert L. Wilburn.[5] *[1]Puget Sound Blood Center, Seattle, WA; [2]Swedish Hospital Medical Center, Seattle, WA; [3]University of Washington, Seattle, WA; [4]Children's Hospital and Regional Medical Center, Seattle, WA; [5]Virginia Mason Medical Center, Seattle, WA.*

Purpose: To determine the value of identifying the HLA specificity of alloantibodies in predicting negative crossmatches in highly sensitized renal transplant candidates.

Methods: Serum samples from renal wait list candidates were tested using FlowPRA™ Specific beads. Individual HLA A, B, DR, DQ and DP antigens were identified as Acceptable Mismatches if no antibody was detected. The ability of these assignments to predict negative cytotoxicity crossmatches was tested retrospectively using results of crossmatches performed using AHG-CDC with T lymphocytes or CDC with B lymphocytes from 100 local donors. The predictive value for flow cytometry crossmatches was tested using crossmatch results with T and B lymphocytes from 86 donors. A crossmatch was included in the analysis if all the mismatched donor antigens were Acceptable Mismatches for the patient. Patients were divided into 2 cohorts: PRA ≥80% or PRA between 20 and 80%. The analysis was limited by: 1) the set of HLA-DQ antigens in the FlowPRA beads was incomplete, 2) sera were not tested for antibody to HLA-C locus antigens, 3) donors were not typed for HLA-DP.

Results: For patients with PRA ≥ 80%, Acceptable Mismatches of 91 patients were compared with 2867 cytotoxicity crossmatches. For donors with Acceptable A and B Mismatches, 93% of T cell crossmatches were negative. When Acceptable DR and DQ Mismatches were included, only 52% of T and B cell crossmatches were negative. However, if patients with antibody to HLA-DP were censored, 81% of T and B crossmatches were negative. For patients with PRA 20%-80%, Acceptable Mismatches of 78 patients were compared with 2158 cytotoxicity crossmatches. For donors with Acceptable A and B Mismatches, 98% of T cell crossmatches were negative. When Acceptable DR and DQ Mismatches were included, 96% of T and B cell crossmatches were negative. In the analysis of flow cytometry crossmatches, Acceptable Mismatches predicted 90% of the negative crossmatches for patients with PRA ≥80% and 88% of the negative crossmatches for patients with PRA between 20% and 80%.

Conclusion: Identification of Acceptable Mismatches accurately predicted negative T and B crossmatches for patients with PRA 20-80% and negative T cell crossmatches for patients with PRA ≥80%. As over 50% of highly sensitized patients had antibody to HLA-DP, typing donors for HLA-DP antigens may increase the predictive value for B cell crossmatches.

Abstract# 942

ORGAN DONATION INCREASES AT LEVEL 1 TRAUMA CENTERS AFTER IN-HOUSE COORDINATORS IMPLEMENTED: COMPARISON OF HOUSTON TO NEW YORK CITY AND LOS ANGELES. Teresa J. Shafer,[1] Charles T. VanBuren,[1] Ronald N. Ehrle,[1] Kimberly D. Davis,[1] Roger E. Durand,[2] Samuel M. Holtzman,[1] Nicholas J. Crafts,[1] Phillip J. Decker.[2] *[1]LifeGift Organ Donation Center, Houston, TX; [2]University of Houston Clear Lake, Clear Lake, TX.*

Purpose: Because large numbers of U.S. potential organ donors (PODs) are located in Level 1 Trauma Centers, resources should be invested in these institutions to maximize conversion of potential donors to actual donors. Daily interaction with hospital staff and direct management of all PODs with early family contact and interaction, can significantly increase conversion rates.

Description: A large OPO replicated a successful In-House Coordinator (IHC) program already in place in two Houston L1TCs in two other large metropolitan areas: New York City and Los Angeles. These cities comprise 3 of the 4 largest cities in the country. The Houston experience, a longer experience of approximately 6 years with the program was compared to NYC and LA. 1999-2000 was compared to 2001-2002.

Results Summary: All L1TCs showed significant improvements in consent and conversion rates after placement of an IHC in the facility. The Houston L1TCs achieved significantly higher donation rates to during both the pre and post periods due to the Houston L1TCs' longer experience with the IHC program. (See Table)

Conclusions: The IHC model improves the donation process by: (1) interacting with families and staff earlier and on a more extended basis during POD cases, (2) improving donation systems through closer OPO-hospital staff relationships and (3) providing on-site management of the hospital's donation system. This intervention may be particularly effective in addressing the long-standing problem of lower consent rates among minority populations. Donation programs benefit from the daily interaction accomplished by having OPO staff located directly within the hospital.

Level 1 Trauma Centers: Pre and Post Implementation of In-House Coordinator Program

Pre = 1999-2000 Post = 2000-2001	Houston N=2 Annual Average : PRE	Houston N=2 Annual Average : POST	New York City N=2 Annual Average : PRE	New York City N=2 Annual Average : POST	Los Angles N=3 Annual Average : PRE	Los Angles N=3 Annual Average : POST
Total Referrals	396	519	118	118	238	302
Percent MINORITY Referrals	55%	58%	58%	47%	85%	89%
Medically Suitable Potential Organ Donors	112	132	55	52	133	119
Actual ORGAN DONORS	70	94	18	23	45	49
Increase in ORGAN DONORS		34%		28%		9%
Conversion Rate	63%	71%	33%	44%	34%	41%
Increase in Conversion Rate		13%		33%		21%

Abstract# 943

THE IMPACT OF A NATIONAL MINORITY COMMUNITY BASED TRANSPLANT EDUCATION PROGRAM: A NATIONAL COMPARATIVE ANALYSIS. Clive O. Callender,[1] Patrice V. Miles,[1] Gwendolyn D. Maddox.[1] *[1]National MOTTEP, Howard University, Washington, DC.*

Purpose:

This is a comparative analysis of a national community-based program to improve minority donation rates. In 1988, minorities represented 15% of the donor population. Previously minorities, especially Blacks, were thought not likely to become donors.

Methodology:

The methodology was based upon the 1982 African American (A.A.) donor education program. Between 1982 and 1988, A.A. donor card signings increased from 20/month to 750/month and Black donations doubled. A nationwide Black donor media campaign was launched from 1986 to1992. Gallup Polls in 1985 and 1990 indicated a tripling of Blacks awareness of transplantation and the number of Blacks signing donor cards. Thereafter, a national community based education program was established that applied the successful methodologies to each targeted ethnic group.

Results:

National program objective measures are: 1) National minority organ donors per million (O.D.M.) increased from 8-10 O.D.M. (1982) to > 40 O.D.M. (A.A. and Latino/Hispanics) in 2002.; 2) National minority donor percentages increased from 15% to 28.5% of donors in 2002 (minorities comprised 25% of the USA population in 2000).

A comparison was made of Organ Procurement Organizations (OPO) donation rates in OPO areas with national program activities and OPO regions without national activity.

Cadaveric Donors per 1,000 Evaluable Deaths and Number of Donors by Ethnicity and OPO's, 1995-1998

Ethnicity	Natl. Program Activities Donation Rates	Natl. Program Activities Donors	No Natl. Program Activities Donation Rates	No Natl. Program Activities Donors	P-value
White, Non-Hispanic	59.3	4,928	59.2	11,279	0.02
White, Hispanic	106.9	1,055	47.4	886	<.01
Black	43.4	1,263	32.9	1,286	<.01
Other	50.7	228	42.4	272	<.01

Conclusions:

1). Minorities now donate at or above the level of their representation in the U.S. population.

2). Comparison data strongly suggest that the national program's methodology has a statistically significant effect in the increase of national minority donation rates.

Abstract# 944

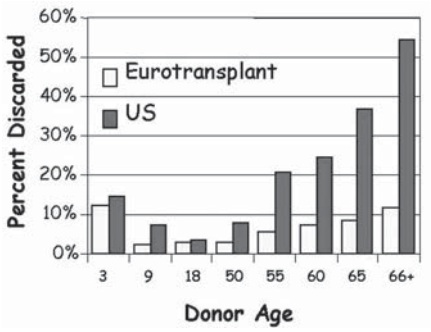
INCREASING THE KIDNEY DONOR POOL BY MORE EFFICIENT USE OF KIDNEYS RECOVERED FROM DECEASED DONORS. Michael Cecka,[1] Bernard Cohen,[2] Mike Smith,[2] John Rosendale.[3] *[1]UCLA Immunogenetics Center, University of California, Los Angeles, CA; [2]Eurotransplant Foundation, Leiden, Netherlands; [3]United Network for Organ Sharing, Richmond, VA.*

Background: Despite the continuing shortage of kidneys from deceased organ donors, many kidneys that are recovered in the US are not transplanted.

Methods: We compared the rate of kidney procurement and transplantation between January 2000 and June 2003 in the US and in the Eurotransplant (ET) region (Austria, Belgium, Germany, Luxemburg, the Netherlands, Slovenia), for different donor age groups.

Results: In the US, 38,275 kidneys were recovered for transplantation between 2000-2003 and 32,809 (85.7%) were transplanted. During the same period, 11,630 kidneys were recovered in the ET region and 11,033 (94,9%) were transplanted. The discard rates according to donor age shown in the figure reveal that the US discards 37% of kidneys from donors aged 61-65 and 54% of kidneys from donors over age 65 that were recovered for transplantation, whereas ET discards 7% and 8% of kidneys from these age groups, respectively. "Biopsy findings" was the most commonly cited reason for discarding older kidneys (44% of discards) in the US. Only 30% of kidneys from donors aver age 65 were transplanted to recipients over age 65 in the US compared with more than two-thirds in ET. The over age 65 donor accounted for 6% of kidneys procured in the US and 11% of those procured in ET.

Conclusions: The US discards more than 1,500 deceased donor kidneys each year that were recovered for transplantation but subsequently judged to be unsuitable. Eurotransplant makes better use of the older donor kidneys probably by encouraging participation in the Eurotransplant Senior Program, in which over age 65 donor kidneys are preferentially allocated to over age 65 recipients. (Am J Transpl. 2002; 2: 664- 670.)



PEDIATRIC CARDIAC TRANSPLANTATION AND POST-TRANSPLANT COMPLICATIONS

Abstract# 945

INFANTS BECOME SENSITIZED TO DONOR HLA ANTIGENS BUT NOT TOLERISED TO INCOMPATIBLE DONOR ABO ANTIGENS FOLLOWING IMPLANTATION OF TISSUE ALLOGRAFTS WITH THE NORWOOD PROCEDURE FOR SINGLE VENTRICLE PALLIATION. Natalia E. Lobach,[1] Lisa Hornberger,[1] Jeffrey F. Smallhorn,[1] Anne I. Dipchand,[1] Neal denHollander,[2] Glen VanArsdell,[1] Stacey M. Pollock-BarZiv,[1] Lori J. West.[1] *[1]Pediatrics, Hospital for Sick Children, Toronto, ON, Canada; [2]Regional Histocompatibility Lab, Toronto General Hospital, Toronto, ON, Canada.*

Background: In heart transplantation, pre-transplant HLA-sensitization increases risk of antibody-mediated rejection and other post-transplant problems. Tissue allografts (homografts) used for aortic arch reconstruction and blood products used in the Norwood procedure may cause HLA sensitization in infants, some of whom will need subsequent heart transplantation. This study aimed to determine the infant immune response to tissue allograft placement. **Methods:** In this cross-sectional analysis, serum from patients who underwent the Norwood procedure in infancy (n=11) were tested post-surgery and compared with control patients who received blood products during infant cardiac surgery without allograft placement (n=4). HLA sensitization was detected using Panel Reactive Antibody screening tests (PRA) and ELISA assays to detect antibody to HLA Class I & II antigens. Development of anti-blood group antibodies (isoagglutinins) was also investigated in patients' serum by reverse blood typing. **Results:** Median age at surgery was 6 days (0-62d) in allograft recipients, and 9 days (0-41d) in controls. Median age at testing was 10 months (4mo-4yrs) in allograft recipients and 4 years (2-6yrs) in controls. 10/11 (91%) allograft recipients were sensitized (PRA≥10%), with 82% highly sensitized (PRA≥4/12); no control patients were sensitized. All allograft recipients with elevated PRA showed positive ELISA to HLA Class I & II antigens. Two allograft recipients have undergone subsequent heart transplantation. Their HLA antibodies were shown in antibody-specificity assays to be directed against the HLA type of their homograft donors and cross-reactive with their heart graft donor. Anti-blood group antibodies developed normally, even in patients whose tissue allografts were from ABO-incompatible donors (n=4). **Conclusions:** HLA sensitization develops in infants following tissue allograft placement, but not after exposure to blood products during cardiac surgery. ABO incompatible allografts did not affect normal development of isoagglutinins. These results show divergent effects on the infant immune system by exposure to T-dependent vs T-independent antigens, and have important implications for infants eventually needing heart transplantation after Norwood palliative surgery.

Abstract# 946

LATE ACUTE CARDIAC REJECTIONS IN PEDIATRIC HEART TRANSPLANTATION: INCIDENCE AND IMPACT ON LONG-TERM OUTCOME. Michael Dandel,[1] Dagmar Kemper,[1] Hans B. Lehmkuhl,[1] Rudolf Meyer,[1] Christoph Knosalla,[1] Onnen Grauhan,[1] Manfred Hummel,[1] Roland Hetzer.[1] *[1]Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, Berlin, Germany.*

Previously we showed that acute rejection (AR) surveillance by intramyocardial electrogram (IMEG) recordings, make routine biopsies in children unnecessary and reduce their mortality due to ARs early after heart transplantation (HTx) close to zero. Due to the lower incidence of late ARs and their usually harmless course, IMEG recordings were discontinued after the second post-transplant year. However, the impact of late ARs on the long-term outcome, especially in children, is controversial. Therefore, we reviewed the late ARs in our pediatric patients to evaluate their clinical relevance. **Methods:** All children transplanted between 1986-1999 with post-HTx time >2 years

were analysed to evaluate prevalence and severity of late ARs occurring after we discontinued IMEG monitoring. Attention was also focused on the temporal relationship between late ARs and the new appearance or aggravation of transplant coronary arteriopathy (TCA).

Results: Of the 68 patients included in the study (age 8.3± 5.4 years at HTx), 22 (32.4%) showed between 1 and 5 biopsy proven and clinically relevant late ARs, which occurred for the first time at 5.0± 3.0 years post-HTx. Of all reviewed patients, 16 (23.5%) died during the study period. Of these deaths, 7 (43.7%) occurred during AR, 3 (18.8 %) were sudden deaths shortly (2-6 weeks) after an AR episode, 3 (18.8%) were related to severe TCA and 1 (6.2%) to infection. In 2 patients (12.5%) the cause of death was unknown. Of the totally 10 patients who died in connection with biopsy-proven AR, 6 also had TCA that developed after the second post-HTx year. In 11 patients with relevant late ARs, but without TCA during their first episode of late AR diagnosed at 4.6± 3.0 years after HTx, the angiogram showed significant TCA lesions at 2.4± 1.3 years after their first late AR episode. The mean number of late ARs/patient/year was higher in those with angiographic TCA that developed after the second post-HTx year than in those without TCA after more than 2 years since HTx (p<0.01).

Conclusions: ARs which occurred beyond the second post-HTx year are the major cause of acute and chronic allograft dysfunction in children later after HTx and are also linked to the development of TCA. Late ARs and TCI are the dominant cause of death after the second post-HTx year in this less compliant age group and thus more careful rejection surveillance also late after HTx is justified to improve the long-term outcome.

Abstract# 947

INFLUENCE OF PRE- AND POST-TRANSPLANT ELISA-DETECTED ANTI-HLA ANTIBODIES ON PEDIATRIC TRANSPLANT OUTCOME. Sylvie Di Filippo,[1] Steven A. Webber,[2] Alin Girnita,[1] Sabrina Tsao,[2] Gerard J. Boyle,[2] Susan A. Miller,[2] Sanjiv K. Gandhi,[2] Adriana Zeevi.[1] *[1]Transplant Pathology, Biomedical Science Tower, Pittsburgh, PA; [2]Pediatric Cardiology, Childrens Hospital of Pittsburgh, Pittsburgh, PA.*

Background: Previous studies showed that ELISA is a sensitive method for detection of antiHLA antibodies.

Aims: In this study, we investigated allosensitization after pediatric heart transplantation (Tx) to determine whether the presence of ELISA-detected antiHLA antibodies (ab) pre- and/or post-transplant, correlate with acute and chronic rejection.

Material and methods: 45 patients, who had serial ELISA pre- and post-Tx were studied. Age at Tx was 8 ± 7 years. Acute rejection (AR) was defined as ISHLT grade > 2. Patients were defined as rejectors (group A= 22 cases) if they had recurrent AR (more than 2 episodes), steroid-resistant AR or graft dysfunction within the first year post-Tx; the other cases (group B= 23) were defined as non-rejectors. Eight developed graft coronary artery disease (CAD). All had at least 2 determinations for post-transplant alloantibodies. Overall 229 samples were analyzed.

Results: Twenty-two of 45 had pre- and/or postTx antiHLA-ab: 77% in group A (17/22) and only 22% in B (5/23), p= 0.0002. PreTx HLA-ab were present in 12 cases (27%): 8 class I+II and 4 class I antiHLA-ab. Presensitization was more frequent in group A (11/ 22= 50%) than in group B (1/ 23= 4%, p= 0.0005). Thirteen cases retained (8 cases) or developed (5 cases) antiHLA-ab during the first year postTx: 10 in group A (50%) and 3 in group B (13%), p= 0.0008. Late after Tx (>1year), antiHLA-ab were present in 8/18 (44%) in group A and 3/22 (13.6%) in group B (p= 0.03). Eleven exhibited de novo antiHLA-ab (6 class I+II, 4 class I and 1 class II): 7/22 in group A (32%) and 4/23 in B (17%, p= 0.0004). Four of 8 cases with CAD (50%) had preformed antiHLA-ab (3 retained post-Tx) compared to 8 of 37 without CAD (25.6%); the small sample size limiting the power of statistical analysis (p= 0.09).

Conclusion: Preformed, persistent and de novo ELISA-detected antiHLA-ab were correlated with first-year acute rejection profile; further investigations with larger number of patients are needed to determine correlation with graft coronary disease.

Abstract# 948

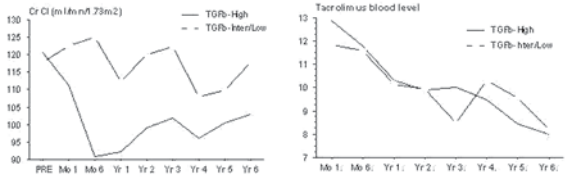
GROWTH FACTOR GENE POLYMORPHISMS AND RENAL FUNCTION IN PEDIATRIC HEART TRANSPLANTATION. Sylvie Di Filippo,[1] Adriana Zeevi,[1] Anat Tambur,[2] Robert Ferrel,[3] Gil Burkart,[4] Kevin McDade,[1] Gerard J. Boyle,[5] Susan A. Miller,[5] Sanjiv K. Gandhi,[5] Steven A. Webber.[5] *[1]Transplant Pathology, Biomedical Science Tower UPMC, Pittsburgh, PA; [2]Pathology, Rush Medical Center, Chicago, IL; [3]School of Public Health, University of Pittsburgh, Pittsburgh, PA; [4]University of South California, Los Angeles, CA; [5]Cardiology, Childrens Hospital of Pittsburgh, Pittsburgh, PA.*

Aim: The aim of this study was to determine whether growth factor and ACE gene polymorphisms are associated with renal outcome in pediatric heart recipients.

Material. Methods: 89 pts underwent 1st heart transplant (Tx) at the age of 7.4 ± 6.9 years, under tacrolimus-based immunosuppression and were followed for at least 1 year postTx (6.7 ± 3.2 years). Renal function was assessed by Schwarz formula-calculated Creatinine Clearance (CrCl ml/mn/1.73m2), preTx, at 1 mo, 6 mo, 1 year, and yearly up to 7 years. Impaired renal function was defined as CrCl < 80 ml/mn/1.73m2. We evaluated genetic polymorphisms of TGFβ1 (codon 10 and 25), VEGF (-2578 AC), PDGF (+1135 AC) and ACE (I/ID/DD) using SSP-PCR method.

Results: Mean CrCl decreased from 120±53 preTx, to 98±40, 96±37, 102±30 and 101±38 at respectively 6 mo, 1 year, 5 years (58 pts) and 7 years (33 pts). TGFβ1 high producers had worse renal function than intermediate/low producers at every postTx time point, despite similar preTx CrCl (preTx= 120±53 vs 118±55 (p= 0.8), at 1-year= 92±38 vs 113±30 (p= 0.03) and similar tacrolimus blood levels (figure). TGFβ1 high-expression was more frequent in patients with 1-year postTx CrCl< 80: 94% vs 68% in low and intermediate-producers, p= 0.004. No statistical difference was found with the other polymorphisms; however, allele AA for VEGF tended to be associated with impaired renal function (1-year CrCl = 85±30 vs 104±36 (p= 0.07); there was a trend to late renal impairement associated with allele I for ACE gene (II and ID): 6-year CrCl= 99±26 with II/ID, vs 115±26 (p= 0.07) with DD.

Conclusion: This study shows that TGFβ1 high-expression is associated with renal dysfunction in pediatric heart recipients; further studies are needed to determine ACE and VEGF impact on renal outcome.

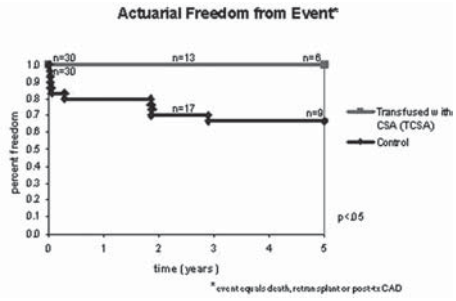


Abstract# 949

NEGATIVE IMMUNIZATION PRIOR TO INFANT HEART TRANSPLANTATION: RBC TRANSFUSION UNDER CYCLOSPORINE COVER. Mark M. Bouck,[1] Biagio A. Pietra,[1] Erin Kunz,[1] Christine Mashburn,[1] D. Dunbar Ivy,[1] Max B. Mitchell,[1] David N. Campbell.[1] *[1]Pediatric Cardiology, UCHSC/TCH, Denver, CO.*

Transfusion of red blood cells (RBC) during the administration of calcineurin inhibitors has been shown to enhance regulatory T-cell populations to exogenous antigens. Infants with complex congenital heart disease who have prolonged waiting for heart transplantation frequently require transfusion for hemodynamic stability. We reviewed the post-transplant clinical results of infants who were transfused pre-transplant with random donor RBCs while also receiving cyclosporine (TCSA) and compared the results to control infants without transfusion. Two doses of cyclosporine were administered orally (2 mg/kg every 12 hrs) prior to and post transfusion. Thirty infants were in the TCSA group and 30 infants in the control group. Infants in the TCSA group received 1.6 ± 1 (range 1 - 6) transfusions with cyclosporine while awaiting trasplant. The groups were contemporaneous with similar induction protocol and followup to 3.6 ± 2.8 vs 2.6 ± 2.3 years (p=ns). The mean age at transplant was 2.4 months in control and 3.9 months in TCSA and wait time was longer in TCSA group (108 vs 60 days) (p<0.05). Infants were followed identically. Ninety percent of TCSA group were on cyclosporine as the only immunosuppressant by 1 year post transplant versus 80% for the control group. Freedom from rejection and rejection frequency (0.63 vs 0.33 episodes/patient) was less in TCSA infants. No untoward events related to transfusion were seen. PTLD was not seen in either group. There was no difference in incidence of infections. A composite end point of death, retransplantation or coronary vasculopathy was compared as shown in the figure. TCSA group had 100% freedom from event to 5 years which was significantly different from control.

We conclude that pre-transplant transfusion with cyclosporine coverage was safe and associated with marked improvement in clinical outcome post infant heart transplantation.



Abstract# 950

SIROLIMUS CAUSES TESTOSTERONE DEFICIENCY IN MALE HEART TRANSPLANT RECIPIENTS. Ingo Kaczmarek,[1] Ioannis Adamidis,[1] Bruno Meiser,[1] Peter Landwehr,[1] Jan Groetzner,[1] Markus Mueller,[1] Peter Ueberfuhr,[1] Bruno Reichart.[1] *[1]Cardiac Surgery, Ludwig-Maximilians-University, Klinikum Grosshadern, Munich, Germany.*

Background: Sirolimus is an immunosuppressive agent of increasing importance for prevention of acute and chronic allograft rejection in organ transplantation. We investigated the impact of sirolimus on hormone levels involved in the hypothalamus-pituitary-gonad axis in male heart transplant recipients.

Methods: A pair-matched analysis with 132 male heart transplant recipients being either on a sirolimus based- or calcineurin inhibitor based immunosuppression was performed. Matching criteria were age, years after transplantation and creatinine levels. Measured parameters were testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), sexual hormone binding globulin (SHBG) and free androgen index (FAI).

Results: Mean testosterone was 3.86 ± 1.41 ng/ml in the sirolimus group and 4.55 ± 1.94 ng/ml in the control (p = 0.025). Serum LH was 12.82 ± 21.19 mlU/ml in sirolimus patients and 6.2 ± 5.25 mlU/ml in the control (p = 0.015). FSH levels were 13.31 ± 18.4 mlU/ml versus 7.32 ± 5.53 mlU/ml, respectively (p = 0.015). The analysis revealed a significant decrease in testosterone and a significant increase in FSH and LH in the sirolimus group. The duration of sirolimus treatment correlated positive with SHBG (p < 0.01), LH (p<0.05) and FSH (p<0.05) and negative with the FAI (p<0.05). Sirolimus trough levels correlated with LH and FSH levels (p<0.01).

Conclusion: Our study demonstrates that heart transplant recipients treated with sirolimus revealed significantly lower testosterone levels and a significant increase in gonadotropic hormones. These effects were trough level dependent. All candidates awaiting organ transplantation should be informed about these adverse effects.

Abstract# 951

SUBCLINICAL INFLAMMATION AND PROTHROMBOTIC STATE IN STABLE LONG-TERM HEART TRANSPLANT RECIPIENTS WITH TREATED BUT PERSISTENT MILD DYSLIPIDEMIA. Michel White,[1] Haissam Haddad,[2] Jacques Genest,[3] Marie Helene LeBlanc,[4] Normand Racine,[5] Peter Pflugfelder,[6] Nadia Giannetti,[7] Ross Davies,[8] Eduardo Azevedo,[9] Debra Isaac,[10] Jeffrey Burton,[11] Ralph Ferguson,[12] Heather Ross.[13] *[1]Research Center, Montreal Heart Institute, Montreal, QC, Canada; [2]Research Center, QE II Health Sciences Centre, Halifax, NS, Canada; [3]Division of Cardiology, McGill University Health Center/Royal Victoria Hospital, Montreal, QC, Canada; [4]Hopital Laval, Ste Foy, QC, Canada; [5]Montreal Heart Institute, Montreal, QC, Canada; [6]London Health Science Centre, London, ON, Canada; [7]Royal Victoria Hospital, Montreal, QC, Canada; [8]The University of Ottawa Heart Institute, Ottawa, ON, Canada; [9]Section of Cardiology, Health Sciences Centre, Winnipeg, MB, Canada; [10]Foothills Medical Centre, Calgary, AB, Canada; [11]University of Alberta Hospitals, Edmonton, AB, Canada; [12]Fujisawa Canada Inc., ON, Canada; [13]The Toronto Hospital, Toronto, ON, Canada.*

Background: Cardiac allograft vasculopathy, and accelerated atherosclerosis are significant causes of morbidity and mortality in heart transplant recipients. We reported that despite the use of lipid lowering agents, stable cardiac recipients may nevertheless exhibit persistent dyslipidemia not satisfying the current guidelines for high-risk patients.

Objectives: To investigate the changes in hemostatic and inflammatory parameters, homocysteine, and adhesion molecules in a large cohort of stable dyslipidemic heart transplant recipients treated with Neoral. The observations from transplant patients were compared with dyslipidemic but otherwise healthy control subjects.

Methods: One hundred twenty-nine stable heart transplant recipients aged 56.7±10.1 years, 78±42 months post-cardiac transplantation, had blood drawn for lipid profiles, C-reactive protein, Lp(a), homocysteine, ICAM, and hemostatic parameters. The observations were compared with 26 age- and sex-matched healthy dyslipidemic subjects taking no medications and presenting no concomitant medical conditions.

Results: (See Table).

Conclusions: Compared with healthy subjects with similar LDL-levels, stable heart transplant recipients exhibit a biochemical profile consistent with subclinical inflammation. Such abnormalities may contribute to accelerate the atherosclerotic process, and may play a role in cardiac allograft vasculopathy following cardiac transplantation.

	LDL-C (mmol/L)	Lp(a) (mg/L)	Fibrinogen (g/L)	ICAM (ng/mL)	CRP (mg/L)	Hcys (µmol/L)	Factor VIII (U/mL)
Transplants (n=129)	3.11±0.78	510*±511	4.46*±1.12	550*±167	4.11*±6.25	19.2*±8.8	2.63*±0.98
Controls (n=26)	3.21±0.59	230±248	2.97±0.76	328±113	2.10±2.22	9.7±2.5	1.40±0.59
P value	0.2087	0.0125	<0.0001	<0.0001	0.0195	<0.0001	<0.0001

Lp(a)=lipoprotein (a), ICAM=inter-cellular adhesion molecules, CRP=high sensitivity, C-reactive protein, Hcys=homocysteine.

Abstract# 952

ACTIVATION OF PURINE NUCLEOTIDE SALVAGE PATHWAY IN MONONUCLEAR CELLS OF CARDIAC TRANSPLANT RECIPIENTS TREATED WITH MYCOPHENOLATE MOFETIL. Elena Devyatko,[1] Andreas Zuckermann,[1] Daniela Dunkler,[1] Ernst Wolner,[1] Michael Grimm,[1] Guenter Weigel.[1] *[1]Department of Cardio-Thoracic Surgery, University of Vienna, Vienna, Austria.*

Background: The objective of the present study was to investigate purine nucleotide metabolism in peripheral blood mononuclear cells (PBMC) of cardiac transplant recipients after switch to mycophenolate mofetil (MMF) therapy.

Methods: Twenty-seven stable heart transplant recipients were switched from azathioprine to MMF 7.1±3.9 years after transplantation. Blood samples were collected before, 3, 6, and 12 months after onset of MMF therapy.

Intracellular concentrations of guanosine 5'triphosphate (GTP) and adenosine 5'triphosphate (ATP) in PBMC were determined by means of HPLC. Inosine monophosphate dehydrogenase (IMPDH) activity was detected by measuring the conversion of inosine monophosphate (IMP) to xanthosine monophosphate and guanosine monophosphate (GMP) in PBMC.

The activities of the salvage pathway enzymes guanine phosphoribosyltransferase (GPRT) and hypoxanthine phosphoribosyltransferase (HPRT) were determined by measuring the formation of GMP from guanine (for GPRT) and IMP from hypoxanthine (for HPRT).

Results: A significant decline of IMPDH activity was observed 3 and 6 months after switch to MMF with 48.5% (p=.001) and 18.6% of enzyme activity (p<.0001) compared to initial value. Twelve months after onset of MMF-therapy activity of IMPDH was partially restored to 48% (p<.0001) compared to 6 months value, but still remained significantly lower, than initial rate.

Intracellular GTP and ATP levels did not change significantly during the entire observation period. To explain this unexpected finding we investigated the activities of the salvage pathway enzymes GPRT and HPRT. The activity of GPRT increased from 7.06 nmol/10^6PBMC before MMF to 12.03 nmol/10^6PBMC (p<.0001), 11.15 nmol/10^6 PBMC(p<.0001) and 9.44 nmol/10^6PBMC (p=.006) after 3, 6, and 12 months of MMF therapy, respectively. HPRT activity was also elevated (p=.003) 6 months after onset of MMF.

Conclusion: Significant decrease of IMPDH activity in PBMC of stable cardiac transplant recipients was demonstrated 3 and 6 months after onset of MMF therapy with partial restoration after 12 months.

Unexpectedly, intracellular GTP levels were not affected during the observation period. We showed for the first time that MMF therapy induces the activation of purine salvage pathway in PBMC which accounts for the maintenance of intracellular purine nucleotide pools.

Abstract# 953

BK VIRAL REACTIVATION IN CARDIAC TRANSPLANT PATIENTS: A DOUBLE-HIT HYPOTHESIS? Shona S. Pendse,[1] Eric Knight,[1] Emilio Ramos,[2] Tania T. Von Visger,[1] Anil K. Chandraker.[1] *[1]Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA; [2]Department of Transplantation, University of Maryland School of Medicine, Baltimore, MD.*

BK nephropathy has emerged in recent years as a significant cause of renal dysfunction in renal allograft recipients. It is currently the most common viral disease affecting renal allografts, with detectable viruria in 10 to 60% of recipients in the post-transplant period. Without treatment, progression to allograft failure can be seen in up to 45% of all patients. The pathogenesis of nephropathy in these patients, however, remains poorly defined.

Renal failure in cardiac transplant patients is common, and has been attributed to a variety of causes. The question as to whether renal disease associated with BK virus plays a role in the renal dysfunction seen in cardiac transplant patients remains to be answered. There is limited data available on the incidence of BKV reactivation in the setting of non-renal solid organ transplantation. Given the correlation of BK infection with the potency of immunosuppression, and the fact that cardiac transplant patients are subjected to high levels of immunosuppression, one expects to see a high level of reactivation in this setting.

This is the first study to prospectively examine the prevalence of BK viral reactivation in the setting of cardiac transplantation. We preformed a cross-sectional analysis of 111 cardiac transplant patients and found decoy cells in 28 patients (25%). Of these, we have thus far tested 16 for the presence of BK viral DNA in the blood and urine by PCR-based assay, and of these, 10 patients have evidence of BK viral DNA in the urine. None of these patients, however, have evidence of BK viremia. The mean serum creatinine of patients with and without BK viruria was 2.01 and 1.71, respectively, with a p-value of 0.31. Mean levels of age, gender, pre-transplant creatinine, cardiopulmonary bypass time, and ischemic time were not significantly different between the two groups.

From these findings we conclude that there is evidence of BKV reactivation in the setting of cardiac transplantation, at a percentage which is similar to that seen in renal allograft recipients. However, it remains latent and does not appear to be a cause of renal dysfunction in these patients. Furthermore, none of these patients had evidence of

viremia, which is noticeably different from findings in renal transplant recipients. Thus, even in the setting of established BK viruria, immunosuppression alone is insufficient to cause BK viremia and nephropathy, and a second, organ-specific hit is necessary, such as kidney inflammation/ischemia or donor-recipient HLA mismatch.

Pediatric Liver and Intestinal Transplantation

Abstract# 954

BILIARY ATRESIA: CURRENT PRACTICES AND OUTCOMES. John C. Magee,[1,2] Tempie E. Hulbert-Shearon,[1,2] M. James Lopez,[1] Friedrich K. Port,[2] Robert M. Merion.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI.*

Management of children with biliary atresia (BA) has improved with respect to both Kasai portoenterostomy and transplantation. We examined if changes in practice have led to improved outcomes. **METHODS:** Using SRTR/OPTN data, patients <35 years old who were listed for or were recipients of primary liver transplants from 1995 to 2002 were identified. Kaplan-Meier estimates of waitlist survival and posttransplant survival by diagnosis were calculated for those transplanted 1995-99. Cox regression models following patients for up to 1 year posttransplant were fitted to determine the risk of mortality and graft loss by graft source in BA recipients <2 years old (520 whole deceased donor [DD-W], 288 split or partial deceased [DD-S], and 279 living donor [LD]), adjusted for recipient race, ethnicity, sex, life support, status 1 at transplant, ABO compatibility, year of transplant, and center. **RESULTS:** BA accounted for 1910 of 9872 wait-listed candidates and 1547 of 5441 transplant recipients. Most BA candidates were <1 year of age (Table). The annual number and age distribution of newly registered candidates and recipients has not changed over time. However, mean age at waiting list death decreased from 5.8 years in 1996 to 2.1 years in 2002. Over two-thirds of transplant recipients with BA were <2 years of age (Table).

All Biliary Atresia	Age: <1 yr	1 yr	2-5 yrs	6-10 yrs	11-17 yrs	18-35 yrs
Listing for Transplant (%)	63.2	10.2	9.1	7.3	6.7	3.4
Transplant (%)	47.2	22.8	13.8	7.5	6.1	2.7

Unadjusted graft survival rates for BA recipients <18 years at 3 months, 1 year, and 5 years were 84.4%, 80.4%, and 74.4%, respectively. Unadjusted patient survival rates were 90.5%, 87.2%, and 83.1%, respectively. Among the 1087 biliary atresia recipients <2 years of age, adjusted analyses indicated that DD-S grafts were associated with 93% higher mortality and 62% higher graft failure risk compared to DD-W grafts ($p<0.01$ for both) during the first year. DD-S grafts were associated with 80% higher graft failure risk compared to LD grafts ($p=0.01$). Mortality risk was 47% higher for DD-S than LD, but the difference was not statistically significant (HR=1.47, $p=0.17$). There was no difference between LD and DD-W grafts for patient or graft survival ($p>0.35$ for both). **CONCLUSIONS:** Listing and transplant practices for patients with BA appear stable over the last 8 years. Age at listing and transplant is younger than generally appreciated. Waiting list mortality has been stable, but the mean age of death has declined, suggesting the youngest children remain underserved.

Abstract# 955

RENAL FUNCTION IN PEDIATRIC LIVER TRANSPLANT SURVIVORS – A REPORT FROM THE SPLIT DATABASE. Kathleen M. Campbell,[1] John C. Bucuvalas,[1] Ravinder Anand,[2] Lan Zeng,[2] The SPLIT Research Group. *[1]Pediatric Liver Care Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]The EMMES Corporation, Rockville, MD.*

Background: Acute and chronic nephrotoxicity are well recognized complications of calcineurin inhibitor use following solid organ transplantation. While single center studies have described the prevalence of renal dysfunction in pediatric liver transplant survivors, attempts to identify risk factors have been hampered by the limitations and biases associated with small populations and single centers. In an effort to validate previous observations we examined multi-center data from a population representing pediatric liver transplant recipients cared for at centers throughout North America.

Purpose: To determine the prevalence and identify the predictors of decreased glomerular filtration rate (defined as calculated GFR < 80 cc/min/1.73m²) in children 3 years post-liver transplantation (LT).

Methods: We queried the SPLIT database to determine the prevalence of GFR < 80 in a population of > 200 pediatric patients at 3 years post-LT. The primary outcome was GFR calculated using the Schwartz formula (cGFR). Independent variables were primary diagnosis, age at LT, race, sex, type of insurance, organ type, PELD score, height Z-score at LT, length of hospitalization and primary immunosuppression. We performed univariate analysis to identify predictors of a cGFR < 80 at 3 years post-LT.

Results: The median age at LT was 1.8 years, with 37.3% of patients transplanted at < 1 year of age. 97.1% of patients received calcineurin inhibitors as primary immunosuppression (51.9% cyclosporine (CSA), 45.2% tacrolimus (TAC)). At 3 years post-LT, 7.9% of patients had a cGFR < 80. In univariate analysis, only CSA primary immunosuppression was associated with a cGFR < 80, with an odds ratio vs. TAC of 8.4 (CI 1.89, 37.3). This association remained significant after adjustment for transplant era (odds ratio 5.6; CI 1.25, 25.1).

Conclusions: Decreased GFR is a frequent complication in pediatric LT survivors. Previous studies have suggested an increased risk of post-LT renal dysfunction associated with CSA compared to TAC. However, this association is frequently confounded by time since transplant and transplant era, which correlate strongly with CSA use. Our data, which controls for these confounding factors, supports the concept of CSA immunosuppression as a risk factor for decreased GFR at 3 years post-LT. These observations are of critical importance as we develop interventions designed to prevent progression from asymptomatic decreased GFR to symptomatic end-stage renal disease.

Abstract# 956

LAPAROSCOPIC LIVING DONOR LEFT LATERAL SECTIONECTOMY FOR PEDIATRIC LIVER TRANSPLANTATION. Daniel Cherqui,[1] Olivier Soubrane,[2] Christophe Chardot,[3] Gauthier Frederic,[3] Fagniez Pierre-Louis,[1] Houssin Didier.[2] *[1]Dept of Surgery, Henri Mondor Hospital, Paris, France; [2]Dept of Surgery, Cochin Hospital, Paris, France; [3]Dept of Pediatric Surgery, Bicetre Hospital, Paris, France.*

We reported the first two cases of laparoscopic harvesting of left lateral liver grafts (Lancet 2002; 359: 392–96). The aim of this study is to report our 2-year experience with this technique.

Methods : From 2001 to 2003, 7 donors underwent laparoscopic left lateral sectionectomy for transplantation in their children. There were 4 mothers and 3 fathers aged 19-37 years (mean 28). Surgical technique included 5 port-laparoscopy (2-12 mm, 1-10mm and 2-5mm) with CO2 pneumoperitoneum, dissection of the left portal pedicle and liver transection without clamping. Grafts were retrieved in a bag through an 8-cm supra-pubic incision. The recipients were 2 girls and 5 boys, with a mean age of 15 months (10-19) and a mean weight of 9.3 kg (7-11). Indication was biliary atresia with previous hepatoportoenterostomy in 6 cases and metabolic disease in 1.

Donors results : Mean operative time was 4.5 hours (4-6) and mean warm ischemia time was 12 minutes (10-15). There was one intraoperative complication due to hemorrhage from the left portal branch which was immediately sutured (case 4) but conversion to laparotomy was undertaken because of suspected stenosis of the left portal vein. There were no other conversions or intraoperative complications. There was one postoperative complication consisting in a pelvic hematoma from the extraction incision, which required no treatment. No patient was transfused intra or postoperatively. Mean hospital stay was 6.2 days (4-10).

Recipients results : All grafts were transplanted and functionned immediately. There were two arterial thromboses. One was asymptomatic (routine Doppler US finding), while the other one was associated with poor portal flow leading to the child's death. One child with cholangitis had a percutaneous biliary drain placed. All children but one are alive with a functionning graft.

Conclusion : This report demonstrates the feasibility and safety of living donor left lateral sectionectomy. The rate of arterial thrombosis in the recipient requires further evaluation.

Abstract# 957

OUTCOME OF ABO INCOMPATIBLE LIVER GRAFTS IN CHILDREN. Sue V. McDiarmid,[1] Ravinder Anand,[2] SPLIT Research Group.[2] *[1]Pediatrics and Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]The EMMES Corporation, Rockville, MD.*

ABO incompatible liver transplantation (LT) is associated with lower patient and graft survivals compared to ABO identical or compatible grafts. We have examined the effect of ABO match on outcome after LT in children enrolled in the Studies of Pediatric Liver Transplantation (SPLIT) - a consortium of 39 pediatric LT centers in the US and Canada. RESULTS: 46 ABO incompatible grafts were transplanted into 45 of 1491children. Of recipients of an incompatible graft, 78.3% were blood type O, 17.4% type B, and 4.3% type A. The blood type of incompatible donors was A in 71.7%, AB in 10.9% and B in 17.4%. Recipient age was <1year in 38%. The most common diagnoses were fulminant liver failure (33.3%) and biliary atresia (24.4%). 13 children had a prior transplant. 55.6% received a whole organ graft. 68.9% were in the ICU at LT, 20% were not hospitalized. The PELD score was ≥20 in 55.6%, but <10 in 11.1%. The # of ABO incompatible LTs has decreased by half comparing 1996 to 2002. Actual patient and graft survival was 60% and 51.1% respectively. There was no significant difference in patient or graft survival for recipients of ABO incompatible grafts for children <1 year, 1-5 years or > 5 years of age, or for children with fulminant liver failure compared to those with biliary atresia. Of the 18 children who died, 8 died within 30 days of LT. Kaplan-Meier analyses of time to death and graft loss for recipients of ABO identical compared to incompatible grafts showed a highly significant difference as early as 3 months after first LT: graft survival 63.8% and 88.7 % respectively, $p<0.0003$. Table 1 shows the risk ratios for death and graft loss for blood type match from a Cox-Regression model (first transplant). 13% of ABO incompatible graft recipients received mono/polyclonal antibody induction therapy and 28.9% had at least 1 episode of rejection. CONCLUSIONS: ABO incompatible LT for children is associated with a significantly lower patient and graft survival, implying this procedure should be performed in only highly urgent patients. Surprisingly in this database only 25 of 45 ABO incompatible recipients had a PELD score >20.

Death and Graft Loss Risk Ratios Comparing Blood type Match

Comparison		Outcome:Death		Outcome: Graft Loss	
		Relative Risk	p-value	Relative Risk	p-value
Identical N=1083	Incompatible N= 32	0.32	0.003	0.37	0.0003
Compatible N=203	Identical	1.75	0.003	1.40	0.035
Compatible	Incompatible	0.56	0.097	0.51	0.028

Abstract# 958

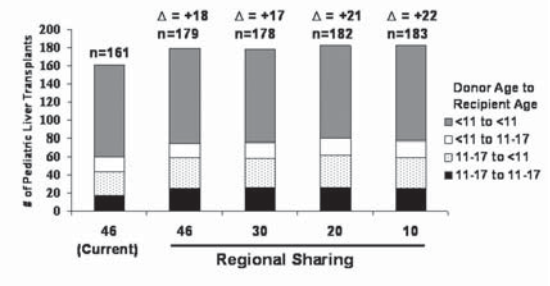
SIMULATION MODELING OF REGIONAL SHARING IN PEDIATRIC DONOR LIVER ALLOCATION. Nathan P. Goodrich,[1] Keith P. McCullough,[1] Sue McDiarmid,[2] Ruth A. McDonald,[3] Ann M. Rodgers,[1] William E. Harmon,[4] John C. Magee,[1,5] Robert M. Merion.[1,5] *[1]SRTR/URREA, Ann Arbor, MI; [2]Department of Pediatrics, UCLA Medical Center, Los Angeles, CA; [3]Children's Hospital, Seattle, WA; [4]Division of Nephrology, Children's Hospital Boston, Boston, MA; [5]University of Michigan, Ann Arbor, MI.*

Background: Under current policy, pediatric deceased donor livers are preferentially allocated to high risk (PELD >46; >50% 3-month mortality) pediatric candidates in the local OPO area. Very few pediatric candidates have high PELD scores in any given OPO. Thus, lowering the PELD threshold alone may not result in many additional pediatric donor to pediatric recipient transplants (PED-PED LTx). Regional sharing may be required to achieve this goal.

Methods: We used the Liver Simulated Allocation Model (LSAM) to study effects of regional and PELD-threshold allocation on PED-PED LTx compared to current policy. Data from 22,323 waitlist candidates and 2,719 deceased donor livers available between 4/1/02 and 9/30/02 were included in the simulation. PELD thresholds (46, 30, 20, 10) were tested with regional sharing. After status 1, LSAM rules offered pediatric livers first to pediatric candidates on the regional list above the threshold, then to adults with MELD >32 (>50% 3-month mortality) in the region, then to children below the threshold, and finally to adults with MELD <32.

Results: Compared to current policy, regional allocation of pediatric donor livers using the existing PELD 46 threshold increased the predicted 6-month number of PED-PED LTx by a mean of 18 (11%) (average of 10 LSAM runs) and was associated with 1 less pediatric waitlist death (Figure). Under regional sharing, lowering the PELD threshold to 10 resulted in 4 additional PED-PED LTx (2%) and 1 more averted pediatric waitlist death.

Conclusions: Regional allocation of pediatric donor livers is predicted to increase PED-PED LTx opportunities and decrease waitlist deaths for children. There is a small incremental effect of lower PELD thresholds for regional sharing beyond that achieved by regional sharing for higher risk children.



Abstract# 959

SOURCES OF DISPARITY IN PEDIATRIC ACCESS TO TRANSPLANTATION. Susan E. Thomas,[1] Valarie B. Ashby,[1,2] Alan B. Leichtman,[1,2] William E. Harmon,[2,3] Robert M. Merion,[1,2] Friedrich K. Port,[2] Robert A. Wolfe,[1,2] John C. Magee.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI; [3]Children's Hospital of Boston, Boston, MA.*

Background: Previous studies have demonstrated disparities in access to deceased-donor (DD) renal transplantation (Tx) among waitlisted adults based upon age, gender, blood type, race, and geography. This study examines whether similar disparities exist in pediatric transplantation once waitlisted.

Methods: First DD relative transplant rates (RR-Tx) for pediatric registrants (age < 18) entering the kidney (N= 3,789), liver (N=6,195), or heart (N=3,418) waiting lists between 1995 and 2002 were calculated using Cox regression models (censored at living donor Tx, removal from the waitlist, or 9/30/03). Each model was adjusted for age, gender, diagnosis group, blood type, insurance coverage, year waitlisted, previous transfusions, general comorbid conditions at entry onto the waitlist, geography (OPO), PRA (kidney only), dialysis modality at waitlisting (kidney only), HLA antigens (kidney only), and medical urgency at waitlisting (liver and heart only).

Results: There were no statistically significant differences for any organ by race or gender. Compared to the national average, there are significant geographic differences in access to DD kidney transplantation by OPO (RR-Tx range=0.27 to 3.24; significant at p<0.05 in 35 out of 58 OPOs). In addition, blood types A and AB have higher RR-Tx than do blood types O and B for all organs. Insurance was significant for kidney transplantation only. Children with Medicaid, Medicare primary, or multiple types of insurance had better access to a transplant (RR-Tx=1.17, 1.19, 1.24 vs. ref; all p<0.05) than did children with private only or HMO only (RR-Tx=1.00 (ref), 0.88 (p=0.31)) insurance.

Conclusions: In marked contrast to data reported for adults, access of waitlisted children to kidney, liver, and heart transplantation is not influenced by race or gender. Access to pediatric kidney transplantation varies significantly by OPO, insurance and blood type. Access to pediatric liver and heart transplantation varies significantly by blood type and OPO, but not by insurance. However, the OPO effect in pediatric liver and heart transplantation is less than that reported in adults. The effect of insurance on pediatric access to kidney transplantation contrasts with that reported in the adult population where those with Medicare and Medicaid have reduced access when compared to those with private insurance.

Abstract# 960

SAFETY AND EFFICACY OF LIVE VIRAL VACCINES IN POST LIVER TRANSPLANT RECIPIENTS ON MONOTHERPAY IMMUNOSUPPRESSION. Patricia Harren, Robert S. Brown, Jean C. Emond, Steven J. Lobritto. *Pediatrics and Surgery, NY Presbyterian Hospital, New York, NY.*

Introduction: Solid organ transplant recipients have not been permitted to receive attenuated live vaccines such as measles, mumps, rubella (MMR) and varicella (Varivax) post-transplantation. Concerns are that immunosuppression will predispose the patients to contracting a severe viral illness from the vaccines and that the vaccines will not result in active immunization. This common practice predisposes the recipients to contracting the wild-type virus throughout their lives. We report our experience with live virus vaccination in liver transplant recipients on monotherapy immunosuppression (cyclosporine or tacrolimus) for at least 3 months. We assessed adverse reactions to vaccination and serologic response rates.

Methods: We retrospectively reviewed 59 pediatric liver transplants at our center between Jan 1998 and June 2002. Ages ranged from 16 days to 16 years at transplantation. The average vaccination time from transplant was 30 months. Immunization records and viral serologies were gathered. Eleven patients had been vaccinated prior to transplant. Three patients died prior to any live vaccines. No data was found on 5 patients. Data reported on the remaining 40 patients. At the time of analysis 40 patients had received MMR and 36 had received Varivax. Complete titer information was collected on 33 patients.

Results: When vaccinated, 17/40 patients were on tacrolimus with a mean trough level of 5.2 ng/ml and 23/40 were on cyclosporine with a mean trough of 182 ng/ml. Four of 40 patients (10%) after MMR reported fever > 102°F and rash (3-14 days). All 33 patients with titer information had active immunization after MMR. Two of 36 patients (5%) after Varivax reported fever > 102°F and localized rash at the injection site (1-7 days). Five of 33 patients (15%) with titer information needed to be revaccinated. Three of 5 patients revaccinated (60%) developed titers. Thirty-one of 33 patients (94%) with titer information had active immunization after Varivax. No serious adverse reactions requiring changes in medication or morbidity occurred.

Conclusion: Live viral vaccination after pediatric liver transplantation on calcineurin monotherapy is safe and effective. Live viral vaccination should be part of routine maintenance post-operative care in these patients to avoid missed days from school, emergency department visits for immunoglobulin post exposure, and aggressive disease from wild-type viral infection. Vaccination practices in other solid organ recipients should be reviewed.

Abstract# 961

INTESTINAL TRANSPLANTATION FOR CHILDREN– SINGLE CENTER EXPERIENCE OF OVER 100 CASES. Tomoaki Kato,[1] Naveen Mittal,[2] Gennaro Selvaggi,[1] Monica Gonzalez,[1] Barbara Miller,[1] Juan Madariaga,[1] Jang Moon,[1] Seigo Nishida,[1] David Levi,[1] John Thompson,[2] Andreas Tzakis.[1] *[1]Liver and GI Transplant, University of Miami, Miami, FL; [2]Pediatric Gastroenterology, University of Miami, Miami, FL.*

Aim To describe single center experience of pediatric intestinal transplantation (Itx) over 10 years.

Methods Retrospective analysis of children who underwent Itx at our institution since August 1994. Results were compared in 4 different groups: Group 1(8/94-12/97, n=26), Group 2 (01/98-12/00, n=30), Group 3 (01/01-12/03, with no Campath-1H induction, n=38) and Group 4 (01/01-12/03, with Campath-1H induction, n=21).

Results 102 children received 115 Itx during overall study period. The median age was 17.5 months (range 6 months to 17 years). Major causes of intestinal failure were: gastroschisis (n=32), NEC (n=17), dysmotility (n=11), volvulus (n=10), intestinal atresia (n=12) Hirschsprung's disease (n=10), and microvillus inclusion disease (n=4). The types of graft included isolated intestine (n=28), composite liver/intestine (n=23), non-composite liver/intestine (n=4), multivisceral (n=54), and multivisceral without the liver (n=6). The pancreas was included in 16 liver/intestinal grafts and kidneys in 8 multivisceral grafts. Tacrolimus was used as baseline immunosuppression in all patients. Induction with OKT3, cyclophosphamide or MMF was used in Group 1. Daclizumab was used in Group 2 and Group 3. Patients in Group 4 received Campath 1H induction.

Seventy-two (71%) had concomitant liver failure. Presence of liver failure was more common in younger children (87% in age <1.5y, vs 53% in age >1.5y, p=0.0001). Fifty patients are currently alive. Two children survived more than 8 years and additional 8

survived more than 5 years. Actuarial patient survivals at 6-month/1-year/2-year were 48%/44%/32%, 61%/54%/47%, 91%/84%/74%, and 60%/47%/47% in Group 1,2,3 and 4, respectively. Incidences of severe rejection were 36%, 23%, 11% and 0% in Group 1, 2, 3 and 4, respectively. Nine patients (9%)developed PTLD; two died, three required re-transplant and the remaining treated successfully with rituximab. Recent transplant year (p=0.004) and absent of severe rejection (p=0.014) positively influenced patient survival.

Conclusions Itx provided reasonable chance of survival in children with intestinal failure and TPN related complication. Develpment of liver failure was the main indication for Itx in younger age. Patient survival improved significantly in recent years with decreased incidence of severe rejection. Effect of Campath-1H induction has yet to be determined in pediatric Itx recipients.

Abstract# 962

NEURODEVELOPMENTAL AND PSYCHOSOCIAL OUTCOMES WITH LONGITUDINAL FOLLOW-UP OF PEDIATRIC INTESTINAL TRANSPLANT RECIPIENTS. Beverly K. Park,[1] Sunny Z. Hussain,[2] Mary B. Ehmann,[3] Mary B. Hickey,[3] John Campo,[3] George V. Mazariegos,[1] Jorge Reyes.[1] *[1]Department of Transplant Surgery, Children's Hospital of Pittsburgh, Starzl Transplantation Institute, Pittsburgh, PA; [2]Division of Gastroenterology and Nutrition, Children's National Medical Center, Washington, DC; [3]Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA.*

With extended survival and improved function following pediatric intestine transplantation (ITx), evaluating neurodevelopmental outcomes is an integral dimension of post-transplant care. **Methods**: The Vineland Adaptive Behavior Scale, Child Behavior Checklist (CBCL), and Child Health Questionnaire (CHQ) were used to assess somatization, anxiety, behavior, functional impairments, physical development, social competence and daily living skills (DLS). Descriptive statistics, between group comparisons and correlations were completed. **Results**: 35 patients were enrolled (9 ITx, 23 liver-ITx, 3 MVTx); M:F 18:17; mean age 9.4 years (range 1.1-22.7); mean time post-ITx 4.54 years (range 0.25-12.5 years). Weaknesses in all domains of the Vineland were seen for ITx patients compared to the norm (p<.001) with children transplanted at ≤ 4 years of age having greater weaknesses than those > 4 years. There was a significant correlation between age at time of transplant with DLS (r=.368; p=.04) and socialization (r=.442; p=.01). Borderline and/or clinical range scores occurred in all CBCL behavior subscales except anxious/depressed and thought processes. The greatest problems were reported in the domains of school (26.3%), somatic complaints (13.3%), competence (11.8% and 17.6%), internalizing (13.3%) and activities (11.5%). Withdrawn (r=.40; p=.04), internalizing (r-.40; p=.04), and competence (r=-.53; p=.03) behaviors were significantly correlated with age at time of assessment. CHQ results revealed significant differences in the domains of Physical Functioning (p<.001), Social Limitations: Physical (p<.001), General Health Perception (p< .001), Pain (p=.04), Family Activities (p<.001) and Parental Impact: Emotional (p<.001). Improvements were seen over time at ≤ 3 years and > 3 years post-Itx. **Conclusions:** Following Itx, children have significant weaknesses in several neurodevelopmental and psychosocial domains and have greater limitations in phsyical and psychosocial functioning when compared to the norm. At greater risk are children undergoing Itx at a very young age. However, improvements are seen over time in physical functioning, family activities, parental impact, and mental health with family relationships appearing to be stronger. Early identification of deficits and supporting the child's strengths may lead to an improved QOL following ITx.

T-Reulatory Cell Function in Tolerance

Abstract# 963

LOW AVIDITY HA-1 SPECIFIC CD8+ T CELLS WITH REGULATORY PHENOTYPE (TGF-β OR IL-10) MAY BE INFLUENCED BY HA-1 MICROCHIMERISM IN THE DENDRITIC CELL SUBSET. Richard Derks,[1] Junchao Cai,[1] Junglim Lee,[1] Ewa Jankowska-Gan,[1] Jos Pool,[2] Tuna Mutis,[2] Els Goulmy,[2] William Burlingham.[1] *[1]Surgery, University of Wisconsin, Madison, WI; [2]immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands.*

Background: The generation of CD8+ T memory cells cross-reactive with alloantigens has been proposed as a major obstacle to clinical allo-tolerance after transplantation. When CD8+ cells appear in the graft, it is most often in the guise of T cytotoxic effector cells, in association with rejection. This may not always be the case. Using differential HLA-tetramer staining, we have identified a CD8+ memory T cell population with regulatory properties (CD8 TR) in a patient with over 30 years of tolerance. CD8+ memory T effector (TE) cells specific for the same minor H antigen (HA-1) but with much higher apparent avidity for cognate MHC peptide were also present in two-fold lower frequency, and could be suppressed by the TR cells. Because the HA-1 minor antigen is normally leukocyte restricted we hypothesized that HA-1 microchimerism, in particular in the DC subsets, may influence the type of regulation that develops (TGF-β or IL-10).

Methods: We have examined three renal transplant patients who have maintained stable graft function. These patients were closely matched for HLA, but mismatched for the HA-1 minor antigen and were classified as regulators by TGF-β or IL-10 cytokine neutralization using the trans-vivo DTH assay.

T cells, B cells, monocytes, and dendritic cells (CD11c+, CD123+) were separated by flow sorting and examined for microchimerism by nested PCR for HA-1. HA-1 microchimerism existed almost exclusively in the dendritic cell population. In addition, a group of six regulators and six non-regulators as determined by DTH assay where analyzed for percentages of myeloid (CD11c+) and plasmacytoid (CD123+) dendritic cells by four color flow cytometry by gating for CD3,14,19-, HLADR+ cells.

Results: Individuals with a higher percentage of plasmacytoid dendritic cells were more likely to regulate by the production of IL-10, as opposed to TGF-β. There was no difference between TGF-β regulators and non-regulators in proportion of the DC subsets.

Conclusion: The presence of HA-1 microchimerism in the dendritic cell subsets influences the phenotype of the low avidity CD8+ T regulatory cell population seen in these patients; in particular a shift towards the plasmacytoid dendritic cell population (CD123+) may predispose the patient toward IL-10 mediated regulation.

Abstract# 964

RAPID PRESENCE OF CD4+/CD45RC- REGULATORY CELLS IN TOLERATED GRAFTS: EVIDENCE FOR A PROTECTIVE ROLE IN THE TOLERATED TISSUES. M. Kawai, H. Kitade, C. Mathieu, M. Waer, J. Pirenne. *UZ Gasthuisberg, University Hospital, Leuven, Vlaams-Brabant, Belgium.*

Regulatory cells (RegC) play an important role in non-deletionnal tolerance but their phenotype remains controversial as well as the compartment (graft *versus* lymphoid tissues) where they mature, expand and operate. To study that, we developed a RegC–based model of tolerance after heart Tx in a fully mismatched RA-to-PVG rat combination *via* Donor-specific-blood-transfusion (DSBT). Adoptive transfer showed presence&expansion of RegC in spleen and lymph nodes, a phenomenon dependent upon the presence of the thymus, the graft and DSBT. By using selective adoptive transfer (MACS system), we demonstrated that RegC are exclusively CD4+/CD45RC- (similar to RegC involved in control of autoimmune diseases in rats) whereas CD4+/CD45RC- cells acted as effector cells, capable of accelerating rejection. CD4+/CD25+ and CD4+/CD25- evoked identical function. In tolerized grafts, we found rapid (day 5), progressive and sustained (day 5-to-14) infiltration by these regulatory CD4+/CD45RC- cells and a lower infiltration by effector CD4+/CD45 RC+ cells, whereas rejecting grafts displayed an exactly reverse profile (progressive predominance of CD4+/CD45RC+ cells over CD4+/CD45RC- cells). This differential profile between rejecting and tolerant rats was not as clearly seen in the spleen, suggesting that regulatory activity is concentrated in the graft. Finally, exposure of DSBT-treated rats to high-doses CsA (50mg/kg) blocked the generation of RegC (assessed by adoptive transfer), caused rejection and transformed the graft infiltrate profile from a tolerant (CD4+/CD45RC- predominant) into a rejecting one (CD4+/CD45RC+ predominant), suggesting that RegC exert their protective effect in the graft. Intragraft presence of RegC was then unequivocally proven by the observation that $1x10^7$ graft infiltrating cells (at day 14 and 30) or reTx of a tolerized graft (at day 5) could tolerize donor-specific grafts in second set naive recipients (n=6 in each experiment; p <.01=significant). Conclusions. Altogether these data demonstrate that CD4+/CD45RC- RegC are present in tolerated grafts where they protect transplanted tissues from effector CD4+/CD45RC+ cells *via* a direct intragraft mechanism, the nature of which being under investigation.

Abstract# 965

ANTI-CD28 INDUCED REGULATORY CELLS AND A DEVIATED ANTIBODY RESPONSE TO KIDNEY ALLOGRAFTS IN THE RAT. Fabienne Haspot, Celine Seveno, Flora Coulon, Marcello Hill, Karine Renaudin, Claire Usual, Jean Paul Soulillou, Bernard Vanhove. *U437, ITERT INSERM, Nantes, France.*

B7 (CD80, CD86) interaction with CD28 is essential for optimal activation of naive T cells. On the other hand, B7 can interact with CTLA-4 which inhibits T cell activation and proliferation. In addition, it was recently shown that CTLA4-Ig stimulates B7 on APC, resulting in the production of indoleamine dioxigenase (IDO), an enzyme that catabolizes tryptophan, which in turn inhibits T cell proliferation. The activation of IDO has been associated with tolerance induction in rodents. Therefore, selectively inhibiting the B7/CD28 pathway without blocking that of B7/CTLA-4 may be strongly immunosupressive and facilitate tolerance induction. In this study, we monitored the immune responses in a model of acute kidney graft rejection (LEW 1W -RT1A^{u}- to fully mismatched LEW 1A -RT1A^{a}-), after the selective inhibition of CD28/B7 interaction using the modulating anti-rat monoclonal antibody JJ319. This antibody was previously shown to prevent rejection in the F344 to LEW model of chronic rat kidney graft rejection. A short term treatment with 8 doses of 4mg/kg of JJ319 (day 0 to 7) resulted in 55% of grafts surviving long term (150>days). Treated animals had an increased alloantibody response skewed towards a Th-2 type (IgG1 and IgG2a isotypes) and specifically directed against donor MHC II molecules. This was in contrast with the antibody response of the Th1-type (IgG2b) directed against MHC I and II molecules found in rejected untreated recipients. Three to four months after transplantation, kidney graft function was normal and stable (Urea: 10 mmol, Creatinin: 39 μmol) and no signs of chronic rejection could be evidenced according to the Banff classification. In these functionally tolerant animals, PBMC and spleen cells were unable to proliferate against donor cells in mixed lymphocyte reactions but could proliferate against third party cells. The blockade of IDO (using 1D methyl tryptophan) and NO generation (using N-Methyl-L-Arginin) fully restored anti-donor reactivity. T cells purified from the same PBMC and splenocytes were fully reactive. Moreover, depletion of OX42^{+} (CD11b/c) cells did not restore proliferation. In conclusion, the selective blockade of CD28 generates regulatory mechanisms that do not involve classical regulatory T cells and also induces an anti-class II antibody response of the Th2-type. These regulatory mechanisms are associated with a normal kidney graft function in the long term without histological signs of chronic rejection.

Abstract# 966

DIRECT EVIDENCE OF ALLOREACTIVE CD8+ T CELL INHIBITION BY CD4+ T REGULATORY CELLS IN THE MAINTENANCE OF TRANSPLANTATION TOLERANCE. Yuan Zhai, Lingzhong Meng, Ronald W. Busuttil, Jerzy W. Kupiec-Weglinski. *The Dumont-UCLA Transplant Center, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Although CD4+ T regulatory cells (Treg) are critically involved in the induction and maintenance of transplantation tolerance, direct evidence of their physiological role in primary recipients (without concomitant exogenous adoptive cell transfers) remains to be elucidated. In this study, we analyzed the immunological mechanisms of allograft prolongation induced by a single dose of anti-CD154 mAb in fully MHC-mismatched recipients (Balb/c to C57BL/6 cardiac model), and focused on the interaction between CD4+ Treg and alloreactive CD8+ T cells during the transplant maintenance phase (>50 days). Donor-specific immune tolerance was established in long-term cardiac allograft recipients, as evidenced by the acceptance of donor-type (MST>30 days; n=6) but rejection of third-party (C3H, MST±SD = 12 ± 4 days; n=3) test skin grafts. In agreement with the allograft survival data, *in vivo* alloreactive CD8+ T cell activation (% of CD44^{high}CD62L^{low} in total CD8+ T cell population) induced by donor-type skin was inhibited (3.2%). However, third-party skin grafts readily induced CD8+ T cell activation (3.4%→12.3%). To determine whether anti-donor CD8+ T cells were deleted or remained under the dominant CD4+ Treg regulation, a depleting anti-CD4 mAb was administered prior to skin graft challenge. This resulted in prompt rejection not only of donor-type test skin, but also of the original cardiac grafts. Additionally, alloreactive CD8+ T cell activation was also recovered (4%→25%). To further dissect the mechanism of CD4 Treg-CD8 interaction, we used a depleting CD25 mAb or blocking CTLA-4 mAb protocol. Depletion of CD4+CD25+ cells resulted in rejection of secondary skin grafts in ca. 50% of long-term tolerant hosts (MST±SD=10±4 days; n=4). Alloreactive CD8 activation was restored (1.4%→13.2%) in rejecting, but not in non-rejecting tolerant hosts, despite CD4+CD25+ depletion. Blocking of CTLA-4 uniformly resulted in the rejection of test skin grafts (MST±SD=9±3 days, n=4) and subsequent rejection of the original cardiac grafts, in parallel with activation of alloreactive CD8+ T cells (6.3%→17.7%). In conclusion: 1) CD154 blockade did not deplete donor-alloreactive CD8+ T cells in tolerant recipients; 2) CD4+ Treg prevented activation of CD8+ T cells following secondary allogeneic test skin graft; 3) this active regulation of alloreactive CD8+ T cells by CD4+ Treg was donor-specific; 4) it was mediated by CTLA-4 signaling; 5) CD25+CD4+ Treg were only partially responsible for the dominant regulation.

Abstract# 967

LINK BETWEEN PROTOCOLS WHICH INDUCE ALLOGRAFT TOLERANCE AND EARLY AND SELECTIVE HIGH INTRAGRAFT FOXP3 EXPRESSION. Iris Lee,[1] Liqing Wang,[1] Engin Ozkaynak,[2] Wayne W. Hancock.[1] *[1]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA; [2]TolerRx, Inc., Cambridge, MA.*

Genes such CD25, CTLA-4, and GITR can be expressed by both regulatory T cells (T-reg) and other cells, and expression of these genes by even T-reg can vary with activation. By contrast, expression of the Foxp3 transcription factor is restricted to CD4+ CD25+ T-reg, appears stable irrespective of T cell activation, and is necessary for the maintenance and function of T-reg cells. We report in vivo data linking selective Foxp3 expression post-transplant (post-Tx) and specific costimulation blockade protocols which lead to long-term allograft survival and tolerance induction.

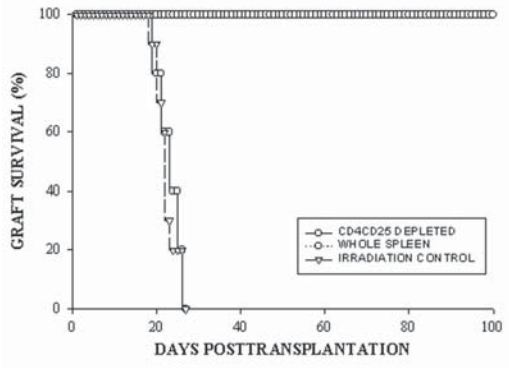
By real-time PCR, levels of Foxp3 are several hundred-fold higher in normal spleen and thymus than other tissues. Analysis of serially harvested cardiac allografts (BALB/c->B6), shows splenic Foxp3 expression decreases in a stepwise manner with developing rejection and rise within allografts; levels at day 5 post-Tx within cardiac allografts are 160-fold higher level than in isografts and, remarkably, 4-fold higher than splenic levels, indicating the migration of T-reg cells to rejecting allografts. However, Foxp3 expression in day 7 allografts harvested from recipients treated with CD154 mAb plus DST were enhanced >15-fold compared to controls or combined CD28/anti-ICOS mAb targeting. Since both costimulation blockade protocols induce permanent engraftment, comparisons at later intervals was possible; despite similar levels of intragraft T cells, only CD154/DST was linked with high Foxp3 expression and only that protocol induced actual donor-specific tolerance. Immunohistologic studies localized Foxp3 expression to infiltrating mononuclear cells post-CD154/DST. CD154/DST therapy was not accompanied by increased TGF-b, IL-10 or indoleamine 2,3 dioxygenase (IDO) expression as compared to levels in control cardiac allografts. Studies of islet allografts (BALB/c->B6) also showed levels of foxp3 expression which were 20-fold higher in conjunction with CD154/DST vs. CTLA4.Ig or combined anti-ICOS/CTLA4.Ig, and again showed no correlation with intragraft expression of TGF-b, IL-10 or IDO, despite similar T cell infiltration.

We conclude that analysis of Foxp3 expression even at early intervals post-Tx indicates key differences between varying protocols which induce long-term engraftment, as well as with rejecting allografts. Ongoing studies are directed towards assessment of (i) whether Foxp3 expression is required for tolerance induction, and (ii) Foxp3 expression in well-functioning vs. rejecting clinical transplants.

Abstract# 968

CARDIAC ALLOGRAFT TOLERANCE ESTABLISHED BY INTRATHYMIC MODULATION IN A RAT MODEL DISPARATE IN A SINGLE CLASS I MOLECULE IS MEDIATED BY CD4^{+}CD25^{+} TREG THAT EXPRESS *FOXP3*. Sadi Koksoy,[1] Esma S. Yolcu,[1,2] Haval Shirwan.[1,2] *[1]Institute for Cellular Therapeutics, University of Louisville, Louisville, KY; [2]Department of Microbiology and Immunology, University of Louisville, Louisville, KY.*

In a rat model disparate for one class I antigen RT.1A^{a}, PVG.R8 to PVG.1U, we previously showed that intrathymic modulation with donor class I allopeptides or splenocytes resulted in prolonged survival of cardiac allografts associated with chronic rejection. Prolongation correlated with the development of regulatory cells in the primary recipients that were able to prevent both acute and chronic rejection following adoptive transfer into secondary recipients. The goal of this study was to characterize these cells with particular emphasis on CD4^{+}CD25^{+} T cells. Tolerant secondary graft recipients had substantially higher percentages of CD4^{+}CD25^{+} T cells in the spleen (23 ± 3%) and blood (28 ± 6%) as compared to naïve rats (11 ± 3% and 9 ± 6%). RT-PCR experiments showed high expression of *Foxp3* in accepted heart grafts compared to no expression in acutely rejected control grafts. CD4^{+}CD25^{+} T cells inhibited donor-specific proliferation responses *in vitro*. Importantly, depletion of these cells from splenocytes of long term secondary graft survivors abrogated their ability to transfer tolerance to tertiary graft recipients.



Furthermore, tolerogenic effect both *in vitro* and *in vivo* was found to be associated with high IL-10 production. These data demonstrate that cardiac allograft tolerance, established through intrathymic immune modulation, is mediated by $CD4^+CD25^+$ Treg that express high levels of *Foxp3* and are induced by indirect allorecognition.

Abstract# 969

METASTABLE TOLERANCE IN THE KIDNEY ALLOGRAFT—PERITUBULAR TGFB(LATENT)+ GRAFT INFILTRATING CELLS INCLUDE BOTH CD4+ T AND NON-T REGULATORY CELLS AND INHIBIT THE LOCAL DTH RESPONSE. Jose Torrealba,[1] William J. Burlingham,[1] John H. Fechner,[1] Ewa Jankowska-Gan,[1] Krista Haanstra,[2] Jacqueline Wubben,[2] Margreet Jonker,[2] Stuart J. Knechtle.[1] *[1]Surgery, University of Wisconsin, Madison, WI; [2]Immunotherapy, Biomedical Primate Research Center, Rijswijk, Netherlands.*

Tolerance to kidney allografts in the Rhesus monkey after anti-CD3 immunotoxin (IT) induction therapy fit the criteria of "metastable tolerance".TGFB(latent)+ infiltrates appear in the kidney allograft between 3 mos-1 yr & correlate with resolution of low grade ARej, and with TGFB-dependent , regulated anti-donor DTH in the periphery. **Methods**: Monkeys either fully MHC mismatched (n=6), or matched for at least one Mamu-DR (n=5) with their kidney donor were induced with anti-CD3 IT , Cyclosporin or anti- CD154 mAb. We investigated by 1- and 2-color IP the subtypes of TGFB(latent)+ "regulatory" as well as "effector" cells in kidney biopsies. Using a trans-vivo DTH assay, we analyzed the function of effector & regulator graft-infiltrating cells(GIC) harvested from 2 ARej grafts using collagenase. **Results**: Graft survival of >1000 days was observed in 6/11 monkeys; 5/6 >1000 days were matched for at least 1 Mamu DR antigen , including 1that has retained its allograft since 1982 (>20 yrs!). As shown in Table 1,

TABLE 1: Immunoperoxidase Analysis of Peritubular Cell Infitrates

Staining for:	Pre-Tx Kidney(n=2)	Acute Rej, 8-17mo(n=5)	Normal/Suspicious 12-30mo(n=5)	Normal 21yr(n=1)	P value (AR v.N/S)
TGFb+	<0.1	0.85 ± 1.0	7.4 ± 2.7	12.1	<.001
TGFb+/CD4+	n.d.	0.32 ±0.25	3.4 ±2.2	1.5	<.02
CD4+	n.d.	32.8 ± 11.8	9.7 ±3.1	2.1	<.01
CD8+	n.d.	14.4 ±10.1	0.6 ±0.5	< 0.1	.05
CD68+	n.d.	12.1 ±10.0	0.7 ±0.3	0.2	.06

mean±SD pos.cells/10 tubules; n.d.=not determined

control kidneys were devoid of TGFB+ cells(<0.1 cells/10 tubules); low nos. were seen in ARej, and a 10-fold higher no.in grafts w/o rejection. Of the TGFB+ GIC, 50% co-stained for CD4 (Table 1), and were roughly equal in no. to the total CD3+ TGFβ+ GIC (not shown). DTH analysis indicated that even during loss of tolerance and onset of rejection, GIC effectors could not mediate DTH unless TGFB1 was neutralized— i.e. the small nos. of TGFβ+ GIL in the peritubular areas (mean=0.85) may retard rejection. **Conclusion**: Metastable tolerance in the Rhesus monkey kidney allograft model is balanced between TGFB(latent)+ regulatory GIC— both CD4+ T and non-T— versus effector CD4+,CD8+ and CD68+ cells. Surprisingly, TGFB(latent)+ GIC may persist for >20 yrs without causing chronic rejection.

Abstract# 970

DIRECT VISUALISATION OF INDIRECT SPECIFICITY OF $CD4^+CD25^+$ REGULATORY T CELLS FOR ALLOPEPTIDES IN VITRO AND IN VIVO. Shuiping Jiang,[1] Dela Golshayan,[1] David S. Game,[1] Robert I. Lechler.[1] *[1]Department of Immunology, Faculty of Medicine, Imperial College London, Hammersmith Hospital, London, United Kingdom.*

Naturally occurring autoreactive $CD4^+CD25^+$ regulatory T cells play a key role in the prevention of autoimmunity and appear to mediate transplantation tolerance. Although $CD4^+CD25^+$ cells are selected on MHC class II-self peptide complexes, data from several transplantation tolerance models indicate that these cells may have indirect allospecificity for donor antigens. $CD4^+CD25^+$ cells with specificity for a defined peptide antigen have not been described. Methods: In order to establish HLA-A2 (103-120) peptide-specific $CD4^+CD25^+$ regulatory T cell lines, purified peripheral blood $CD4^+CD25^+$ cells from $HLA\text{-}DR1^+A2^-$ individuals were primed with autologous dendritic cells pulsed with the A2 peptide. Results: the cell lines were potent inhibitors of proliferation and IL-2 secretion by $CD4^+CD25^-$ T cell lines specific for the same peptide. The antigen-specificity for the A2 peptide was demonstrated in suppression assays and flow cytometry analysis using a fluorescent tetramer composed of DR1:A2 (103-120) peptide complexes. About 9% of $CD4^+$tetramer$^+$ cells were seen in the $CD4^+CD25^+$ cells, while 68% of $CD4^+CD25^-$ cells were $CD4^+$tetramer$^+$, demonstrating that human $CD4^+CD25^+$ regulatory T cells with indirect allospecificity for a defined allopeptide can be selected in vitro. To extend these studies in vivo we have established similar lines from CBA mouse $CD4^+CD25^+$ cells specific for a class I K^b peptide. The indirect allospecificity of the murine lines was tested in the setting of skin transplantation. The $CD4^+CD25^+$ cells prolonged CBK (CBA transgenic for K^b) skin graft survival into CBA mice, but not third party BALB/c skin graft without the use of any other immunosuppression, suggesting that the $CD4^+CD25^+$ cells have specificity for the K^b peptide. Taken together, these data suggest that self-reactive $CD4^+CD25^+$ regulatory cells can be hijacked into allopeptide specific cells in vitro, and these cells are able to limit alloresponses in vivo, thus paving the way for using $CD4^+CD25^+$ regulatory T cells as cell therapy to promote clinical transplantation tolerance.

Abstract# 971

A ROLE FOR NATURALLY OCCURING CD4+CD25+ REGULATORY T CELLS IN SPONTANEOUS ALLOGRAFT ACCEPTANCE. Laurence A. Turka,[1] Elise Chiffoleau, Chuangqi Chen, Zihao Wu, Sigrid Sandner, Mohamed H. Sayegh.[2] *[1]Department of Medicine, University of Pennsylvania, Philadelphia; [2]Brigham and Womens Hospital, Harvard Medical School, Boston.*

We and others have shown that previously unsensitized B6 mice ($H\text{-}2^b$) fail to reject MHC class II mismatched bm12 ($H\text{-}2^{bm12}$) cardiac allografts, although they do reject bm12 skin allografts. Spontaneous cardiac graft acceptance is not a result of low alloreactive precursor frequency, as TCR transgenic mice directly reactive to $I\text{-}A^{bm12}$ (termed anti-bm12, ABM mice), in which over 95% of T cells proliferate in response to bm12 cells in vitro or in vivo, also do not reject bm12 cardiac allografts. As CD4+CD25+ regulatory T cells have been shown to play a role in maintaining self-tolerance and regulating graft rejection, we asked whether or not such cells can mediate spontaneous allograft acceptance.

To determine if regulatory T cells played a role in spontaneous allograft acceptance, B6 mice were thymectomized and depleted in vivo using the anti-CD25 mAb PC61. Mice treated in this fashion rapidly rejected bm12 cardiac allografts whereas mice thymectomized without CD25 depletion accepted bm12 cardiac allografts, as do control untreated mice. Similarly, treatment of B6 recipients with blocking anti-CTLA4 mAb induced bm12 cardiac allograft rejection.

We next studied B6 or ABM mice which were long-term (>100 days) bm12 cardiac allograft recipients. We found that T cells from these mice exhibit reduced proliferative activity (by CFSE dye dilution) and IL-2 secretion against bm12 stimulators compared with naive B6 or ABM responders. Interestingly, the in vitro T cell hyporesponsiveness of long-term cardiac allograft recipients was completely abrogated by depletion of CD25+ T cells from the responding population. Depletion of CD25+ T cells from naive mice had no effect on the anti-bm12 response, suggesting that successful long-term engraftment increased the number and/or potency of CD4+CD25+ regulatory T cells. Strikingly, and consistent with these in vitro findings, B6 mice with spontaneous long-term engraftment of bm12 hearts did not reject bm12 skin transplants, while naive B6 mice rapidly reject bm12 skin (~12-18 days).

To our knowledge, these are the first data showing that pre-existing regulatory T cells can be sufficient to promote spontaneous vascularized graft acceptance. Our results also show that during the process graft acceptance, regulatory T cells expand in number and/or potency, and this is accompanied by the de novo acquisition of skin allograft acceptance. These observations have therapeutic implications for using autologous regulatory T cells to promote transplant tolerance.

Abstract# 972

A SEROPREVALENCE STUDY OF WEST NILE VIRUS IN SOLID ORGAN TRANSPLANT RECIPIENTS. Deepali Kumar,[1] Michael Drebot,[2] Susan Wong,[3] Gillian Lim,[4] Harvey Artsob,[2] Peter Buck,[4] Victoria Edge,[4] Atul Humar.[1] *[1]Infectious Disease and Transplantation, University of Toronto, Toronto, ON, Canada; [2]National Microbiology Laboratory, Health Canada, Winnipeg, MB, Canada; [3]New York State Dept.Health, New York, NY; [4]Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa, ON, Canada.*

Background: West Nile virus causes severe neurological disease in approximately 1 in 150 cases. However, the occurrence of severe disease may be more common in immunosuppressed transplant patients. In the summer of 2002, a West Nile outbreak occurred in the Toronto, Canada area. The area has a large multi-organ transplant program. To determine the spectrum of disease and clinical impact of community acquired West Nile infection and assess public health behavior patterns in transplant recipients, we carried out a seroprevalence study in this patient population.

Methods: Patients were enrolled primarily from outpatient transplant clinics and the transplant inpatient ward. Patients who had not left hospital since transplant were excluded. Sera were initially screened for antibodies to West Nile virus by the hemagglutination inhibition (HI) test. HI reactive sera were then tested using a West Nile virus IgM ELISA and a plaque reduction neutralization test. A questionnaire was provided to patients to assess knowledge and behavior patterns with regards to West Nile virus.

Results: 855 organ transplant patients were enrolled. Type of transplant included kidney (n=419), liver (n=204), lung (n=94), heart (n=83), pancreas (n=46) and others (n=9). Median time from transplant was 50.6 months (range 2 weeks – 410 months). The seroprevalence of IgM antibody to West Nile was 6/855 (0.7%). One patient had IgG antibody alone and likely had remote infection. All 6 patients (100%) had symptomatic disease, and 5/6 (83%) had meningitis, encephalitis, and/or acute flaccid paralysis. The knowledge among patients concerning the risk of West Nile virus infection was incomplete, and behavior patterns reflected a poor rate of compliance. Only 56% knew of at least one protective measure and only 44% had acted on at least one protective measure. Only 33% of patients used insect repellant when outdoors.

Conclusions: Community acquired West Nile virus is an important threat to transplant patients. The rate of severe neurological disease is much higher than reported in the general population. Education regarding personal protection measures is critical in these patients. However, despite high public awareness of West Nile virus, and specific educational attempts by the transplant program, incomplete knowledge and poor rates of compliance were reported.

Abstract# 973

WEST NILE ENCEPHALITIS IN RECIPIENTS OF ORGAN TRANSPLANTS. Kadiyala V. Ravindra,[1] Alison G. Freifeld,[2] Andre C. Kalil,[2] David F. Mercer,[1] Wendy J. Grant,[1] Jean F. Botha,[1] Lucile E. Wrenshall,[1] R. Brian Stevens.[1,3] *[1]Department of Surgery, University of Nebraska Medical Center, Omaha, NE; [2]Department of Infectious Diseases, University of Nebraska Medical Center, Omaha, NE; [3]Department of Microbiology/Pathology, University of Nebraska Medical Center, Omaha, NE.*

Amongst the 9 patients with solid organ transplants infected with West Nile virus reported thus far in literature (see Table), 8 developed encephalitis and 2 died. This is alarming compared to the less than 1% incidence of serious neurological manifestations and 2% mortality in the general population. Over the past year, we treated 3 solid organ recipients who developed West Nile fever on long-term follow-up (see Table). They included a 44-year-old male (kidney-pancreas), a 37-year-old female (pancreas) and a 2½-year-old (living donor kidney). Their immunosuppression is detailed in below. Two of them developed serious meningoencephalitis requiring ventilator support for up to a week. The management strategy included prompt reduction of immunosuppression and supportive care. The fever subsided over a week in all patients, but the neurological recovery was slower. The child recovered to near normalcy in two weeks and was discharged home. In contrast, the adult male who developed encephalitis has significant neurological sequelae and is undergoing rehabilitation. Immunosuppression was restored upon recovery in all.

Transplant physicians, particularly in West Nile virus endemic areas, must be alive to the possibility of West Nile fever in their patients developing fever in the summer months. Prompt reduction of immunosuppression is probably the most vital step in the successful management of these patients.

West Nile Virus Infections in Recipients of Solid Organ Transplants

Citation	Years of Infection	Age/Sex	Organ	Immunosuppression (a)	Clinical Course	Outcome
N Engl J Med	2002	38/M	Kidney	FK, MMF, Steroids	encephalitis	
2003;		63/M	Kidney	FK, MMF, Steroids	encephalitis	one
348:2196		31/F	Heart	ATG, FK, Rapa, Steroids	encephalitis	death
		71/F	Liver	FK, Steroids	fever	
Am J	2000-2001	42/M	Kidney	FK, AZA, Steroids	encephalitis	alive
Transplant. 2003; 3(10):1312		76/M	Kidney	MMF, Steroids	encephalitis	alive
Transplant Infectious Disease, 2002; 4(3):160	2000-2001	42/M	Lung	FK, MMF, Steroids	encephalitis	alive
N Engl J	2002	47/M	Liver	Not Mentioned	encephalitis	alive
Med 2003; 349:1236		62/M	Kidney	Not Mentioned	encephalitis	death
Current Series	2003	44/M	Kidney/ Pancreas	FK, MMF, Steroids	encephalitis	alive
		2.5/M	Kidney	FK, MMF	encephalitis	alive
		37/F	Pancreas	CSA, MMF	fever	alive

(a) FK= Prograf; MMF=mycophenolate mofetil; AZA=Imuran; ATG=anti-thymocyte globulin; Rapa=Rapamune; CSA=Neoral

Abstract# 974

COMMUNITY-ACQUIRED WEST NILE VIRUS ENCEPHALOMYELITIS IN SOLID ORGAN AND BONE MARROW TRANSPLANT RECIPIENTS: CLINICAL, DIAGNOSTIC, AND NEUROPATHOLOGICAL FEATURES. Gregory T. Everson,[1] Brad Marder,[1] Marilyn Levi,[1] Stephen P. Laird,[1] Trevor McNutt,[1] W. John Pape,[1] Lisa Forman,[1] B. K. Kleinschmidt-DeMasters,[2] Kenneth Tyler.[2] *[1]Medicine, University of Colorado Health Sciences Center, Denver, CO; [2]Neurology, University of Colorado Health Sciences Center, Denver, CO.*

Objective: Investigate clinical, diagnostic, pathological features of West Nile Virus (WNV) infection in immunosuppressed bone marrow and solid organ transplant recipients. **Background:** In 2003, >8470 cases of WNV infection occurred in the U.S. and >2745 cases occurred in Colorado (553 meningoencephalitis (MENC), 52 deaths). During this outbreak, we encountered both MENC and acute flaccid paralysis (AFP) due to community-acquired WNV disease in our otherwise stable population of transplant recipients. **Design/Methods:** 10 transplant recipients (4 renal, 1 renal/pancreas, 2 liver, 1 lung, 1 bone marrow) were hospitalized with severe WNV infection documented by WNV IgM in CSF or serum. Clinical features, diagnostic and laboratory studies including CSF examination, neuroimaging, EEG, EMG/NCV, and neuropathology were characterized. **Results:** Estimated incidence of MENC+AFP due to WNV virus infection in Colorado was .014% for the general population of approximately 4 million, and .250% for the approximately 4000 transplant recipients in the state. All patients acquired infection in the community, none had history of recent (<3 mos) transfusions, and all received their transplants 8 mos-15 yrs prior to WNV infection and were on maintenance immunosuppression. 9 of 10 patients had MENC and 3 had associated AFP with quadriparesis. One case had AFP alone. All AFP cases required at least transient mechanical ventilation. CSF examination demonstrated pleocytosis (10/10, counts ranged between 5-5400), elevated protein (10/10), and lymphocytic predominance (9/10). Brain MRIs demonstrated abnormalities of white matter (7/8) and thalamus, basal ganglia and brainstem (3/8). EEGs were abnormal (7/7) and showed generalized slowing (7/7), triphasic slow waves (2/7), and PLEDs (2/7). 3 patients had seizures. Neuropathology of our fatal case demonstrated multifocal necrosis in thalami, s. nigra, pons, cerebellum and anterior spinal cord. Management strategies included reduction in immunosuppression and use of interferon (6), standard IV Ig (4), WNV IV Ig (2), and ribavirin (1). **Conclusions:** The 20-fold increase in MENC+AFP over the general population, indicates unique susceptibility of transplant recipients to severe WNV infection. Pathological studies indicate virus-mediated rather than inflammatory CNS injury. Effective management strategies remain to be defined.

Abstract# 975

SEVERE ACUTE RESPIRATORY SYNDROME (SARS) IN TRANSPLANTATION: CLINICAL AND VIROLOGIC FINDINGS AND IMPLEMENTATION OF A SARS SCREENING TOOL. Deepali Kumar,[1] Gabriella Farcas,[1] Karl Uy,[1] Kevin Kain,[1] Gary Levy,[1] Atul Humar.[1] *[1]Infectious Diseases and Transplantation, University of Toronto, Toronto, ON, Canada.*

Background: SARS is an emerging infection caused by a novel coronavirus (CoV). During the worldwide SARS epidemic, two transplant patients developed infection at our center. SARS posed several problems unique to transplantation. First, transplant patients with SARS may have more severe disease with greater infectivity. Second, SARS could theoretically be transmitted from a donor to a recipient. To prevent the latter, a clinical SARS donor screening tool was implemented. We report a case of SARS and provide our experience with SARS screening.

Methods: SARS CoV viral load was performed using RT-PCR on tissue obtained at post-mortem and compared to a cohort of non-transplant SARS patients (21 patients). The donor SARS screening tool was prospectively utilized and assessed during the outbreak.

Results: A 57 year old man with emphysema received a double lung transplant. Post-operatively he had a stroke and was in a rehabilitation facility. At 4 months post-transplant he developed fever, myalgias, diarrhea, and progressive dyspnea requiring intubation. Despite aggressive treatment, he died two weeks after onset of disease. BAL and stool samples were positive for the SARS-CoV. The illness was transmitted to several others including his wife and three healthcare workers. The CoV viral load was higher in all tissues compared with post-mortem tissues from a non-transplant cohort with SARS (Table 1). Specifically, the lung viral load was 10,000-fold higher in the transplant patient than the cohort mean. SARS resulted in the temporary closure of transplant programs until a clinical screening tool for donors was implemented. During the SARS outbreak, 22 cadaveric donors were screened for SARS with this tool. Two donors were determined high risk and refused. No evidence of SARS transmission was seen with use of the remaining donors.

SARS CoV viral load from a transplant patient vs. mean tissue viral load from non-transplant cohort (n=21) with SARS

Tissue	Transplant patient viral load (copies/g)	Cohort viral load (copies/g)
Lung	8.8×10^9	3.6×10^5
Lymph node	8.9×10^8	7.1×10^4
Heart	2.8×10^7	3.2×10^4
Kidney	7.4×10^5	4.8×10^4
Liver	1.6×10^6	1.8×10^4

Conclusion: Transplant recipients are at risk of severe disease from SARS. Higher viral loads are detected in tissues of transplant recipients potentially making them more infectious. A clinical screening tool was useful in the prevention of SARS CoV transmission by cadaveric donors.

Abstract# 976

AN ASSESSMENT OF HERPESVIRUS CO-INFECTIONS IN PATIENTS WITH CMV DISEASE: CORRELATION WITH CLINICAL AND VIROLOGIC OUTCOMES. Atul Humar,[1] Deepali Kumar,[1] David Safronetz,[2] Graham Tipples.[2] *[1]Infectious Diseases and Transplantation, University of Toronto, Toronto, ON, Canada; [2]National Microbiology Laboratory, University of Manitoba, Winnipeg, MB, Canada.*

Background: In patients with CMV disease, a high rate of viral co-infection with other herpesviruses, specifically HHV-6 and 7, has been reported. The effect of herpesvirus co-infections on the rate of response of CMV disease to therapy is largely unknown. We prospectively analyzed herpesvirus co-infections in a cohort of organ transplant recipients with CMV disease and assessed their effect on clinical and virologic outcomes.

Methods: In solid organ transplant patients with CMV disease, about to start ganciclovir therapy, samples were collected at baseline (disease onset) and then at a minimum of 1-week intervals. Viral load testing for CMV, HHV-6 and HHV-7 was done using real-time PCR assays. Qualitative PCR for EBV was also done.

Results: 50 transplant patients with CMV disease were analyzed. 36/50 (72%) patients had received previous prophylaxis, and therefore the time of CMV disease onset was quite late post-transplant (median 152 days). Herpesvirus co-infection was detected in only 5/50 (10%) of patients and included HHV-6 infection in 2 patients (4%) and EBV infection in 3 patients (6%). HHV-7 co-infection was not detected in any patient. CMV peak viral load, and viral load at onset of disease was similar in patients with and without other herpesvirus co-infections (p=NS). Time to clearance of CMV viremia (after starting ganciclovir) was 20.5 ± 12.4 days in patients with viral co-infection vs. 17.6 ± 8.6 days in patients without (p=NS). In the two patients with HHV-6 co-infection, HHV-6 viral copy number was 100-1000 fold higher than CMV copy number and was unaffected by ganciclovir therapy.

Conclusion: Herpesvirus co-infections were uncommon in patients with CMV disease. Specifically a low rate of HHV-6 co-infection (4%) and no HHV-7 co-infections were seen. This is in contrast to previous reports, and may be due to the late onset of CMV disease in a heavily prophylaxed population. Co-infection did not affect CMV viral clearance rates or clinical response to therapy. In patients with HHV-6 co-infection, HHV-6 viral load was unaffected by ganciclovir therapy.

Abstract# 977

AN ASSESSMENT OF ADENOVIRUS INFECTION IN A LARGE COHORT OF SOLID ORGAN TRANSPLANT RECIPIENTS. Atul Humar,[1] George Moussa,[1] Tony Mazzulli,[1] Raymund Razonable,[2] Carlos Paya,[2] Emma Covington,[3] Emma Alecock,[3] the PV 16000 Study Group. *[1]Infectious Diseases and Transplantation, University of Toronto, Toronto, ON, Canada; [2]Infectious Diseases, Mayo Clinic, Rochester, MN; [3]Medical Science, Roche Products Limited, Welwyn Garden City, United Kingdom.*

Background: Little is known about adenovirus infections in adult solid organ transplant recipients. Since the virus can establish latency, reactivation may be a relatively common event post-transplant. Viral replication may lead to directly attributable symptoms or could have indirect effects on graft function and rejection.

Methods: We assessed adenovirus infection in 263 organ transplant recipients (liver, kidney, heart, and kidney-pancreas) participating in an international trial comparing 100 days of oral ganciclovir vs. valganciclovir for CMV prophylaxis. Adenovirus PCR (LightCycler-based assay; limit of detection ~ 100 copies) was done on plasma samples collected on or around week 1, day 28, 56, 100, 6 months and 12 months post-transplant and correlated with outcomes

Results: Adenovirus DNA was detected in 19/263 patients (7.2%) and in 21/1395 (1.5%) plasma samples up to 12 months post-transplant. Viremia by transplant type was: liver (n=10/121), kidney (n=6/92), heart (n=3/45), and kidney-pancreas (n=0/5). Viremia was detected within the first 10 days post-transplant in 4 patients, on day 28 in 2 patients, on day 100 in 7 patients, and between month 6-12 in 8 patients. The number of patients who were positive while on anti-CMV prophylaxis was 12/263 (4.6%) and was similar to the rate of positivity after discontinuation of prophylaxis. Symptoms were evaluated at the time of adenovirus detection: 11/19 (58%) patients had no symptoms at the time of viremia; 2/19 (10.5%) had gastrointestinal symptoms (predominantly diarrhea), 2/19 (10.5%) had respiratory symptoms, and 4 patients (21%) had vague/inconclusive symptoms. 4/19 (21%) patients were subsequently treated for CMV disease and 1/19 (5%) patients were subsequently treated for biopsy proven acute rejection.

Conclusions: This is among the largest studies assessing adenovirus in adult solid organ transplant recipients, and demonstrates that viremia is relatively common. Anti-viral prophylaxis against CMV did not seem to affect adenovirus. The majority of patients were asymptomatic although some had respiratory or gastrointestinal symptoms. No effect on subsequent acute rejection was observed.

Abstract# 978

DETERMINANTS OF TRANSPLANT SURGEONS' WILLINGNESS TO PROVIDE ORGANS FOR PATIENTS INFECTED WITH THE HEPATITIS B, HEPATITIS C, AND HUMAN IMMUNODEFICIENCY VIRUSES. Scott D. Halpern,[1] David A. Asch,[1] Peter Stock,[2] Abraham Shaked,[3] Emily Blumberg.[1] *[1]Department of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Department of Surgery, University of California, San Francisco, San Francisco, CA; [3]Department of Surgery, University of Pennsylvania, Philadelphia, PA.*

Purpose: To determine whether transplant surgeons believe patients infected with HBV, HCV, or HIV should be candidates for transplantation, and to clarify the factors that influence these views.

Methods: We mailed a 3-page questionnaire to all U.S. transplant surgeons included in the American Society of Transplant Surgeons' (ASTS) mailing list, along with a cover letter explaining the purpose of the study and a $10 incentive. We sent a second questionnaire to all surgeons not responding within 5 weeks. We cross-matched the ASTS list with the American Medical Association's Master File to obtain data on surgeons' practice-related and demographic characteristics, and compared characteristics of responders and nonresponders to assess the potential for nonresponse bias.

Results: Of 619 eligible transplant surgeons, 347 (56%) provided complete responses. Overall, 69%, 71%, 36%, and 6% of surgeons believed that HBV+ patients, HCV+ patients, HIV+ patients, and patients with AIDS, respectively, should be candidates for transplantation. For each patient population, surgeons' perceived post-transplant survival among infected patients was a strong predictor of their willingness to allocate organs to these patients (all $p < 0.001$). In a multivariable logistic regression model, older surgeons (odds ratio (OR) = 1.9; $p = 0.02$), thoracic surgeons (OR=20.0, $p < 0.001$), and surgeons with greater fears of becoming infected with HIV intraoperatively (OR=1.9, $p = 0.02$) were less likely to consider HIV+ patients as transplant candidates. Most surgeons (56%) erroneously believed that the intraoperative patient-to-surgeon transmission risk of HIV was greater than that for HBV, HCV, or both. Nonresponse bias is unlikely to have influenced these results because the only difference between responders and nonresponders was that surgeons board-certified in urology were less likely to respond than surgeons with other certifications ($p < 0.0001$).

Conclusion: The majority of surgeons do not believe that HIV-infected patients should be candidates for transplantation. Surgeons' expressed willingness to allocate scare organs to such patients is most strongly associated with their estimates of post-transplant survival, but is also associated with practice type and fear of patient-to-surgeon transmission. The availability of more clinical data may ultimately influence surgical opinion.

Abstract# 979

USE OF CIDOFOVIR AS A RESCUE AGENT FOR GANCYCLOVIR RESISTANT CMV INFECTION AND DISEASE AND PAPILLOMA VIRUS ASSOCIATED SKIN LESIONS IN TRANSPLANT RECIPIENTS. Hugo Bonatti,[1] Stefan Schneeberger,[1] Claudia Boesmueller,[1] Wolfgang Steurer,[1] Raimund Margreiter,[1] Ferguel Cakar,[1] Paul Hengster.[1] *[1]Department of General and Transplant Surgery, University Hospital, Innsbruck, Tirol, Austria.*

BACKGROUND: Solid organ transplantation is frequently complicated by viral infections. Cidofovir is a broadspectrum antiviral agent with activity against CMV, HSV, VZV, EBV and HHV6, 7, 8 but also against Adeno-, Pox-, BK- and Papilloma viruses. It is also active against UL87 (transphosphorase) mutants of CMV showing Gancyclovir (GCV) resistance.

AIM: To retrospectively review our experience with the use of systemic and topical Cidofovir in transplant recipients.

PATIENTS AND METHODS: Between 1.1.2000 and 30.11.2003 more than 700 solid organ transplants were performed at our centre. Standard immunosuppression consisted of Calcineurin inhibitor based triple drug therapy with or without ATG or IL2 receptor antagonist induction. A total of 6 patients (1% of patients transplanted during the study period) received systemic Cidofovir including 1 kidney, 1 pancreas, 1 lung, 1 small bowel and both limb recipients. In addition three transplant recipients were treated with topical Cidofovir for Papilloma virus associated skin lesions.

RESULTS: In all three cases regression of skin lesions was achieved. Cidofovir was given for prophylaxis in one bilateral hand transplant recipient after development of GCV associated neutropenia. The remaining 5 patients received Cidofovir for CMV infection or disease. Four patients had developed breakthrough CMV disease during GCV prophylaxis, in two cases a UL97 mutant was isolated and in two patients we observed clinically GCV resistant CMV disease. In the remaining patient the indication was GCV associated neutropenia. The patient who received Cidofovir prophylaxis developed CMV infection after withdrawal, but responded to a second antiviral course. All cases of CMV disease responded to therapy. Only in the lung recipient we observed a relapse and one kidney recipient who received only one therapy cycle developed repopulation with a wild type CMV strain and required ValGCV therapy. No severe side effects were observed in this cohort, in specific no sustained renal impairment.

CONCLUSIONS: Cidofovir was found highly active in the treatment of Papilloma virus associated skin lesions and GCV resistant CMV disease in SO recipients and was well tolerated.

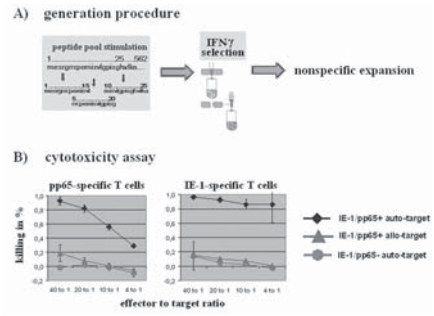
Abstract# 980

GENERATION OF ANTIGEN-SPECIFIC T LYMPHOCYTES FOR ADOPTIVE IMMUNTHERAPY IN TRANSPLANT PATIENTS. M. H. Hammer,[1,2] G. Brestrich,[1] H.-D. Volk,[2] P. Reinke.[1] *[1]Department of Nephrology and Internal Intensive Care; [2]Institute of Medical Immunology, Charité, Berlin, Germany.*

Infectious diseases and cancer development are major complications in immunosuppressed transplant patients. The diminished cellular immunity is thought to be causative for EBV-associated lymphomas, skin cancers or severe HCMV-infections. Restoring immunity by adoptive lymphocyte therapy has been shown to be effective in tumour and infectious diseases. In SOT, the infused lymphocytes are of recipient origin and generated to recognize relevant proteins presented by self MHC-molecules. However, current generation procedures have major limitations.

generation strategy	limitations	advantages
single peptides (9-aa) **(MHC-I tetramers)** (pulsed on PBMCs or DCs)	- HLA-type dependent - dependent on the knowledge of immuno-dominat epitopes	- high level of biosafty - specific CD8⁺ lymphocytes - single specificity (escape mechanism)
viral/tumor lysats **recombinant proteins** (pulsed on PBMCs or DCs)	- reliability (dependent on APC-processing and presentation) - biosafety (purity) - mainly CD4⁺ T cells (MHC-II presentation)	- HLA-type independent - independent of epitope knowledge - specific CD4⁺ lymphocytes - multiple specificities
transgenic APCs **infected APCs** (e.g. B-cells, fibroblasts)	- biosafety (e.g viruses) - time consuming generation - expensive (master cell bank, GMP-suitable virus production…)	- HLA-type independent - independent of epitope knowledge - specific CD4⁺ and CD8⁺ lymphocytes - multiple specificities

In this report we present a novel time and cost effective generation procedure for HLA-type independent production of specific T cells. It is based on short-time stimulation with 15-AA overlapping peptide pools, selection of activated, IFN-γ secreting cells and non-specific expansion (A). We applied the protocol to generate T cells specific for the HCMV proteins pp65 and IE-1 and compared it with current procedures. Generation of pp65 and IE-1 specific T-cells from the same volunteers was successful in 7/8 experiments. Cell lines consisted of $CD8^+$ and $CD4^+$ cells with multiple pp65/IE-1 epitope specificities that showed lysis of autologous $pp65^+$ or IE-1^+ targets (no killing of $pp65^-$/IE-1^+-auto/allo targets) (B).



The presented procedure allows the generation of tumour and pathogen specific T cells, even with unknown epitopes, for a broad patient population.

EARLY POST-TRANSPLANT AND DONOR ISSUES

Abstract# 981

FACTORS PREDICTING THE FUNCTIONAL OUTCOME OF LAPAROSCOPIC DONOR NEPHRECTOMY: AN ANALYSIS. Mahesh C. Goel,[1] Charles S. Modlin,[1] Ithaar H. Derweesh,[1] J. Feng,[1] Stuart M. Flechner,[1] David A. Goldfarb,[1] Inderbir Gill,[1] Andrew C. Novick.[1] *[1]Div of Transplantation and Division of Minimal Invasive Surgery, Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, OH.*

Objective:

We evaluated our laparoscopic donor-recipient database to study the factors influencing the outcome of allografts from laparoscopic kidneys.

Material and Methods:

Record of all laparoscopic donor-recipient data was retrieved from transplant database. Short-term allograft function was determined based on serum creatinine at day 1-5, 10, 20 and 30, serum creatinine >2.5 at day 10 and serum creatinine >1.5 at day 30, delayed graft function (DGF) and long-term outcome were assessed. Donor factors analyzed were age, sex, BMI, side left versus right, warm ischemia time, cold ischemia time, use of heparin, transperitoneal versus retroperitoneal, multiple artery versus single artery. A statistical model was built and logistic regression was used to determine the factors influencing the outcome. Univariate and multivariate analysis were performed to determine the factors influencing the functional outcome.

Results:

There were 207 patients in the study, mean donor age of 42.5± 9.2 years, mean BMI 26.7 ± 4.0 kg/m² mean WIT was 249.3 seconds (range 120-540 seconds). Mean age of recipient was 44.4± 13 years and BMI was 26.2± 4.7 kg/m². The table shows the details of significant results in each category. Higher BMI of recipient, rights kidney and retroperitoneal surgery were found to be risk factors for early graft dysfunction. Older donor and obese recipient were risk factors for DGF. Donor BMI directly influences the long-term allograft function. Also allograft did better in female and white recipients (P=<. 001). Warm ischemia time, total operating room time and cold ischemia time did not play a significant role in the outcome.

Conclusions: Outcome of laparoscopic donor nephrectomy is largely dependent on non-surgical factors: older donor age, higher recipient BMI and male sex of recipient are important for short-term outcome, obese donor is a risk for long-term outcome.

Results

Significant Variable	Univariate analysis	Multivariate analysis
S. Creatinine	Trans versus Retro	Recipient BMI, Trans vs. retro
S. Creatinine Day 10	None	Recipient BMI, trans vs. retro
S. Creatinine Day 20	None	Recipient BMI, trans vs. retro
S. Craetinine Month 1	None	Recipient BMI
Delayed graft Fx	Donor age	Donor age, Rec BMI, Rec. Sex
S. Cr.>1.5 at day 30	Donor age	Donor age, Rec BMI
S. Cr.>2.5 at day10	Donor age	
	Long-term Function	
Acute Rejection		Recipient BMI
Year 1		Donor age, Rec sex and race
Graft Survival	Donor BMI	
Patient survival	Donor BMI	

Abstract# 982

STEROID FREE IMMUNOSUPPRESSION IN KIDNEY RECIPIENTS WITH DELAYED GRAFT FUNCTION(DGF) MONITORED BY PROTOCOL BIOPSIES: A CONTROLLED STUDY TO EVALUATE THE OUTCOME OF ACUTE REJECTION(AR), GRAFT FUNCTION(GF), SUBCLINICAL ACUTE REJECTION(SCAR), CHRONIC ALLOGRAFT NEPHROPATHY(CAN) AND ONE YEAR SURVIVAL.

Mysore S. Anil Kumar,[1] Michael Heifets,[2] Billie Fyfe,[3] Miten H. Parikh,[1] Muhammed I. Saeed,[1] Sheng G. Xiao,[1] Susan Stabler,[4] Michael J. Moritz,[1] Debra Sierka,[4] Aparna Kumar.[1] *[1]Transplant/Surgery, Drexel University College of Medicine, Philadelphia, PA; [2]Nephrology/Medicine, Drexel University College of Medicine, Philadelphia, PA; [3]Pathology, Drexel University College of Medicine, Philadelphia, PA; [4]Pharmacy/ Transplant, Hahnemann University Hospital, Philadelphia, PA.*

DGF is considered a risk factor for higher incidence of AR and inferior one year graft survival. The aim of this study was to analyze the outcome of recipients with DGF maintained on steroid free immunosuppression. 141 consecutive cadaver kidney recipients maintained on steroid free therapy and transplanted between June 2000 and November 2003 were studied; 62(44%) had DGF and 79(56%) did not. Biopsy was completed by day 10 in DGF patients to rule out incidental AR. All recipients were given basiliximab induction. Only 2 doses of steroids were given to all recipients;250 mg on day 0 and 125 mg on day 1 and then discontinued.Maintenance immunosuppression was a calcineurin inhibitor with mycophenolate mofetil (MMF) or sirolimus(SLR). SLR was used in 45% of the recipients in the DGF group and 48% of the non DGF group. All patients had protocol biopsies at regular intervals to assess SCAR, CAN and other pathological conditions. Clinical AR was also confirmed by biopsy. Clinical AR and SCAR were treated with methylprednisolone 1g for 2 days. Maintenance steroid therapy was not initiated in any patients including those with AR. African Americans formed 75% in DGF and 53% in nonDGF groups(p=0.04). The mean cold ischemia time(CIT) was 16.5±10.5 in DGF and 15.1±10.5 hours in nonDGF group(p=ns). Table shows the donors aged ≥60 years, incidence of AR, SCAR, CAN, serum creatinine(SC), creatinine clearance(CCl) in the 2 groups. [table] One year patient and graft survival were 92% and 85% in DGF and 92% and 88% in nonDGF groups respectively. This data shows that donor age, adjunct therapy with SLR or MMF, and CIT are not different in the 2 groups. African American recipient proportion was significantly higher in DGF group. AR, SCAR, CAN and kidney function as measured by SC, CCl and patient and graft survival were similar in the 2 groups for up to12 months. Steroid free therapy did not affect any of the parameters or outcomes measured in the DGF group. We conclude that steroid free therapy is not detrimental in patients with DGF and provides equivalent results up to one year in both DGF and nonDGF groups. Early biopsies in DGF recipients to exclude AR, minimizes the graft loss in this group.Long term follow up is required before steroid free treatment can be recommended on a routine basis in DGF recipients.

	Patients with SLR	Donor age ≥60 years	AR	SCAR	Moderate CAN	SC (mg/dl) at 1 year	C.Cl (mls/min) at 1 year
DGF recipients (N=62)	28(45%)	9(14.5%)	9(14.5%)	17(27%)	10(19%)	2.2±1.1	60±25
Non DGF recipients (N=79)	38(48%)	14(17.7%)	6(7.5%)	21(26%)	14(18%)	2.0±1.7	68±11

Abstract# 983

KIDNEY TRANSPLANTATION IN THE ELDERLY: VARIATIONS OF THE "MATCH" GAME.

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Introduction: The aging donor (D) and recipient (R) population has led to new challenges in kidney transplantation (KT). Controversy exists regarding the optimal approach to the elderly D or R. A number of strategies have been proposed including matching by age, medical risk, serology, HLA, size, or nephron mass. The purpose of this study was to retrospectively review our single center experience in deceased donor (DD) KT with respect to age.

Methods: From 10/1/01 through 11/15/03, we performed a total of 129 DD KTs, including 33 (26%) in Rs ≥60 years and 96 (74%) in Rs 19-59 years of age. The DD pool included 51 expanded criteria donors (ECD; defined and allocated according to UNOS policy) and 78 standard criteria donors (SCD; defined as non-ECD). ECD kidneys were utilized by matching estimated renal functional mass to recipient size (BMI <25 kg/m²), including the use of dual KTs (N=8). ECD kidney Rs were further selected based on age >40 and low immunologic risk. Rs received rATG or alemtuzumab induction in combination with tacrolimus, MMF, and steroids; those ≥60 had lower tacrolimus targets and MMF doses.

Results: The mean age differed significantly between R groups (65 vs 46 years, p<.001), including 7 patients >70. In Rs ≥60, 22 (67%) received KTs from ECDs compared to 29 from ECDs (30%, p<.001) in Rs <60. Other demographic and transplant characteristics were similar among groups. Patient survival is 97% in Rs ≥60 compared to 99% in Rs <60 (p=NS) with a mean follow-up of 12 months. Kidney graft survival rates are 94% in Rs ≥60 vs 89% in Rs <60, p=NS. Initial and subsequent graft function, rejection, infection, re-operations, length of stay, re-admissions, and resource utilization were similar among groups. Nine patients had opportunistic viral infections (5 CMV, 3 polyomavirus nephropathy, 1 EBV-associated PTLD), including 4 (12.5%) in Rs ≥60 compared to 5 (5%, p=NS) in Rs <60. However, all 9 cases occurred in ECD Rs (18% ECD vs 0 SCD Rs, p<.001).

Conclusions: By matching nephron mass with R size and avoiding the use of ECD kidneys in high immunologic risk Rs, short-term outcomes that are comparable to SCD kidneys in younger patients can be achieved with either older Ds or Rs, regardless of age. However, the risk of viral infection is higher in ECD Rs, and long-term follow-up is needed to ultimately determine the risks and benefits of KT in this setting.

Abstract# 984

DOES THE TERMINAL CREATININE VALUE IN A YOUNG DECEASED DONOR INFLUENCE THE LONG-TERM GRAFT SURVIVAL FOLLOWING KIDNEY TRANSPLANTATION ?

Kunam S. Reddy,[1] Darcy Davies,[2] Adyr Moss,[1] Marek Mazur,[1] Raymond Heilman,[1] David C. Mulligan.[1] *[1]Transplantation Surgery and Medicine, Mayo Clinic Hospital, Pheonix, AZ; [2]Research Department, United Network for Organ Sharing, Ricjmond, VA.*

Background: Elevated serum creatinine value in a young deceased donor can be due to multiple reasons including dehydration, acute tubular necrosis, effect of brain death, rhabdomyolysis and drug or contrast nephrotoxicty. There is wide variation in clinical practice regarding the acceptance of kidneys from young donors with elevated creatinine and it is not clear what the impact of this acute insult is on the long-term graft survival following kidney transplantation. **Methods**: Using United Network for Organ Sharing (UNOS) database, all primary kidney transplants (N=27650) performed between 1994-2001 from deceased donors aged 10-39 were analyzed. Kidney transplants from donors with history of diabetes, hypertension, or kidney disease and multi-organ transplant recipients were excluded from this analysis. Other donor and recipient factors which can influence the long-term graft survival were included in the multivariate model along with donor creatinine. **Results**: The distribution of donor creatinine level is as follows: 0-0.9: 48%; 1.0-1.4: 37%; 1.5-1.9: 9%; 2.0-2.4: 3% and >2.4: 3%. Unadjusted Kaplan-Meier graft survival rates are shown in the following table. In the multivariate Cox regression analysis, the relative risk (RR) of 6 month graft survival between a donor with a creatinine level 0.5 unit more than another donor is 1.12. The RR for long-term graft survival for those kidneys functioning at 6 months with a 0.5 unit change more in donor creatinine is 1.04 when death was treated as graft failure and 1.05 when death with functioning graft was censored. **Conclusions**: Kidneys from young deceased donors with elevated creatinine provide excellent short and long-term graft survival. Terminal creatinine value in young donors is not a risk factor for long-term graft survival. Although these results represent a select group of kidneys actually transplanted, further evaluation for expanding use of this group of donors is warranted.

Terminal creatinine value (mg/dl)	Year Post Tx	Graft Survival Rate (95% Confidence INterval)	Number Followed in each Group
0-0.9	1	91.4 (90.9-91.9)	12730
	5	74.0 (73.1-74.9)	6711
1.0-1.4	1	91.2 (90.6-91.7)	9557
	5	72.3 (71.2-73.3)	5335
1.5-1.9	1	90.5 (89.3-91.6)	2364
	5	70.7 (68.6-72.8)	1315
2.0-2.4	1	90.1 (88.0-92.3)	719
	5	67.9 (63.9-72.0)	390
>2.4	1	91.0 (89.0-93.0)	802
	5	73.7 (70.3-77.2)	461

Abstract# 985

RESOURCE UTILIZATION IN CADAVER RENAL TRANSPLANTION. Prabhakar Baliga,[1] Sylvia Odom,[1] Greg Gilbert,[1] Kathy Turissi,[1] Angello Lin,[1] Fuad Afzal,[1] Osemwegie Emmovon,[1] Jeffrey Rogers,[1] P. R. Rajagopalan,[1] Kenneth Chavin.[1] *[1]Department of Surgery, Division of Transplant, Medical University of South Carolina, Charleston, SC.*

A predominant economic focus in renal transplantation has been to reduce costs for the initial hospitalization and first 90 days. We determined factors which effect early readmission, and their financial impact. Methods: We performed a retrospective analysis of all kidney transplants performed from July 1, 2001 to June 30, 2003. The following variables were analyzed: Demographics, CIT, HLA mismatches, ATN, Induction therapy, Diabetes, Coronary artery disease, BMI, education level, and insurance status. Chi-square and Student's t-test were performed for all variables under investigation versus a dependent variable of readmission within 30 days. Logistic regression was performed with demographic variables and variables considered to be clinically significant. All variables were dichotomized at clinically important levels. Causes of readmission, hospital days, number of readmits, and time from discharge were also collected. Charges were captured for the first time readmissions in ATN recipients. Results: 195 kidney transplants were performed and a 28 % readmission rate was observed.

Multivariate analysis revealed ATN and African American to be significant statistical predictors for readmission within 30 days (p=0.0017 and p=0.0412), respectively. CIT greater than 420 minutes had a 2.1 odds ratio of ATN. Odds Ratio and p-values for Variables and Readmission within 30 days:

VARIABLE	OR	p-value
African American (AA)	2.5	0.0412
Age Greater than 55	0.9	0.7106
Female	1.5	0.2743
BMI Greater than 30	1.1	0.7771
CIT Greater than 420 minutes	1.1	0.8939
ATN	3.3	0.0017
Induction	0.9	0.8111
Diabetic (DM)	1.8	0.2147
CAD	2.0	0.1644
Medicare as primary insurance	1.0	0.9364

Using the parameter estimates from the logistic regression mode, the highest odds ratio of readmission is AA with a primary diagnosis of DM (4.5), with ATN complicating the post transplant course the odds ratio increased to 14.8. ATN recipients incurred a charge of $15,300/patient for the first time readmit (n=45). Conclusion: The focus in kidney transplantation should extend beyond the transplant admission to reducing early rehospitalization. Specific recipient populations such as AA and DM are at high risk and utilize more resources. Recipients with ATN have the highest readmission rate. Efforts to impact ATN by donor selection, decreasing CIT or introducing pulsatile preservation are important strategies to consider.

Abstract# 986

INCREASED RISK OF PERI-OPERATIVE CARDIAC EVENTS IN KIDNEY TRANSPLANT RECIPIENTS WITH DELAYED GRAFT FUNCTION. John S. Gill,[1,2] Craig Solid,[3] Brian J. G. Pereira,[2] Allan J. Collins.[3] *[1]Nephrology, University of British Columbia, Vancouver, BC, Canada; [2]Nephrology, Tufts-New England Medical Center, Boston, MA, Canada; [3]Nephrology Analytical Services, Minneapolis, MN.*

Delayed graft function (DGF) may be associated with volume overload and increased cardiac work load. In this study we determined the association of DGF with death and major cardiac events (acute myocardial infarction, cardiac arrest, congestive heart failure, CABG, PTCA) in first 30 days after transplantation. Study patients included adult, cadaveric, first, kidney only transplant recipients between 1995-2000 who were Medicare eligible for at least 12 months prior to transplantation. Death and major cardiac events were determined by transplant year in patients with and without DGF. A multivariate logistic regression analysis was performed to determine the independent association of DGF with the combined endpoint of death or major cardiac event. The table shows that the incidence of death and major cardiac events was relatively constant over time and that major cardiac events were more frequent in patients with DGF. In a multivariate analysis which included patient age, gender, race, cause of ESRD, comorbid conditions, duration of dialysis exposure, cardiac investigations in the year prior to transplantation, pre-transplant hemoglobin level and the requirement for blood transfusion after transplant, patients with DGF had an increased odds of mortality or major cardiac event (odds ratio =1.6, 95% confidence interval 1.4-1.7, p <0.0001). Because delayed graft function may be predictable prior to transplantation, it may be preferable to avoid the allocation of kidneys at risk for DGF to transplant candidates with a high cardiovascular disease burden.

Year	1995	1996	1997	1998	1999	2000
N	2844	2976	3147	3255	3095	3210
With cardiac event N (%)	446 (16)	505 (17)	563 (18)	535 (16)	545 (18)	557 (17)
Died N (%)	60 (2.1)	71 (2.4%)	91 (2.9%)	59 (1.8%)	67 (2.2%)	64 (2.0%)
DGF N (%)	798 (28)	826 (28)	822 (26)	914 (28)	838 (27)	842 (26)
% of patients with DGF who had cardiac event N (%)	177 (22)	195 (24)	200 (24)	187 (21)	179 (21)	208 (25)

Abstract# 987

SEVERE DYSBALANCE OF ANTIOXIDATIVE CAPACITY IN OLDER RECIPENTS IS ASSOCIATED WITH POORER OUTCOME IN KIDNEY TRANSPLANTATION. Susanne Kolodziej,[1] Michael Marzinzig,[1] Alexander Schneider,[1] Jens M. Mayer.[1] *[1]Visceral-& Transplantation Surgery, University Hospital, Ulm, Germany.*

Oxidative stress associated with ischaemia/ reperfusion and operative trauma influences clinical outcome in kidney transplantation. Older recipients tend to have more complications and a less favourable outcome in kidney transplantation. While this may be associated with increased oxidative stress, little is known about a possible dysbalance of the antioxidative capacity in these patients. Blood samples were drawn from 31 patients undergoing kidney transplantation pre- and perioperatively. Markers of oxidative stress and the antioxidative system were measured. Median age was 55 years (21-67), nine were older than 60 years. Antioxidative capacity TORC and vitamine E equivalent Trolox were lower in older recipients perioperatively and at any other time point up to six hours after reperfusion. From 15min after reperfusion, 4-HNE and GSSG were higher in older recipients and increased in the effluate from the renal vein compared to systemic values. GSH, malondialdehyde MDA and thiobarbiturat- reactive substances TBARS increased after reperfusion with even higher values in the venous effluate. They were normal again after 6h with no difference between the two groups. Irrespective of age, patients with a delayed graft function had lower preop TORC than those with primary function. In these patients 4HNE was higher from reperfusion to the end of the observation period. Patients with acute rejection had higher MDA, 4HNE and PLA2 activity in the renal vein blood than patients without acute rejection. After reperfusion, markers of oxidative stress and lipid peroxidation are increased in systemic blood and blood drawn locally from the renal vein. They are further increased in patients with delayed graft function and acute rejection. However, this does not cause a noticeable decrease of the systemic antioxidative capacity. In contrast to this, older recipients have a lower antioxidative capacity even preioperatively. This patient-dependent dysbalance of the antioxidative system could be responsible for the poorer outcome in older patients and offers the possibility of prophylactic treatment.

Abstract# 988

RESOLUTION OF NATIVE KIDNEY PROTEINURIA IN THE IMMEDIATE POST RENAL TRANSPLANT PERIOD. Prakas T. D'Cunha,[1] Ravi Parasuraman,[1] K. K. Venkat.[1] *[1]Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

The magnitude of proteinuria correlates with poor outcome in native kidney disease and after renal transplantation. Since preemptive renal transplantation (PETx) is on the rise, many patients receive renal transplants with residual urine output and proteinuria from the native kidneys. Delineation of the source of proteinuria (native vs allograft) in the immediate post-transplant period (IPTP) is important for appropriate interventions. However, It is not known if native kidney proteinuria persists post transplantation (postTx).

Methods: We prospectively evaluated 11 Live donor Tx recipients with urine output pre transplantation (preTx), immediate graft function and stable creatinine postTx. Random urine protein: creatinine (UPr:Cr) ratio was measured immediately preTx and weekly thereafter. Group A (n=5) included PETx recipients and Group B (n=6) were on dialysis preTx. All recipients had UPr:Cr > 0.5 pre-Tx and total resolution was defined as UPr:Cr <0.2.

Results: Demographic features included a mean age of 45 years, 4 Female, 7 Male, 3 Caucasians and 8 African-Americans. The causes of ESRD were diabetic nephropathy (n=4), hypertension (n=3), reflux nephropathy (n=1), and chronic glomerulonephritis (n=3). The mean serum creatinine preTx was 8.8 ± 2.3 mg/dl in the patients on dialysis, 6.2 ± 1.7 mg/dl in the preemptive patients and 1.3 ± 0.37 mg/dl at 5 weeks post-Tx. The immunosuppressive regimen was based on tacrolimus, mycophenolate mofetil and corticosteroids ± induction with Thymoglobulin®.The mean tacrolimus level at 4 weeks postTx was 10.4 ± 2.7 ng/ml. No patient was on ACE inhibitors, angiotension receptor blockers or nondihydropyridine calcium channel blockers during the study period.One patient not included in the above analysis had proteinuria that did not resolve and allograft biopsy at 3 wks showed acute humoral rejection with glomerulopathy. The evolution of UPr:Cr in the IPTP is shown in the table.

Conclusion: Our results show complete resolution of native kidney proteinuria at a mean duration of 5 weeks post Tx and Persistent proteinuria beyond 8 weeks postTx is of allograft in origin and cannot be attributed to the native kidneys.

Group	Immidiate Pre Tx UPr/Cr	Nadir Post Tx UPr/Cr	No. Of Weeks Post Tx Nadir Reached	P value Pre:Post Nadir
Total n=11	2.94 ± 2.60	0.131 ± 0.54	4.7 ± 2.5	0.005
Group A- Preemptive n=5	3.41 ± 3.29	0.136 ± 0.60	3.4 ± 2.3	0.037
Group B - Dialysis n=6	2.54 ± 2.10	0.127 ± 0.053	5.8 ± 2.2	0.088

Abstract# 989

MATHEMATICAL MODELS DO NOT PREDICT EARLY RENAL ALLOGRAFT DYSFUNCTION POST LIVING DONATION.

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Introduction: Without protocol biopsies early allograft dysfunction in live renal donation is difficult to detect. We asked whether or not mathematical models such as: Cockcroft-Gault, MDRD and Nankivell could be used to predict post-donation creatinine as a mechanism of identifying early allograft dysfunction.

Methods #1: Retrospective chart review of living donor renal transplant patients at our University Hospital from 2001 to 2003 (76 donor pair charts) was performed. Exclusion from the study was based on lack of available information (9 donor pair charts). We applied the above mentioned mathematical equations to predict early serum creatinine in the recipient by using anthropomorphic information from the recipient and the known transplanted renal function from the donor. Linear regression analysis was performed to evaluate for possible correlation between early post-transplant serum creatinine measurements in the recipient and estimated serum creatinine from the mathematical equations in all patients and in patients without identified allograft dysfunction.

Results #1: We found no correlation between estimated and measured serum creatinine in recipients of live donor kidneys using either Cockcroft-Gault, MDRD or Nankivell equations. R^2 = 0.270, 0.082, 0.034 respectively in patients without allograft dysfunction.

Methods #2: Compensatory hypertrophy and hyperfiltration are known to occur in solitary and post-transplant kidneys. Hence, we used the donor as a control and asked whether or not the estimated clearance or GFR of the donor correlated with the estimated clearance or GFR of the recipient 1 week after transplantation using anthropomorphic data from each coupled with measured laboratory values.

Results #2: We found no correlation between recipient and donor estimated creatinine clearance or GFR using Cockcroft-Gault, MDRD or Nankivell equations. R^2 = 0.041, 0.033, $3.86e^{-4}$ respectively in patients without allograft dysfunction.

Conclusion: Neither Cockcroft-Gault, MDRD or Nankivell mathematical equations were able to reliably predict serum creatinine, clearance or GFR in recipients. Possible explanations include: 1) 24 hour creatinine clearance is not a reliable indicator of GFR or may be over/under collected. 2) Medications used early post-transplantation may alter creatinine clearance or GFR. 3) Variable muscle catabolic rate related to medications or early post-operative status. 4) Demographics used to derive these equations were different.

EXPERIMENTAL IMMUNOSUPPRESSION: NEW INSIGHTS II

Abstract# 990

ASSESSMENT OF REGULATORY T CELL FUNCTIONS IN KIDNEY TRANSPLANT PATIENTS ON MMF BASED IMMUNOSUPPRESSION.

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The majority of current immunosuppressive protocols for solid organ transplant recipients are based on calcineurin inhibitors, increasingly combined with the anti-proliferative agent mycophenolate mofetil (MMF). We have recently demonstrated that indirect T cell recognition of donor-specific HLA peptides plays an important role in the immunopathogenesis of chronic allograft rejection (CR). We have also previously generated HLA allopeptide specific T cell lines and clones from renal transplant recipients with CR treated with cyclosporine (CsA), azathioprine (Aza) and corticosteroids which were of Th1 phenotype (IFN-γ), while lines and clones from stable patients were of a Th2 (IL-10) phenotype. For this study we generated T cell lines using peripheral blood lymphocytes (PBLs) from renal transplant recipients treated with MMF+CsA (n=12) or MMF+Tac (n=6) with either CR (biopsy, serum creatinine > 2 mg/dl) or stable renal function (SRF, serum creatinine ≤ 2 mg/dl). Generated lines showed a significant response to donor-specific peptide (CR: 24.125± 3.256 cpm vs SRF: 4.283 ± 731 cpm). T cell lines from patients with CR produced IFN-γ, but only minimal amounts of IL-10 (548±94 vs. 58±12 spots/10^6 cells) in response to the donor specific peptide. In contrast, T cell lines from patients with SRF produced IL-10 but minimal IFN-γ assessed using ELISPOT (533±37 vs 52±11 spots/10^6 cells, respectively) and confirmed by ELISA and were CD4/CD25 positive (FACS-analysis). In this study on MMF-based immunosuppression we confirm that CR is associated with a Th1 pattern of cytokine production, while stable renal function is associated with a Th2 phenotype regardless of the immunosuppressive regimen used (CsA/Tac+MMF vs CsA+Aza). This analysis may provide an invaluable tool to clarify the potential of current immunosuppressive protocols with MMF enabling the induction of T regulatory cells.

Abstract# 991

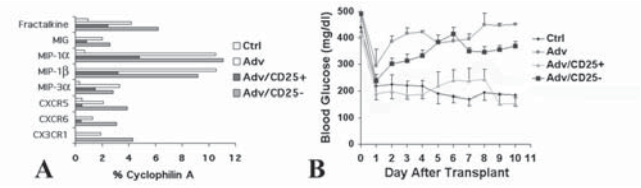
Abstract has been withdrawn.

Abstract# 992

CD4+CD25+ T CELLS INHIBIT INNATE IMMUNE RESPONSES OF MURINE ISLETS.

Nan Zhang,[1] Shuang Fu,[1] Dongmei Chen,[1] Haojiang Zhang,[1] Yaozhong Ding,[1] Jonathan S. Bromberg.[1] [1]*Recanati Miller Transplantation Institute, Mount Sinai School of Medicine, New York, NY.*

Background: Previous work demonstrated that surgical manipulation, ischemia, or adenovirus vector infection induced innate immune responses in mouse islets, resulting in production of chemokines and impaired islet engraftment. CD4+CD25+ T cells with regulatory functions participate in immune tolerance and the regulation of inflammatory responses. Aims: To evaluate the role of CD4+CD25+ T regulatory cells to modulate adenovirus vector induced chemokine expression in murine islets, and to determine their effect on islet engraftment. Methods: CD4+CD25+ T cells were sorted from BALB/c splenocytes. Murine islets were isolated by stationary digestion method with collagenase from BALB/c mice. Freshly isolated islets were transduced with AdCMVLacZ vector at multiplicity of infection (MOI) of 100 for 1 hour, and then cocultured with CD4+CD25+ or CD4+CD25- T cells at ratio of islet cells: T cell of 2:1 (assuming 1000 cells/islet). Cultures were harvested after 48 hours for determination of chemokine gene expression profiles. Additionally, groups of islets and T cells were cotransplanted in a marginal islet mass model to diabetic recipients (150 islets/mouse). Group I: control; group II: AdCMVLacZ transduced; group III: CD4+CD25+ T cells + AdCMVLacZ; group IV: CD4+CD25- T cells + AdCMVLacZ. Results: AdCMVLacZ transduction induced a wide variety of chemokines in islets. CD4+CD25+ T cells inhibited chemokine expression, including fractalkine, MIG, MIP-1α, MIP-1β, MIP-3α, CXCR5, CXCR6 and CX3CR1. The marginal islet mass transplants showed that vector transduction prevented islet engraftment and diabetes cure, while CD4+CD25+ T regulatory cells, but not CD4+CD25- control T cells permitted engraftment and cure. Conclusion: CD4+CD25+ T cells modulate innate immune responses in murine islets by inhibition of chemokine expression and improve transduced islet engraftment.



Abstract# 993

B LYMPHOCYTE DIRECTED INDUCTION IMMUNOTHERAPY INDUCES LONG-TERM ISLET ALLOGRAFT SURVIVAL IN NON-HUMAN PRIMATES. Chengyang Liu,[1] Ergun Velidedeoglu,[1] Trayn Green,[1] Les Rolf,[1] Susan Y. Rostami,[1] Brigitte Koeberlein,[1] Hooman Noorchashm,[1] Clyde F. Barker,[1] Ali Naji.[1] *[1]Harrison Department of Surgical Research, University of Pennsylvania Medical Center, Philadelphia, PA.*

B Lymphocytes are the most abundant antigen presenting cell (APC) population of the immune system. By virtue of their clonally expressed surface immunoglobulin, these lymphocytes are capable of highly specific alloantigen uptake and presentation. Here, we determined the impact of disrupting cognate T-B collaboration on the fate of islet allografts in a non-human primate (NHP) model. Cynomolgus monkeys were rendered diabetic (150mg/kg-BW STZ) and developed a stable state of hyperglycemia (>300mg/dl) and insulin-dependence. All recipients were transplanted, intra-portally (day 0), with 20000-35000 IEQ/kg of isolated allogeneic islets. Recipients in the control group (n=4) were treated with an induction regimen consisting of 5mg/kg Thymoglobulin (on days 0, 2, 5 and 10) followed by maintenance monotherapy with Rapamycin (to achieve a therapeutic level of 8-15ng/ml). Recipients in the experimental group (n=5) were treated with a combined induction regimen including 5mg/kg Thymoglobulin (on days 0, 2, 5 and 10) and the B lymphocyte depleting mAb, Rituxan (375mg/m2 on days 0 and 5), followed by Rapamycin maintenance. All control recipients rejected their islet grafts within 14-23 days. On the other hand, the experimental group enjoyed long-term allograft survival: 266d, >300d, >400d, >40d (x2). Rapamycin was discontinued at day 200 post islet transplantation in three long-term islet allograft survivors; 2/3 of these long-term survivors continue to remain euglycemic for >300 days. Kinetic flow cytometric analysis of the recipient PBL following induction immunotherapy demonstrated that Thymoglobulin alone promotes transient T cell depletion for up to 3 weeks, as expected, without depleting B lymphocytes. On the other hand, induction immunotherapy with combined Rituxan and Thymoglobulin led to a complete depletion of B lymphocytes lasting for 50-60 days; in addition, to the expected degree of Thymoglobulin mediated T cell depletion. These results indicate that combined transient T- and B-lymphocyte depletion can induce long-term islet allograft survival, while minimizing the need for chronic immunosuppression with Caclineurin inhibitors and steroids in NHPs. Collectively, this preclinical study indicates that early abrogation of cognate T-B lymphocyte interaction may induce long-terms allograft acceptance by disrupting the APC function of B cells and/or preventing the development of anti-donor antibodies.

Abstract# 994

SHORT TERM ANTI-CD4 PLUS ANTI-TNF-α RECEPTOR TREATMENT IN ALLOGENEIC SMALL BOWEL TRANSPLANTATION RESULTS IN LONG TERM ALLOGRAFT SURVIVAL. Jan M. Langrehr,[1] Markus H. Hammer,[2] Kathrin Gube,[2] Andreas Pascher,[1] Dietrich Polenz,[1] Manfred Lehmann,[2] Petra Reinke.[2] *[1]Surgery, Charité, Humboldt University, Berlin, Berlin, Germany; [2]Nephrology, Charité, Humboldt University, Berlin, Berlin, Germany.*

Background: Rejection in up to 70% remains the main problem in clinical small bowel transplantation, even with modern immunosuppression. Treatment with the non-depleting anti-CD4mab induces indefinite survival in heart and kidney transplantation, however, in small bowel transplantation only a marginal improvement in survival could be detected. Differing from specific immune reactions, the early inflammation after transplantation, is due to unspecific ischemia-reperfusion injury. We hypothesized that abrogating the early non-specific inflammation - by blocking the TNF-a receptor activation - in combination with anti-CD4 treatment would increase survival.

Material and Methods: Orthotopic small bowel transplantation was performed in the strongly mismatched DA (RT1a) to Lewis (RT1l) combination. Group I were untreated controls (n=7), group II was treated 10 times (20 mg/kg iv, day –1 to +21) with the non-depleting anti-CD4mab (Rib5/2) (n = 7). Group III received 4 times anti-TNF-a receptor mabs (0,3 mg/kg iv; 60 min pretransplant – day +9) (n = 6). Group IV was treated with the combination of both antibodies (n = 12). The follow-up included survival time, clinical outcome, histology and cytokine quantification by Real-Time PCR (TaqMan).

Results: Non-treated controls were rejected after median of 8.3 days. TNF-a blockade alone showed similar median survival (8.8 days). The anti-CD4mab treatment resulted in moderately prolonged median graft survival (19.7 days) and the combination anti-TNF and anti-CD4 resulted in significantly prolonged graft survival of 103.4 days (range 12-387) with 2/3 of the animals surviving longer than 50 days. acute rejection was observed in group I, II and III, whereas only moderate cellular infiltration could be found after combined treatment. At day 3 grafts were harvested and analysed for cytokine expression. The lowest level of IFN-g and CD25 - a marker of activated T-cells - was found in the combination group, representing a diminuished cellular infiltration due to missing non-specific inflammation. A shift towards a Th2 response was not responsible for the beneficial effect (no differences in IL-4, IL-10 and TGF-b expression).

Conclusions: The data clearly showed that early treatment of non-specific inflammation resulted in long time survival in a highly immunogenic transplant situation. The use of available TNF-a inhibitors may deliver substantial benefit to clinical small bowel transplantation.

Abstract# 995

LONG-TERM CARDIAC ALLOGRAFT SURVIVAL AFTER DUAL THERAPY WITH ANTI-CD40L AND ANTI-LFA-1 ANTIBODIES. Biagio A. Pietra,[1] Robert J. Plenter,[1] Susan O. Cushing,[2] An Doan,[1] Ronald G. Gill.[1] *[1]Dept. of Pediatrics, The Children's Hospital, Denver, CO; [2]Barbara Davis Center, University of Colorado, Denver, CO.*

MR-1 (anti-CD40L, anti-CD154) has been shown to be effective in prolongation of heart allografts in mice. The combination of MR-1 and KBA (anti LFA-1) has been shown to prolong islet allograft rejection and leads to tolerance. Recently, LFA-1 has been shown to be capable of intracellular signaling independent of CD28 costimulatory signals. We asked if the combination of MR-1 and KBA monoclonal antibodies could induce long-term cardiac allograft survival in mice. Balb/c were heterotopically transplanted into C57BL/6 mice which received control Abs, MR-1 alone, KBA alone, or both MR-1 and KBA. KBA was administered at 200micrograms on days 0, 1, 7, 14 (4 doses) by I.P. injection. MR-1 250 micrograms was administered on days -1, then Q3-4days x9 doses by I.P. injection. Rat IgG and Hamster IgG was administered in control mice in identical fashion, respectively. Rejection was defined as cessation of heart beat by daily palpation. Rejection was confirmed by H&E microscopy. Balb/c hearts were rejected in C57BL/6 in 6.3 days (n=3, 4, 7, 8) which was not different from mice treated with control antibody alone, Hamster IgG, 8.7days (n=3, 7, 9, 10), Rat IgG 11 days (n=3, 7, 8, 18), nor both 8.3 days (n=3, 7, 9, 9). However, immunotherapy with MR-1 resulted in prolonged allograft survival 2/6 >60 days (n=6, >60x2, 19, 20, 22, 42), KBA alone resulted in 4/6 >60days (n=6, >60x4, 28, 33), but combination therapy KBA+MR-1 resulted in 5/5 >60days. No data is available on whether long-term mice demonstrate donor specific tolerance. We conclude that combination therapy with KBA and MR-1 is extremely effective in promoting long term cardiac allograft survival in mice. These results support the findings that LFA-1 could transmit co-stimulatory signals and is attractive target for cardiac transplantation.

Abstract# 996

SANGLIFEHRIN A, A NEW SELECTIVE IN VIVO INHIBITOR OF BIOACTIVE IL-12 PRODUCTION. Holger Hackstein,[1] Christoph Steinschulte,[1] Timucin Taner,[2] Angus W. Thomson,[2] Gregor Bein.[1] *[1]Clinical Immunology and Transfusion Medicine, Justus Liebig University Giessen, Giessen, Germany; [2]Thomas E. Starzl Transplantation Institute and Department of Immunology, University of Pittsburgh Medical Center, Pittsburgh, PA.*

Background: Recently, we have discovered that the novel cyclophilin-binding immunosuppressant Sanglifehrin A (SFA) blocks bioactive IL-12 production by human dendritic cells in vitro. Sanglifehrin A is structurally related to cylosporine, but unlike the latter, does not inhibit calcineurin activity. The molecular mechanism of SFA is currently unknown. Here, we have analysed the capacity of SFA to impact on proinflammatory (IL-12p70, TNF-α) and immunomodulatory (IL-10) cytokine production in vivo. By using independent in vivo models that employ different IL-12 inducers we provide evidence that SFA abrogates IL-12p70 production in vivo while having minor or no effects on IL-10 and TNF-α production.

Methods: Mice (C57BL/10, $H2K^b$) were injected intraperitoneally (i.p.) for 3 days with SFA (10 mg/kg/d) or drug vehicle to study drug effects under steady-state conditions. Additionally, to explore SFA's effects specifically on dendritic cells (DCs) under dynamic conditions, we expanded DCs 20-30 fold in vivo by injecting the endogenous growth factor Flt3L (10 mg/d; 10d, i.p.) and co-injecting SFA (10 mg/kg/10d, i.p.). Subsequently, under either conditions, animals were stimulated with CpG DNA or LPS and IL-4 i.p. Four hours later, peripheral blood was drawn after cardiac puncture and splenic and bone marrow DC subsets were analysed by flow cytometry.

Results: The data show that a 3-day course of SFA inhibited 70% of in vivo IL-12p70 production, induced by either CpG or LPS/IL-4 stimulation. Under dynamic conditions, a 10-day course of SFA blocked 95% of LPS/IL-4 induced IL-12p70 and 98% of CPG-induced IL-12p70 production in vivo. These effects are not due to suppressive effects of SFA on total DC numbers or DC subsets, as indicated by four colour flow cytometry. In direct contrast, the production of TNF-α and the immunoregulatory cytokine IL-10 were only moderately (<20% compared to vehicle-injected controls) affected by SFA.

Conclusion: In conclusion we propose that SFA represents functionally a novel class of immunophilin-binding immunosuppressants that has a high selectivity and potency to abrogate production of the major proinflammatory and Th1-skewing cytokine IL-12p70.

Abstract# 997

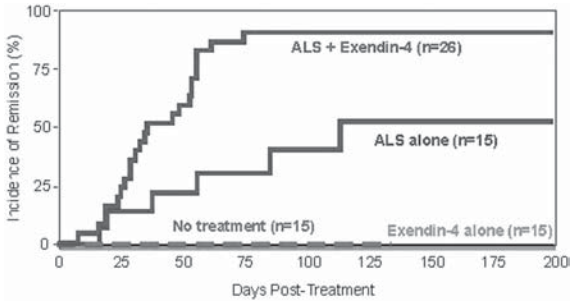
MULTIPLE MECHANISMS OF B CELL AND PLASMA CELL APOPTOSIS INDUCED BY POLYCLONAL RABBIT ANTI-THYMOCYTE GLOBULIN (rATG). Martin S. Zand,[1] Thuong Vo,[3] Jennifer Huggins,[2] Tina Pellegrin.[1] *[1]Nephrology Unit; [2]Rheumatology Unit; [3]Department of Surgery, University of Rochester Medical Center, Rochester, NY.*

Polyclonal rabbit anti-thymocyte globulin induces rapid apoptosis of human B cells *in vitro*, and has been used succesfully to treat alloantibody mediated renal allograft rejection. Antibodies directed at B cell antigens in rATG are likely due to the presence of thymus resident B cells in the immunizing preparation. Apoptosis induced by rATG occurs within 2 hours in the absence of complement. In this study, we identify several B cell surface proteins linked to specific apoptotic pathways, demonstrate caspase dependent and independent mechanisms of rATG induced apoptosis in naive and activated (CpG or CD40L) B cells, and normal human bone marrow resident plasma cells (PC). **Methods**: Naive and memory human B cells were obtained by phlebotomy followed by CD19 magnetic bead isolation. Normal human PC's were obtained by bone marrow aspiration from volunteers followed by CD138 magnetic bead isolation. B cell blasts were obtained culture with CpG DNA or CD40L in the presence of IL-2, IL-10, and IL-15. PC's were also generated *in vitro* by secondary culture with IL-6, IL-15, IFN-α, and hyalurinic acid. Apoptosis was assessed after 18h incubation in rATG (Thymoglobulin; 100 µg/ml) by annexin-V / TOPRO-3 staining, caspase 3/8/9 activation by fluorochrome-tagged caspase substrate labelling, and mitochondrial membrane depolarization by Mitotracker red dye. rATG binding specificities were assessed by rATG compatative inhibition of monclonal antibody binding (CD5, CD19, CD20, CD27, CD38, CD40, CD80, Fas, HLA-ABC, HLA-DR). **Results:** rATG induced rapid apoptosis of naive (94%) and activated (98%) B cells, as well as PC's (87%). Activation of caspase 3, 8, and 9 could be identified in all cell types after 3-6 hours of incubation in rATG. The pan-caspase inhibitor zVAD-fmk reduced, but did not eliminate, rATG apoptosis. T cell adsorbed rATG had high anti-B cell activity, suggesting that apoptosis may be specific for B cell antigens. Compatative binding experiments indicate the presence of antibodies to several pro-aptotic surface signalling molecules including: CD20, CD38, CD95, HLA-DR, and CD27. Similar results were obtained at all stages of B cell differentiation using an *in vitro* B cell-to-plasma cell differentiation system. **Conclusion**: rATG contains antibodies to multiple apotosis surface signaling proteins (CD20, CD27, CD5, HLA-DR, CD27, CD95), and activates multiple apoptotic pathways, in B and plasma cells. These findings support the clinical use of rATG to treat alloantibody mediated allograft rejection.

Abstract# 998

TRANSIENT SHORT COURSE OF POLYCLONAL ANTI-T CELL ANTIBODY FOR CURE OF FULL BLOWN DIABETES. Takashi Maki,[1] Norihiko Ogawa,[1] James F. List,[2] Joel F. Habener.[2] *[1]Surgery, Beth Israel Deaconess Medical Center, Boston, MA; [2]Medicine, Massachusetts General Hospital, Boston, MA.*

Treatment of diabetes-prone, yet clinically healthy individuals, with immunosuppressive drugs is not a desirable form of therapy because of potential complications associated with chronic use of the drugs. However, transient use of immunosuppression in already diabetic patients may be well justified if the therapy has the potential to cure diabetes. We investigated whether antilymphocyte serum (ALS), a polyclonal anti-T cell antibody, effectively cures full blown diabetes in NOD mice, a mouse model of type 1 diabetes. Methods: NOD mice with >300 mg/dl blood glucose in 3 consecutive measurements were considered diabetic and divided into 4 treatment groups: ALS alone; ALS + exendin-4, an agent that stimulates beta cell replication and differentiation; exendin-4 alone; and no treatment. ALS (0.5 ml, i.p.) was given twice three days apart immediately after diagnosis of diabetes. Exendin-4 was given i.p. at 12 nmol/kg for 4 consecutive days twice with 3 days of rest in-between. No further treatment was given except insulin that was given to all diabetic mice. Persistent <200 mg/dl blood glucose was considered a cure. Results: ALS alone achieved reversal of overt diabetes in 50% of mice within 115 days. Addition of exendin-4 to ALS increased the cure rate to 89% within 75 days. Cure of diabetes was accompanied by progressive normalization of glucose tolerance, improvement of islet histology, increased insulin in the pancreas, and release of insulin in response to glucose challenge, all of which indicate an increased beta cell mass. Syngeneic NOD/SCID islets transplanted in cured mice remained intact, suggesting long-term abrogation of autoimmunity. No treatment or exendin-4 alone failed to produce disease remission. Conclusion: A transient short course of polyclonal anti-T cell antibody combined with beta cell growth factors such as exendin-4 presents a novel approach to the cure of new onset type 1 diabetes.



IMMUNOSUPPRESSION COMPLICATIONS AND MEDICAL COMPLICATIONS

Abstract# 999

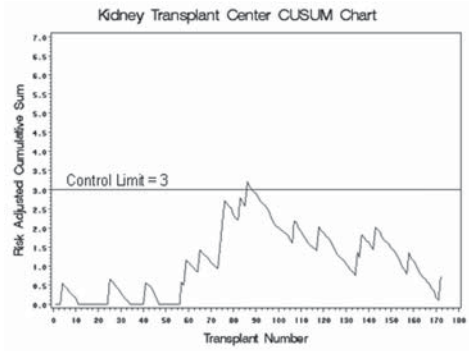
TRANSPLANT CENTER MONITORING USING A CONTINUOUSLY UPDATABLE, RISK-ADJUSTED TECHNIQUE (CUSUM). David A. Axelrod,[1] Mary K. Guidinger,[2] Robert A. Metzger,[3] Russell H. Wiesner,[4] Randall L. Webb,[2] Robert M. Merion.[1,2] *[1]Northwestern University, Chicago, IL; [2]URREA, Ann Arbor, MI; [3]TransLife-Florida Hospital Medical Center, Orlando, FL; [4]Mayo Clinic, Rochester, MN.*

Assessing and monitoring transplant outcomes is an important public policy goal. Current outcomes monitoring is episodic and time-delayed. We explored the use of the cumulative summation technique (CUSUM) to provide real-time, continuously updated, risk-adjusted monitoring of outcomes.

Methods: Data from the Scientific Registry of Transplant Recipients were reviewed for adult kidney and liver transplants performed between 1/97 and 12/01. Multivariate logistic regression models were fitted to predict graft failure (kidney) and death (liver) risk. These models were used to construct a CUSUM outcome chart for 258 renal and 114 liver transplant centers. Using a risk-adjusted cumulative sum of 3 as a control limit, we compared centers flagged by CUSUM for potentially substandard performance to centers flagged by the current SRTR/OPTN method.

Results: During the study period, 60,470 kidney transplants were performed with a 1-year graft failure rate of 9.2%. CUSUM signaling flagged 57 centers and identified 20 of 22 centers identified by the current methods. CUSUM-flagged centers, which were not identified by the SRTR/OPTN method, had deteriorating performance as demonstrated by an increasing CUSUM score (Figure). During the study period, 18,277 liver transplants were performed with a 1-year mortality rate of 13.9%. The CUSUM method identified 27 centers as having potentially declining performance, including 9 of 11 centers identified using the SRTR/OPTN method.

CUSUM monitoring can identify transplant centers with significant changes in performance over time. This technique appears to be more sensitive than existing methods because it includes data from all previous transplants. Adopting CUSUM monitoring techniques may lead to improved transplant outcomes by rapidly identifying significant performance changes at transplant centers.



Abstract# 1000

FTY720 WITH REDUCED DOSE NEORAL (RDN) MAY OFFER A SAFETY/TOLERABILITY PROFILE ADVANTAGE OVER CONVENTIONAL IMMUNOSUPPRESSIVE THERAPIES. G. Russ,[1] R. M. Ferguson,[2] S. Mulgaonkar,[3] H. Tedesco-Silva,[4] F. Oppenheimer,[5] R. Walker,[6] A. Knoflach,[7] U. Kunzendorf,[8] Y. Patel,[9] R. Preiss.[10] *[1]The Queen Elizabeth Hospital, Woodville, SA, Australia; [2]Ohio State University Medical Center, Columbus, OH; [3]Saint Barnabas Medical Center, Livingston, NJ; [4]Hospital do Rim e Hipertensao, Sao Paulo, SP, Brazil; [5]Hospital Clinico Y Provincial de Barcelona, Barcelona, Spain; [6]Royal Melbourne Hospital, Parkville, VIC, Australia; [7]Universitatsspital Zuerich, Zuerich, Switzerland; [8]University of Schleswig, Kiel, Germany; [9]Novartis Pharmaceuticals Corporation, East Hanover, NJ; [10]Novartis Pharma AG, Basle, Switzerland.*

Introduction: FTY720 is the first in a new class of immunomodulators and may represent a better tolerated therapy for post-transplant maintenance regimens. A phase 2 study reported equivalent efficacy for FTY720 5 mg/RDN vs MMF/full dose Neoral (FDN) in *de novo* renal transplant recipients. The safety/tolerability profile for FTY720 at 12 months post-transplant was analysed. **Methods:** Adult patients undergoing a primary cadaveric or living donor transplant were randomised to: daily doses of FTY720 5 mg/RDN, FTY720 2.5 mg/RDN, FTY720 2.5 mg/FDN or MMF 2.0 g/FDN. All patients received corticosteroids but no antibody induction therapy. For the RDN regimens, the Neoral dose (C2 level) was adjusted to achieve exposure at 50% less than C2 targets for FDN regimens. **Results:** 261 patients who received at least one dose of maintenance medication were included. Patient demographics were similar across treatment groups except for the inclusion of more male subjects who received FTY720. Although the overall incidence of adverse events and serious adverse events were comparable, the metabolic profile for FTY720 5 mg/RDN showed a trend towards a potential safety advantage compared with MMF/FDN: for patients without lipid-lowering therapy HDL >1.55 mmol/L (48.3% vs 30.0%, respectively) and LDL ≤3.35 mmol/L (51.7% vs 60.0%, respectively). The incidence of clinically relevant infections is reported below. Owing to the expected pharmacodynamic effect on heart rate, bradycardia was reported for all FTY720 groups (28.9–29.2%); this was transient, self limiting and not associated with a relevant increase in cardiac morbidity. No difference in malignancies was reported for FTY720 5 mg/RDN and MMF treatments (2.8% vs 2.6%). **Conclusions:** FTY720 5 mg/day plus RDN is well tolerated in renal transplant patients and may confer safety advantages over conventional regimens with respect to its improved metabolic profile and reduced incidence of infections.

%	5 mg FTY720/RDN (n=72)	2.5 mg FTY720/FDN (n=74)	MMF/FDN (n=39)
Bacterial infection	25.0	39.5	38.5
UTI	18.1	27.6	30.8
H simplex	5.6	6.6	10.3
CMV	1.4	9.2	7.7

FTY720 2.5 mg/RDN not included beyond month 6 (switched to standard therapy)

Abstract# 1001

CALCIUM CHANNEL BLOCKADE AND PREVENTION OF RENAL GRAFT FUNCTION DETERIORATION IN CYCLOSPORINE-TREATED RECIPIENTS: A MULTI-CENTRE PROSPECTIVE, RANDOMIZED, PLACEBO-CONTROLLED, 2-YEAR STUDY. Dirk R. J. Kuypers,[1] Hans H. Neumayer,[2] Lutz Fritsche,[2] Klemens Budde,[2] Yves Vanrenterghem.[1] *[1]Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [2]Department of Nephrology and Renal Transplantation, University of Berlin, Charité, Berlin, Germany; [3]for the Lacidipine Study Group.*

Studies have provided conflicting results as to the protective role of calcium channel blockers (CCB) in cyclosporine(CsA)-treated patients with regard to blood pressure control and preservation of renal graft function.

We conducted a multicentre prospective, randomised, placebo-controlled study in *de novo* recipients of a primary cadaveric renal graft on CsA therapy. The aim of this 2-year study was to demonstrate that lacidipine could prevent deterioration of renal graft function and to asses the effect of lacidipine on graft function (plasma iohexol clearance), renal plasma flow (plasma PAH clearance), anastomotic arterial blood flow (doppler), systolic/diastolic blood pressure, acute rejection and hospitalisation rate.

A total of 118 recipients were available for an intention-to-treat analysis on efficacy (lacidipine: n=59; placebo: n=59). Three patients on lacidipine therapy and 4 on placebo experienced treatment failure defined as an increase in serum creatinine from baseline of more than 60% (p=0.57). Graft function assessed by serum creatinine concentration and measured GFR, was better in lacidipine-treated patients from 1 year onwards (p<0.01 and p<0.05). RPF and anastomotic blood flow were not persistently higher in lacidipine-treated patients. Study groups did not differ in acute rejection rate, trough blood CsA concentrations, systolic /diastolic blood pressure, number of anti-hypertensive drugs, hospitalisation rate and adverse event rate.

The use of lacidipine in CsA-treated renal recipients results in a significantly better graft function at 2 years and this effect is independent of blood pressure.

Plasma iohexol clearance (GFR) and PAH clearance (RPF), anastomotic blood flow and serum creatinine at 1,12 and 24 months post-transplantation.

	Month 1	Month 12	Month 24
GFR (mL/min/1.73m²):			
Placebo group:	46.7 ± 16	42.6 ± 15.2	42 ± 15.4
Lacidipine group:	50.2 ± 13.9	50 ± 14.6*	50.1 ± 16.2*
Renal Plasma Flow (mL/min/1.73m²):			
Placebo group:	358 ± 83	349.8 ± 87	348.5 ± 94
Lacidipine group:	378.5 ± 96	375.1 ± 90*	378.3 ± 82
Anastomotic blood flow (cm/sec):			
Placebo group:	110.9 ± 39	113.9 ± 37	115 ± 51
Lacidipine group:	123.8 ± 49	143.9 ± 73*	120.3 ± 49
Serum creatinine (mg/dL):			
Placebo group:	1.96 ± 0.87	1.8 ± 0.55	1.72 ± 0.47
Lacidipine group:	1.75 ± 1.2	1.54 ± 0.54*	1.47 ± 0.5*

*p<0.05: statistically significantly different between placebo group and lacidipine group.

Abstract# 1002

PREGNANCIES FATHERED BY MALE KIDNEY TRANSPLANT RECIPIENTS ON NEWER IMMUNOSUPPRESSIVE MEDICATIONS. Scott W. Cowan,[1] Antonella Lavelanet,[1] Lisa A. Coscia,[1] Michael J. Moritz,[2] Vincent T. Armenti.[1] *[1]Surgery, Thomas Jefferson University, Philadelphia, PA; [2]Surgery, Drexel University College of Medicine, Philadelphia, PA.*

This study analyzed 126 outcomes of 120 pregnancies (including twins and triplets) fathered by 97 kidney transplant recipients on newer immunosuppressive medications reported to the National Transplantation Pregnancy Registry (NTPR). Data were collected via questionnaires, phone interviews and hospital records. In the general U.S. population, birth defects occur in approximately 3-5% of children. Of the 126 outcomes, there were 115 livebirths (91%) and 11 spontaneous abortions (9%, one due to Turner's syndrome). There were no ectopic pregnancies, therapeutic abortions or stillbirths. The mean gestational age was 39 ± 2.4 weeks, range 31-43 weeks, with a mean birthweight of 3244 ± 649 grams, range 1417 - 4848 grams. Included in the table are the immunosuppressive regimens administered around the time of conception. One recipient received two doses of Campath 1H post-transplant. Another recipient was participating in an investigational drug trial (ERL).

Regimen	Number of fathered pregnancies	Newborn problems
Neoral®, aza, pred	31 (25.8%)	tongue tied (1)
Neoral®, MMF, pred	28 (23.3%)	polydactyly (1)
Neoral®, pred	14 (11.7%)	ureteral reflux (1)
Neoral®, aza	3 (2.5%)	tongue tied (1)
Neoral®, MMF	1 (0.8%)	
Neoral®, ERL	1 (0.8%)	ureteral reflux (1)
Gengraf®, MMF, pred	3 (2.5%)	
tacro, MMF, pred	16 (13.3%)	undescended testicle (1)
tacro, pred	10 (8.3%)	inherited kidney disease (1)
tacro, aza, pred	2 (1.7%)	
tacro, MMF	1 (0.8%)	
tacro alone	2 (1.7%)	spina bifida (1)
Sandimmune®, MMF, pred	4 (3.3%)	
MMF, pred	3 (2.5%)	
sirolimus, pred	1 (0.8%)	

aza=azathioprine, MMF=mycophenolate mofetil, pred=prednisone

Current paternal graft function was adequate for 87.6%, reduced in 2.1%, not functioning in 4.1% and unknown in 6.2%. Among the 115 children, the majority were developing well with 7 children reporting continuing problems. There was 1 major malformation (spina bifida) and 4 minor anomalies (tongue-tied (2), polydactyly, undescended testicle) for an overall rate of 4.3% (5/115).

CONCLUSIONS: Compared to the general population, reports to the NTPR do not reveal an increase in the incidence of birth defects in the newborn of pregnancies fathered by kidney transplant recipients on newer immunosuppressive medications.

Abstract# 1003

IMMUNOSUPPRESSIVE CHANGES BEFORE OR DURING PREGNANCY: DO THEY AFFECT NEWBORN AND GRAFT OUTCOMES? William J. Gaughan,[1] Lisa A. Coscia,[2] Stephen R. Dunn,[1] Michael J. Moritz,[3] Vincent T. Armenti.[2] *[1]Medicine, Thomas Jefferson University, Philadelphia, PA; [2]Surgery, Thomas Jefferson University, Philadelphia, PA; [3]Surgery, Drexel University College of Medicine, Philadelphia, PA.*

This study analyzed pregnancy outcomes in female kidney recipients who had changes in adjunctive immunosuppression before conception or during pregnancy. Data were collected via questionnaires, phone interviews and hospital records. Analysis was by 1-sided Fisher's Exact test or, for continuous variables, by 2-sided independent t-test. Of the female kidney recipients reported to the National Transplantation Pregnancy Registry, there were 24 kidney recipients (29 pregnancies, 31 outcomes, group A) with an adjunctive immunosuppressive discontinuation or switch before or during pregnancy. In group A, changes were: discontinuing azathioprine (aza) or mycophenolate mofetil (MMF), switching MMF to aza, or switching sirolimus to aza. These recipients were compared to 80 recipients (99 pregnancies, 103 outcomes, group B) where adjunctive immunosuppressive therapy was not switched or changed. All recipients in both groups were receiving Neoral® or tacrolimus in combination with other agents. MMF was the adjunctive therapy in A 19 and B 7 pregnancies; sirolimus was the adjunctive therapy in A 2 and B 1 pregnancies. Significant outcomes included: transplant to conception interval p=0.03 (A 3.6 vs. B 5.2 years), prematurity p=0.01 (<37 wks; A 69.6% vs. B 40%), and neonatal complications p=0.03 (A 64% vs. B 39%).

Graft loss within 2 years of delivery approached significance: p=0.08, A 17% (4/24) vs. B 5% (4/80). Mean serum creatinine was similar before, during and after pregnancy between the groups. There were no significant differences in hypertension, rejection (A 3.5% vs. B 2%), and diabetes mellitus during pregnancy, livebirths, stillbirths, spontaneous abortions, and birthweights. Structural malformations were reported in 4% A and 5.1% B newborn (p=not significant). There was one neonatal death reported in Group A.

CONCLUSIONS: When compared to recipients who continued their adjunctive immunosuppressive therapy, female kidney recipients who switched or discontinued their adjunctive therapy before or during pregnancy did not have a significant increase in the incidence of rejection during pregnancy, although somewhat poorer graft outcomes were reported. Whether graft survival is related to adjunctive immunosuppressive changes before or during pregnancy requires further study. While the incidence of newborn structural malformations was similar between groups, pregnancy exposures remain limited with the newer agents.

Abstract# 1004

NEPHROTIC-RANGE PROTEINURIA AND OTHER SEVERE ADVERSE EVENTS AFTER CONVERSION TO SIROLIMUS IN RENAL TRANSPLANTATION: IS HIGH EXPOSURE TO SIROLIMUS RESPONSIBLE? Karine Hadaya,[1] Martin Wissing,[1] Nylufer Broeders,[1] Daniel Abramowicz.[1] *[1]Nephrology, Hopital Erasme, ULB, Brussels, Belgium.*

Sirolimus (SRL) is an immunosuppressive drug with little or no nephrotoxicity. Conversion to SRL some time after renal transplantation (RT) is most often undertaken as rescue therapy for chronic allograft dysfunction (CAD). The efficacy and the side effects of this strategy are still largely unknown. We have retrospectively analyzed a cohort of 23 renal transplant recipients converted to SRL as main immunosuppressive agent in our center.

Between March 2000 and October 2002, 23 patients were converted to SRL at a median of 667 days (range, 44-4995) after RT. In 20 patients, SRL replaced a calcineurin inhibitors (CNI) because of: CAD (n=18), repeated acute rejection episodes (AR) (n=1) or severe tremor (n=1). In 3 patients, SRL replaced MMF because of: gastrointestinal intolerance (n=2) or depression (n=1). At the time of conversion, SCr was 2.43±1.07 mg/dL (mean ± SD). On March 2003, 7/23 patients (30.5%) are still on SRL with a stable renal function (SCr 2.36±1.18 mg/dL; p= NS vs preconversion). In 16/23 (69.5%) patients, SRL was discontinued after a mean of 125 days (range, 28-335) because of death (N=2; MOF and cerebral hemorrhage), graft failure (N=2) , and severe adverse events (one or more) in 12 patients: 1 acute pancreatitis, 1 hepatitis, 1 abdominal abscess, 1 delayed wound healing, 1 stroke, 1 AR, 1 severe hypertriglyceridemia, 1 raised SCr and 7 de novo nephrotic-range proteinuria (median 2.9g/day; range, 2.5-9.8) that occurred 9 days after conversion (mean; range, 5-117). In the univariate Cox analysis, SRL discontinuation was associated with a previous AR (RR=2.5), a retransplantation (RR=2.8), the number of HLA ABDR mismatches (R=1.6) and the highest SRL through level recorded during the first month post-conversion (Max SRL) (RR=1.36). In the multivariate Cox analysis, only the Max SRL was a risk factor for SRL discontinuation (RR=1.36). The nephrotic-range proteinuria was reversible after SRL discontinuation in 6/7 patients. A previous AR was the only risk factor associated.

We conclude that conversion to SRL as main immunosuppressive drug in RT was associated with a considerable incidence of major side effects leading to SRL discontinuation in more than 2/3 of the patients. A nephrotic-range proteinuria, that seems reversible after SRL discontinuation, occurred in almost 1/3 of the patients. High exposure to SRL after conversion might have contributed to the high incidence of side effects.

Abstract# 1005

DE NOVO ANORECTAL CANCERS IN RENAL TRANSPLANT RECIPIENTS. S. M. Rudich,[1] J. F. Buell,[1] T. M. Beebe,[1] T. G. Gross,[1] M. J. Hanaway,[1] R. R. Alloway,[1] J. Trofe,[1] E. S. Woodle.[1] *[1]Division of Transplantation, Univ of Cincinnati, Cincinnati, OH.*

Anal cancer has been associated with viral stimulation including HPV and anal condyloma. It is usually a limited malignancy compared to de novo malignancies of the colon and rectum. Squamous cell cancer of the anorectum is known to occur with higher frequency in transplant recipients. **Purpose:** To examine anal cancers in renal txp recipients. **Methods:** We examined all cases of anal canal cancers arising de novo in patients reported to our database for both patient demographics and tumor characteristics. **Results:** 30 renal allograft recipients presented with anal canal cancers. In txp recipients, there was a 12% incidence of anal canal cancers (30/234), which is significantly higher than the 2% noted by SEER. The incidence of higher stage malignancies was considerable: stage I (n=9), stage II (n=10), stage III (n=4), and stage IV (n=7). The average age at cancer diagnosis (55.6 yrs) was significantly less than that of the general (non-txp) population as reported by the SEER registry (74 yrs). The majority of patients were male 23/30 (77%), which is in direct contrast with SEER data, which notes a 2/3 majority of females (p<0.001). The time from txp to cancer diagnosis was shorter in anal canal tumors than in rectal SCC or colorectal tumors.

	Anal canal CA	SCC of the rectum	Colorectal CA	p value
No. of cases	30	54	150	ns
Age at dx (yrs)	55.6	49.5	54	ns
Months from txp to CA dx	86.0	100.8	103.5	< 0.05

Stage I tumors were treated with local excision in the majority of cases, with colonic sleeve resection reserved for 2 cases; all with universal survival. Stage II cancers were also treated conservatively with local resection, except for 2 cases treated with APR; with one death due to malignancy in an APR pt. Stage III disease was treated in 75% of cases with APR, with universal survival. However, of the 7 stage IV renal txp recipients, only 2 pts had colonic sleeve resection, with the remaing receiving only paliative therapy; all succumbed to disease within 14.0 months following diagnosis.

Conclusions: Compared to the general population, anorectal cancers in renal txp recipients were found more frequently at a younger age, in males much more so than females, at a much advanced stage, and at a markedly greater incidence. As these lesions have a strong viral etiology, more thorough and intense surveillance of viral infection should enable better prognostication of disease and allow for treatment prior to the development of cancer.

Abstract# 1006

SKIN MALIGNANCY IN TRANSPLANT RECIPIENTS. J. F. Buell,[1] T. M. Beebe,[1] T. G. Gross,[1] M. J. Hanaway,[1] J. Trofe,[1] R. R. Alloway,[1] E. S. Woodle.[1] *[1]Israel Penn International Transplant Tumor Registry/Div. of Transplantation, University of Cincinnati, Cincinnati, OH.*

Skin cancer is the most common malignancy identified among transplant recipients. Considerable differences exist in tumor distribution and biologic activity between transplant recipients and patients in the general population. This study seeks to highlight some of those significant differences

Methods: A retrospective review of all transplant recipients with skin malignancies was performed. Patient demographics and tumor characteristics were examined.

Results: 2018 transplant recipients from the United States with skin malignancies were examined. Three main groups were identified: squamous cell cancer (SCC), basal cell cancer (BCC), and concomitant malignancies (BCC/SCC). Squamous cell to basal cell cancer ratio was found to be 1.9 to 1. Mean patient age was similar among all groups. The ratio of extra-renal to renal allograft recipients was identical for all three groups (3:1). The interval from transplant to malignancy was long (>50mos) compared to other solid organ or lymphotogenous malignancies, with the greatest interval identified among those with isolated SCC lesions. In the SCC group, there was a 9% incidence of nodal or secondary site involvement affecting the cervix, perineum, or lung. The highest recurrence rate was demonstrated in the combined malignancy group. The interval to recurrence was greatest in the mixed tumor group (41.0 ± 38.6 months), followed by the BCC (29.3 ± 27.5 mos) and SCC (27.3 ± 28.5 mos) groups. Cancer specific deaths were significantly higher in the SCC (8%) and BBC/SCC (6.8%) group compared to BCC (3.6%).

	N	Age	Time Post Tx	Recurrence	CA Specific Mortality
BCC	556*	53.5	49.1	12%*	11%*
SCC	1037	52.7	69.1	20%	18%
BCC/SCC	425	54.1	57.3	48%	15%
p-value	<0.05	NS	<0.05	<0.01	<0.05

Conclusions: In the patients reported to the Registry, skin cancer was the most common malignancy. SCC was observed with greater frequency in reported transplant recipients compared to that seen in the general population. Skin malignancies occur after long interval of immunosuppression. SCC alone or in combination with BCC appears aggressive, and often carries with it significant mortality. However, the highest incidence of recurrence was demonstrated in the mixed tumor group.

Abstract# 1007

CORTICOSTEROID AVOIDANCE AMELIORATES LYMPHOCELE FORMATION AND WOUND HEALING COMPLICATIONS ASSOCIATED WITH SIROLIMUS THERAPY: 2 YEAR FOLLOW-UP IN 109 PATIENTS. C. C. Rogers,[1] J. W. Alexander,[1] R. R. Alloway,[1] T. J. Metze,[1] R. E. Boardman,[1] J. Trofe,[1] M. Gupta,[1] T. Merchen,[1] M. J. Hanaway,[1] J. F. Buell,[1] M. Cardi,[1] P. Roy-Chaudhury,[1] E. S. Woodle. *[1]Div of Transplantation, U of Cincinnati, Cincinnati, OH.*

Both sirolimus (RAPA) and corticosteroids (CS) have been shown to adversely impact wound healing. Moreover, since RAPA and CS may have additive effects on wound healing; we sought to determine if corticosteroid avoidance (CSAV) would ameliorate the wound healing complications associated with RAPA. **METHODS**: 109 patients (pts) treated with a CSAV regimen (no pre or post transplant CS) were compared to a historical control group (n=72) that received cyclosporine (CsA), mycophenolate mofetil (MMF) and CS. The CSAV regimen included thymoglobulin (mean 2.6 doses), RAPA (8-12 ng/ml), MMF (2 grams/day), low-dose CsA, (trough 100 ng/ml, discontinued at 4-6 months) along with arginine and canola oil nutritional supplements. Complications were classified as: wound healing complications (WHC) or infectious wound complications (IWC). WHC include lymphocele, hernia, dehiscence, and skin edge separation. IWC include wound abscess and empiric antibiotic therapy for wound erythema. **RESULTS:** The CSAV group was largely CS free: 11% of pts received CS for rejection, 12% received CS for recurrent disease and 85% of pts are currently off CS. 5% of patients who received CS develped wound complications however, CS were started after the development of all wound complications.

	CSAV (n=109)	Control (n=72)	p-value
Mean age at transplant	48.5	46.8	0.44
Pre-transplant diabetes	34 (31%)	21 (29%)	0.68
Males:Females	75:34	42:30	0.09
Mean BMI	26.6	26.8	0.79
Time to WHC (median, days)	297	42	**0.04**
Time to IWC (median, days)	19	29	0.39
WHC incidence	15 (13.7%)	19 (28%)	**0.03**
IWC incidence	13 (12%)	6 (8%)	0.44
Lymphocele	6 (5.5%)	12 (16%)	**0.04**
Wound dehiscence	0	3 (4%)	**0.03**

In a subgroup analysis the reduction in lymphocele formation was seen primarily in patients with a BMI > 30 (3.5% vs. 31.8%, p=0.006). Additional subgroup analyses within groups found a higher incidence of lymphoceles in the BMI > 30 control group than in the BMI< 30 control group (31.8% vs. 10%, p=0.02) and a significantly higher incidence of wound abscesses in the BMI > 30 CSAV group than in the BMI < 30 CSAV group (14.2 vs. 2.4%, p=0.02) **CONCLUSIONS:** CSAV in a RAPA based regimen results in: 1) marked reduction in lymphoceles and WHC and 2) a reduction in lymphoceles primarily in obese patients. CSAV provides a promising approach for addressing WHC associated with RAPA therapy.

Innate and Adaptive Immunity and Cytokines in Ischemia-Reperfusion

Abstract# 1008

T AND B CELLS INTERACT TO MODULATE KIDNEY ISCHEMIA REPERFUSION INJURY. Melissa J. Burne-Taney,[1] Naoko Yokota,[2] Hamid Rabb.[1] *[1]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Nephrology and Hypertension, Seirei Yokohama Hospital, Yokohama, Japan.*

T and B cells have recently been identified as mediators of ischemia reperfusion injury (IRI) in kidney and other organs. The relationships, if any, between T and B cells in mediating IRI are unknown. We used Recombinase Activating Gene-1 (RAG-1) knockout mice, deficient in T and B cells, to study T and B cell interactions in renal IRI. Renal IRI was performed using 30 min bilateral renal clamping. RAG-1 deficient mice on a C57BL/6 or BALB/CJ background were not protected from renal injury compared to wild type mice from matched backgrounds and actually had a worse course of renal IRI. This contrasts to previous data from our laboratory showing that T cell deficient (*nu/nu*)(*J Clin Invest* 108:1283, 2001) and B cell deficient (μMT)(*J Immunol* 171:3210, 2003) mice are protected from renal IRI. Histologic analysis of kidney tissue was performed and no differences were observed between RAG-1 deficient and wild type mice. PMN infiltration was also similar in RAG-1 deficient and wild type mice. We then performed T and B cell specific adoptive transfers separately into RAG-1 deficient mice. T or B cells were purified using negative selection columns and transferred 3 weeks prior to renal IRI. Adoptive transfer of either T or B cells significantly protected RAG-1 deficient mice from renal IRI compared to RAG-1 deficient mice that did not receive a transfer. Confirmation of reconstitution and purity was performed by FACS analysis.

T or B cell adoptive transfer into RAG-1 deficient mice protects from renal IRI

Hours Postischemia	0	24	48	72
RAG deficient	0.84±0.14	2.72±0.29	3.20±0.42	2.67±0.48
RAG + T cells	0.76±0.05	1.62±0.26*	1.60±0.46*	0.94±0.25*
RAG + B cells	0.97±0.16	1.95±0.19*	1.79±0.51*	0.97±0.14*
Wild type	0.80±0.05	1.91±0.27*	1.88±0.35*	0.88±0.21*

These data demonstrate the novel finding that T and B cells interact for full expression of tissue injury after an ischemic insult. Though interactions between T and B cells are well established, this is the first evidence for T and B cell interactions in an allo-antigen independent model of acute tissue injury. T and B cell-directed therapy can not only improve the course of ischemic injury, but by decreasing tissue injury could in turn decrease subsequent cellular and antibody-mediated rejection.

Abstract# 1009

THE TLR4 SIGNALING PATHWAY LINKS INNATE AND ADAPTIVE IMMUNITY IN LIVER ISCHEMIA/REPERFUSION INJURY. Yuan Zhai,[1] Xiu-Da Shen,[1] Feng Gao,[1] Ryan O'Connell,[2] Ronald W. Busuttil,[1] Genhong Cheng,[2] Jerzy W. Kupiec-Weglinski.[1] *[1]The Dumont-UCLA Transplant Center, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Ischemia-reperfusion injury (IRI) represents a serious problem affecting transplantation outcomes. Both innate (e.g., PMNs, complement) and adaptive (T lymphocytes) immunity contribute to the development of liver IRI. However, the molecular mechanisms interlocking these cellular cascades remain to be elucidated, in particular: (1) which innate immune system is triggered by IR? and (2) how its downstream mechanism triggers T cell activation? We identified TLR4 as a possible initiating innate immune system, responsible for hepatic IRI. By microarray analysis of IR-injured livers, we also identifed a single chemokine, IP-10, significantly upregulated in and associated with irreversible IRI. Indeed, post-ischemic neutralization of IP-10 did ameliorate hepatocellular injury. Here, we determined the intracellular signaling pathway of TLR4 activated by IR, which led to IP-10 upregulation/hepatocellular injury in a mouse partial liver warm IR model (90 min. ischemia, followed by 6 h of reperfusion). At least three distinct intracellular signaling pathways have been identified downstream of TLR4 activation, each of which produceing somewhat overlapping but different pro-inflammatory profiles. To differentiate between these pathways, TLR4-, MyD88-, and IRF3-KO mice were used. Liver damage was evaluated by sALT levels and histology (Suzuki criteria). Intrahepatic induction of IP-10 and TNF-α were measured by quantitative RT-PCR. While WT B6 mice suffered severe IRI, as evidenced by increased sALT levels and liver pathology, TLR4 KO mice were IRI resistant (sALT IU/L=276±50 vs. 2149±485 WT controls). MyD88 KO mice remained IRI sensitive (722±479 vs. 1122±306 WT controls), whereas IRF3 KO mice were IRI resistant (396±158 vs. 4467±1737 WT controls). Correlated with the liver damage, intrahepatic IP-10 induction was abolished in TLR4 KO, and IRF3 KO, but sustained in MyD88 KO mice. As a putative effector molecule, intrahepatic TNF-α expression was comparable with IP-10 levels in all the groups. Thus, liver IR activates TLR4 through MyD88-independent, but IRF3-dependent pathway to induce IP-10, which may then be responsible for recruitment/activation of T cells, leading to full scaled IRI. Our study identifies a key signal transduction pathway during liver IRI, which links innate and adaptive immunity in liver IRI. Targeting molecules along this pathway may provide us with much needed and novel anti-IRI therapeutic approaches in the clinics.

Abstract# 1010

HO-1 CYTOPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS IN HEPATIC ISCHEMIA/REPERFUSION INJURY ARE TOLL-LIKE RECEPTOR-4 INDEPENDENT. Sei-ichiro Tsuchihashi, Constantino Fondevila, Jeffrey Ma, Bibo Ke, Yuan Zhai, Ronald W. Busuttil, Jerzy W. Kupiec-Weglinski. *The Dumont-UCLA Transplant Center, David Geffen School of Medicina at UCLA, Los Angeles, CA.*

Background: Overexpression of heme oxygenase (HO)-1, hsp32, protects against cellular stress in many inflammatory events, including ischemia/reperfusion (I/R) injury. The toll-like receptor (TLR) system provides a triggering signal in the pathogenesis of infection and inflammatory diseases, and can promote activation of intracellular pathways leading to cytokine/chemokine expression. This study was designed to explore cytoprotective effects of HO-1 induced by cobalt protoporphyrin (CoPP), and its relationship with the TLR system in a model of mouse hepatic warm I/R injury. **Methods**: Partial warm ischemia was produced in the left/medium hepatic lobes for 90 min followed by 6 h reperfusion in C57BL/6 mice. Experimental animals were divided into 4 groups: 1) sham (n=3); 2) mice treated with saline 24 h before ischemia (n=5); 3) mice treated with CoPP (5.0 mg/kg i.p.) 24 h before ischemia (n=5); 4) mice treated with CoPP as in group 3 plus ZnPP (1.5 mg/kg i.p.) 25 h and 1 h before ischemia, resp. (n=5). Western blot analysis of HO-1 and TLR4 protein expression in the liver were performed before and after I/R. Serum GOT/GPT levels were measured, and liver samples were collected for histology. The mRNA expression of cytokines (TNF-α, IL-1β, INF-γ, and IL-10), INF-γ-inducible protein (IP-10), and TLR4 were analyzed by RT-PCR. **Results**: CoPP treatment significantly increased hepatic expression of HO-1 protein, as compared with untreated/sham controls. Adjunctive ZnPP diminished HO-1 levels before and after reperfusion. TNF-α, IL-1β, INF-γ and IP-10 mRNA expression were significantly inhibited in animals treated with CoPP ($p<0.05$), whereas IL-10 mRNA expression was upregulated ($p<0.05$). Although TLR4 mRNA and protein expression were upregulated by I/R, they were not significantly inhibited by CoPP monotherapy. I/R-induced hepatocellular damage, as measured by sGOT/GPT release (IU/L) was significantly lower in animals treated with CoPP (439±24 and 354±45), as compared with saline (2713±108 and 2672±211, $p<0.05$) or CoPP + ZnPP treated (1960±237

and 2164±496, p<0.05) groups. Histological damage was ameliorated selectively in livers with increased CoPP-induced HO-1 expression. **Conclusion**: This study is the first to document that HO-1 induction protects mice against warm hepatic I/R injury, and in parallel it downregulates the expression of pro-inflammatory cytokines. Unexpectedly, HO-1 cytoprotective mechanism remains TLR4 independent.

Abstract# 1011

A NEW MOUSE MODEL OF PROLONGED HEPATIC COLD ISCHEMIA FOLLOWED BY ORTHOTOPIC LIVER TRANSPLANTATION (OLT). Xiu-Da Shen,[1] Feng Gao,[1] Bibo Ke,[1] Yuan Zhai,[1] Sei-Ichiro Tsuchihashi,[1] Charles R. Lassman,[2] Douglas G. Farmer,[1] Ronald W. Busuttil,[1] Jerzy W. Kupiec-Weglinski.[1] *[1]Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA; [2]Pathology, Dumont-UCLA Transplant Center, Los Angeles, CA.*

Background: The current mouse models of liver injury, resulting from ischemia and reperfusion (IRI) are largely limited to in situ "warm" ischemic component. Indeed, the "cold" IRI model in mice requires a demanding OLT. As "cold" ischemia is critical for a full-blown liver IRI sequel in the clinics, a reliable mouse model of "cold" hepatic ischemia followed by OLT is warranted. Here, we report on the development of such a mouse "cold" IRI/OLT model. **Methods and Results**: Syngeneic OLTs were performed in Balb/c mice. Donor livers preserved in UW solution at 4°C for either 1h (Gr. 1) or 24h (Gr. 2) were transplanted with rearterialization (AOLT), in which a hand-sutured end-to-side anastomosis of the donor's hepatico-aortic-mesenteric arterial segment to the recipient's abdominal aorta was carried out. In Gr. 3, livers were cold preserved for 24h prior to transplantation without arterialization (NOLT). The anhepatic phase was ca. 18 min and hepatic artery patency was >96%. The 100d survival rate was 91.7% (11/12) in Gr. 1; 33.3% (4/12) in Gr. 2 (AOLT); and 8.3% (1/12) in Gr. 3 (NOLT). The sALT, and TNF-α, IL-2, and IFN-γ OLT levels in Gr. 2 were markedly increased at 1h, 6h, 1d and 3d after reperfusion, as compared with Gr. 1 (P<0.05-0.01). In parallel, the expression of TLR4, caspase-3 and IFN-γ-inducible protein 10 (IP-10), a chemoattractant for activated T cells, were all dramatically enhanced in Gr. 2 at d1 and d3 post-OLT. This data was correlated with histological studies of hepatic I/R injury. Thus, in Gr. 2, increased number of apoptotic hepatocytes/endothelial cells was detected at 6h after perfusion, with visible sinusoidal congestion/hepatocyte vacuolization. The hepatocellular necrosis (10-20%) was prominent at d1-3 post-OLT. In contrast, Gr. 1 livers showed good preservation of lobular architecture and no necrosis.**Conclusion**: This is the first study, to report on the development of a new and reproducible mouse model of "cold" hepatic IRI followed by OLT. Indeed, hepatic arterialization is essential for long-term follow-up. Extended "cold" liver ischemia in mice enhanced local TLR4 expression, promoted apoptosis and induced Th1 type proinflammatory response, the cardinal features of hepatic IRI insult. By employing an array of genetically manipulated mouse strains, this model should help us in addressing the key questions related to the mechanism of liver IRI.

Abstract# 1012

SIGNIFICANCE AND THERAPEUTIC POTENTIAL OF PROSTAGLANDIN E2 RECEPTORS IN LIVER ISCHEMIA REPERFUSION INJURY. Yukiyasu Kuzumoto,[1] Masayuki Sho,[1] Naoya Ikeda,[1] Kaoru Hamada,[2] Takashi Mizuno,[1] Satoru Akashi,[1] Yoshikazu Tsurui,[1] Hisanori Kashizuka,[1] Yoshiyuki Nakajima.[1] *[1]First Department of Surgery; [2]Second Department of Internal Medicine, Nara Medical University, Kashihara, Japan.*

Background: Prostaglandin E2 (PGE2) mediates a variety of both innate and adaptive immunity through four distinct receptors; EP1, EP2, EP3 and EP4. Recent studies have suggested the critical role of EP2 and EP4 in several inflammatory disease models. We investigated the significance of EP2 and EP4 receptor and the efficacy of each selective agonist in hepatic ischemia/reperfusion injury (I/R injury).

Methods and Results: Seventy percent partial hepatic ischemia was performed for 90 or 120 minutes in male C57BL/6 mice. First, we evaluated the local expression of EP2 and EP4 in the liver. Both EP2 and EP4 expressed in the naïve liver. EP4 expression was significantly up-regulated after 6h of reperfusion following 90 minutes of ischemia, while EP2 expression was not changed. Furthermore, EP2 agonist treatment did not show any protective effect on liver function. By contrast, EP4 agonist treatment significantly inhibited hepatic injury compared to control at 6 h of reperfusion (sAST: 854±117 vs.4683±1043, sALT:1535±292 vs.6252±1638, sLDH:3884±569 vs.19653±4917). Histological analysis also confirmed this protective effect of EP4 agonist. Massive cellular infiltration and extensive hepatic cellular necrosis was observed in control mice, while the lobular architecture was well preserved and there was few necrosis in EP4 agonist treated mice. To address the underlying mechanism, we evaluated local expression of cytokine, chemokine and adhesion molecule using real-time quantative PCR. EP4 agonist treatment significantly down-regulated the local expression of several pro-inflammatory cytokine (TNF-α, IL-1β and IFN-γ), chemokine (MCP-1 and IP-10) and adhesion molecule (E-selectin and ICAM-1) after 2h of reperfusion following 90 minutes of ischemia. By contrast, IL-10, an anti-inflammatory cytokine, was significantly up-regulated. Finally, the removal of shunt liver after 120 minutes of ischemia resulted in the death of 86 % of mice treated with saline within 48 h. By sharp contrast, 80% of mice treated with EP-4 agonist survived (P=0.016 compared to control).

Conclusion: This study demonstrates for the first time the inhibitory role of EP4 receptor in hepatic I/R injury and therapeutic efficacy of selective EP4 agonist for protection of the liver.

Abstract# 1013

CD40Ig GENE TRANSFER DOWNREGULATES THE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PROTECTS RAT LIVERS FROM ISCHEMIA/REPERFUSION INJURY. Bibo Ke,[1] Xiu-Da Shen,[1] Feng Gao,[1] Douglas G. Farmer,[1] Ronald W. Busuttil,[1] David M. Briscoe,[2] Jerzy W. Kupiec-Weglinski.[1] *[1]Dumont-UCLA Transplant Ctr, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Children's Hospital, Harvard Medical School, Boston, MA.*

Background: Vascular endothelial growth factor (VEGF), a potent angiogenesis factor induced by activated T cells, stimulates angiogenesis in the process of inflammatory response. Our previous studies have shown that CD4+ T cells play an important role in Ag-independent proinflammatory response in ischemia/reperfusion (I/R) injury. This study explores the role of VEGF in rat liver inflammatory injury triggered by I/R and modulated by gene transfer of CD40Ig. **Methods:** Sprague-Dawley (SD) rats were infused with Ad-CD40Ig or Ad-βgal reporter gene ($2.5x10^9$ pfu i.v.). One day later, livers were harvested, preserved for 24 h at 4°C in UW solution, and then transplanted orthotopically (OLT) into syngeneic SD recipients. Animals were followed for survival; separate groups of OLTs were harvested at day 1, 3, 7 and 14, and analyzed histologically. VEGF and cytokine gene expression pattern was screened by RT-PCR. Intragraft expression of anti-oxidant HO-1 and anti-apoptotic Bcl-2/Bcl-xl genes was assessed by Western blots. **Results:** 100% of Ad-CD40Ig pretreated OLTs survived >14 days (vs. 50% in Ad-βgal controls and untreated group; n=6 rats/gr). Unlike Ad-βgal controls, which showed significant edema, moderate sinusoidal congestion, and moderate to severe necrosis (>60%), the Ad-CD40Ig group revealed minimal sinusoidal congestion/necrosis. These correlated with decreased sGOT levels in Ad-CD40Ig group. Ad-CD40Ig gene transfer significantly reduced apoptosis in OLTs treated with Ad-CD40Ig (p<0.005). Intragraft expression of mRNA coding for VEGF in Ad-βgal and untreated controls was consistently increased, as compared with sham controls. However, Ad-CD40Ig gene transfer significantly decreased VEGF expression. Unlike in controls, TNF-α, IL-2/IFN-γ remained depressed, whereas that of IL-4/IL-10 reciprocally increased selectively in the Ad- CD40Ig group. The expression of HO-1 and Bcl-2/Bcl-xl was increased throughout in Ad-CD40Ig transduced OLTs, as compared with Ad-βgal group. **Conclusion:** This is the first report, which documents that VEGF is associated with the process of hepatic inflammatory injury induced by I/R. Ad-based CD40Ig gene therapy downregulated VEGF expression and protected rat OLTs against otherwise severe cold I/R injury. Ad-CD40Ig gene transfer prevented hepatic apoptosis, facilitated Th1 to Th2 shift, and triggered the expression of anti-oxidant/anti-apoptotic genes with cytoprotective functions.

Abstract# 1014

17β-ESTRADIOL DIFERENTIALLY ACTIVATES THE MITOGEN-ACTIVATED PROTEIN-KINASES AND IMPROVES SURVIVAL FOLLOWING REPERFUSION INJURY OF REDUCED-SIZE LIVER. Mario Vilatoba,[1] Guadalupe Bilbao,[1] Christopher Eckstein,[1] Irshad H. Chaudry,[2] Devin E. Eckhoff,[1] Juan L. Contreras.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL; [2]Center for Surgical Research, University of Alabama at Birmingham, Birmingham, AL.*

Isquemia/Reperfusion-Injury (I/R-I) of the liver is a common ocurrence in resectional surgery and liver transplantation and may impair regeneration, ultimatly leading to liver failure. The three major mitogen activated protein kinases (MAPK): ERK, p38 and c-Jun N-terminal kinase (JNK) are critical in transmission of signals triggered by proinflammatory cytokines, stress, and growth factors. Previous studies demonstrated that Estradiol reduces I/R-I. In this study, we assessed the effects of estradiol on liver function, survival and activation of MAPK in a murine model of reduced size liver I/R-I. **Methods**: 70% of liver mass in C57BL/6 mice were subjected to ischemia for 45 minutes. After reperfusion, the non-ischemic lobes were resected. Estradiol or the estrogen antagonist ICI-182780 were given 1 hour before the injury (n=7). ALT was assessed at 6 hours and apoptosis by ELISA at 24 hours. The activation of JNK, p38 and ERK was assessed by Western Blots. **Results**: Females presented lower initial hepatocellular injury (ALT=714±110) and 70% had indefinite survival after a reduced-size I/R-I, whereas no significant changes were observed in males or ovariectomized mice. Higher incidence of apoptosis was observed in male animals given saline (enrichment factor=7.22 ±0.8) versus males treated with estradiol (5.85±0.3, P,0.05). Conversely, estradiol promoted p38β (5.8±1.2 normalized to actin) and ERK (7.12±2.6 normalized to actin) activation compared with controls (1.28±0.8 and 3.36±1.12 normalized to actin, respectively, P<0.05). **Conclusion**: Estradiol limited hepatocellular injury and promoted survival following reduced size I/R-I to liver. Estradiol inhibited the activation of proapoptotic JNK pathway and induced the activation of the antiapoptotic p38β pathway, and proliferation through ERK. Estrogen therapy may be important in clinical conditions associated with I/R-I, specially split or living donor liver transplantation.

Abstract# 1015

CYCLIC RGD PEPTIDES WITH HIGH AFFINITY FOR α5β1 INTEGRIN PROTECT GENETICALLY FAT ZUCKER RAT LIVERS FROM COLD ISCHEMIA/REPERFUSION INJURY. Constantino Fondevila,[1] Xiu-Da Shen,[1] Carolina Moore,[1] Judy Melinek,[2] Ronald W. Busuttil,[1] Ana J. Coito.[1] *[1]Department of Surgery, The David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Department of Pathology, The David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Ischemia/reperfusion (I/R) injury often results in dysfunction of steatotic orthotopic liver transplants (OLT). Expression of fibronectin (FN) by sinusoidal endothelial cells is an early event after liver injury. We hypothesized that FN-leukocyte interactions are critical in liver I/R injury. We have recently shown that α4β1-FN interactions preferentially regulate T cell recruitment in OLT. The present work was designed to assess the function of α5β1-FN interactions in steatotic OLT. **Methods and Results:** cyclic RGD (cRGD) peptides (500 μg/rat), with high affinity for α5β1 integrin, were administered through the portal vein of steatotic Zucker rat livers before and after the 4h cold ischemic storage. Lean Zucker recipients of steatotic OLTs received an additional 3d-course of cRGD peptides (1mg/rat, i.v.). Administration of cRGD peptides significantly increased the 14d OLT survival rate as compared with respective controls (100% vs. 50%, n=8 rats/gr; p<0.001). cRGD treated OLTs showed no signs of vascular congestion or necrosis, contrasting with extensive centrilobular pallor and necrosis in control livers (score: 0.3 ± 0.4 vs. 2.5 ± 0.5; p<0.008), at day 1 post-OLT. Hepatic function was improved in cRGD treated recipients as evidenced by decreased levels of sGOT (717±33 vs. 2437±903; p<0.02), and MPO (0.96±0.12 vs. 1.89±0.35; p<0.03). Moreover, cRGD therapy diminished intra-graft expression (mRNA) of iNOS (~8 fold), without affecting eNOS expression, and of pro-inflammatory cytokines such as TNF-α (~1.4 fold), and IFN-γ (~4 fold). Interestingly, blockade of α5β1-FN interactions had a reduced effect on the initial recruitment of T lymphocytes (7±0.8 vs. 11±2.8; p<0.06); however, it strongly inhibited intra-graft infiltration of monocyte/macrophages (74±5 vs. 173±34; p<0.0006). Leukocyte migration requires adhesion and focal matrix degradation. Metalloproteinase-9 (MMP-9), a gelatinase implied in FN breakdown, was profoundly depressed in cRGD peptide treated livers (MMP-9/actin mRNA: 0.2 vs. 1.8). Indeed, MMP-9 was highly expressed by macrophages in control steatotic OLTs. **Conclusion:** cRGD peptide therapy down-regulated MMP-9 expression, decreased monocyte/macrophage recruitment, and inhibited the expression of iNOS and pro-inflammatory cytokines. Importantly, it significantly improved steatotic liver function and recipient survival. This is the first study to document a function for α5β1 -FN interactions in OLT.

Abstract# 1016

IL-13 PROTECTS MOUSE INTESTINES FROM ISCHEMIA AND REPERFUSION INJURY BY ACTIVATION OF STAT6 PATHWAY. Bibo Ke,[1] Xiu-Da Shen,[1] Ian C. Carmody,[1] Feng Gao,[1] Ronald W. Busuttil,[1] Jerzy W. Kupiec-Weglinski,[1] Douglas G. Farmer.[1] *[1]Dumont-UCLA Transplant Ctr, David Geffen School of Medicine, Los Angeles, CA.*

Background: Ischemia/reperfusion (I/R) injury is one of major factors leading to dysfunction or loss graft function following small intestinal transplantation (SITx). IL-13 has been shown to modulate the inflammatory response by down-regulating the production of proinflammatory cytokines. This study was to evaluate the cytoprotective effects and putative mechanisms of IL-13 against injury in a mouse intestinal I/R model. **Methods:** Male C57BL6 (WT) mice were anesthetized and underwent 100 minutes of warm ischemia induced by clamping the superior mesenteric artery. Mice received either sterile saline or 1 μg recombinant murine IL-13 (rIL-13) via the lateral tail vein before the induction of ischemia. Separate groups were performed for survival and analysis. For the latter group, intestinal tissue was harvested at 4 hr, 24 hr, day 3 and day 7 and assessed for histology, myeloperoxidase, expression of Stat6 and anti-oxidant (HO-1) genes by Western blots, cytokine gene expression by competitive-template RT-PCR. **Results:** 100% of mice pretreated with rIL-13 survived >7 days (vs. 50% in saline controls; n=6 rats/gr). rIL-13 treatment resulted in near normal histopathological architecture. In contrast, controls demonstrated mucosal erosion, villous congestion/ hemorrhage, and apoptosis. rIL-13 treatment also significantly decreased intestinal myeloperoxidase (surrogate marker for neutrophil accumulation) as compared with saline controls (1.72±0.14 vs. 4.55±0.34). The expression of Stat6 and HO-1 were markedly increased in WT mice treated with rIL-13, as compared with that of saline controls. Unlike in controls, the expression of mRNA coding for TNF-α/IL-1β and IL-2/IFN-γ remained depressed, whereas that of IL-13/IL-4 reciprocally increased in the WT treated with rIL-13. **Conclusion:** IL-13 treatment plays a protective role in mouse intestinal warm I/R injury leading to reduced tissue injury and improved survival. IL-13 treatment appears to protect the intestine by reducing inflammatory and Th1 type cytokine expression while fascilitating Th2 type cytokine expression via a Stat6 pathway. Upregulation of anti-oxidant HO-1 by IL-13 may exert synergistic cytoprotection against intestinal inflammatory injury and these results may suggest a HO-1 dependent regulation of intestinal Stat6 inflammation after I/R injury.

Abstract# 1017

LIVER TRANSPLANTATION WITH NEOADJUVANT CHEMORADIOTHERAPY FOR HILAR CHOLANGIOCARCINOMA. Charles B. Rosen,[1] Julie K. Heimbach,[1] Michael G. Haddock,[2] Steven R. Alberts,[3] Scott L. Nyberg,[1] Michael B. Ishitani,[1] Gregory J. Gores.[1] *[1]William J von Liebig Transplant Center, Mayo Clinic, Rochester, MN; [2]Department of Radiation Oncology, Mayo Clinic, Rochester, MN; [3]Department of Oncology, Mayo Clinic, Rochester, MN.*

We developed a protocol combining neoadjuvant radiotherapy, chemosensitization and orthotopic liver transplantation (OLT) for patients with operatively confirmed stage I and II hilar cholangiocarcinoma (CCA) in 1993. We reviewed our experience with specific **AIMS** to 1) delineate the role of the staging operation, 2) assess efficacy, and 3) determine whether results warrant use of donor livers. **METHODS:** All patients had unresectable CCA without evidence of metastases or CCA arising in the setting of primary sclerosing cholangitis (PSC). Diagnostic criteria were intraluminal brush cytology or biopsy or a CA 19.9 level >100ng/ml with a radiographically malignant stricture. Neoadjuvant therapy included external beam irradiation, transcatheter Iridium-192 brachytherapy, and thereafter, protracted IV 5-FU infusion or oral capecitabine. A staging abdominal operation was performed prior to OLT. We compared survival after OLT for patients with CCA versus hepatitis C (HCV), hepatocellular carcinoma (HCC), and PSC whom underwent OLT at our institution during the same time period. **RESULTS:** Fifty-six patients were enrolled in the protocol. Four patients had disease progression and another four patients died prior to completion of neoadjuvant therapy. Fourteen of 48 patients (29%) whom underwent operative staging had findings precluding OLT.

Patient survival from initiation of protocol (% ± SE)

	N	1 Year	3 Year	5 Year
All patients	56	75± 6	54 ± 8	54 ± 8
Staged patients	48	86 ± 5	64 ± 8	64± 8
Negative staging	*34	97 ± 3	84 ± 8	84 ± 8
Positive staging	14	58 ± 14	10 ± 9	10± 9

*28 transplanted, 6 await OLT

Twenty-eight patients underwent OLT. Six patients are awaiting OLT. Three patients died from perioperative complications. Four patients developed recurrent disease 22 - 63 months after OLT; 3 died at 24 - 76 months and one remains alive at 79 months.

Patient survival from OLT (% ± SE)

	N	1 Year	3 Year	5 Year
CCA	28	88 ± 6	82 ± 8	82 ± 8
HCC	67	89 ± 4	83± 7	73 ± 11
HCV	147	91 ± 2	83 ± 3	75 ± 5
PSC	131	96 ± 2	92 ± 3	90 ± 3

CONCLUSIONS: Neoadjuvant chemoradiotherapy with subsequent OLT achieves excellent survival for patients with stage I and II hilar cholangiocarcinoma. The abdominal staging operation is essential. Results with the intention-to-treat analysis compare favorably with published surgical experiences. Patient survival after transplantation is comparable to results of OLT for other chronic liver diseases and justifies use of donor livers for selected CCA patients.

Abstract# 1018

ARE TOO MANY PEDIATRIC LIVER RECIPIENTS TRANSPLANTED AT STATUS 1? Sue V. McDiarmid,[1] Ann M. Harper.[2] *[1]Pediatrics and Surgery, David Geffen School of Medicine, UCLA, Los Angeles, CA; [2]Statistics, United Network of Organ Sharing, Richmond, VA.*

Status 1 for children awaiting liver transplantation differs from adults by including children with chronic liver disease, a policy unchanged with the introduction of PELD/ MELD. This study reports the number of children transplanted with deceased donor grafts at status 1 in the UNOS database, either meeting the standard criteria, or 'by exception' (i.e. approved by the regional review board). Comparisons of demographics between the pre and post-PELD eras, regional differences, PELD scores of status 1 patients, and patient and graft survivals are examined. RESULTS: Data from 18 month periods pre-PELD (8/27/00-2/26/02) and post-PELD (2/27/02-8/27/03) showed that pre-PELD 339 of 710 (47.7%) children were transplanted at status 1 compared to 306 of 741 (41.3%) children post-PELD. The most common diagnoses were acute hepatic necrosis (23.1%) and biliary atresia (22.3%). Of pts with biliary atresia 31.2% were at status 1 by exception. The % of pts at status 1 by exception between the 2 eras were: 23.3% pre-PELD and post-PELD 29.7%. For 9 of 11 regions performing >20 pediatric LT, the % of children transplanted at status 1 was 29.6-61.2% pre-PELD and 22.4-65.7% post-PELD. Of these 6.2-43.3% were at status 1 by exception. The % of retransplants was 21.8% pre-PELD, and 21.2% post-PELD. Between the 2 eras the median age of status 1 by exception pts was the same (1.0 yrs) and was lower than for the standard status 1 pts: 2.0 yrs pre-PELD, 5.0 post-PELD). The mean PELD score of status 1 by exception pts was 14.0 compared to 22.6 for standard status 1 patients (p<0.0001). Table 1 compares pt and graft survivals at 6 months for deceased donor recipients pre and post-PELD for: all children (overall), status 1 (standard), status 1 (exception) and non status 1. There were no significant differences between groups either within or

between eras. CONCLUSIONS: In this analysis pt and graft survival was not effected by status 1 vs nonstatus 1 at LT. However, the practice of transplanting almost half of pediatric recipients at status 1 has an important effect on donor liver allocation. Questions to be addressed are time waiting and death on the list for status 1 and non status 1 children, and the appropriate definition of status 1.

6 month patient and graft survivals.

Era	Overall Patient; Graft Survival	Status 1 standard Patient; Graft Survival	Status 1 exception Patient; Graft Survival	Non-status 1 Patient: Graft Survival
Pre-PELD	90 .3% : 83.2%	86.1%: 77.9%	93.0%: 86.4%	92.5%: 85.1%
Post-PELD	88.6%: 83.0%	81.5%: 72.5%	80.7%: 76.1%	92.8%: 88.6%

Abstract# 1019

HAS THE PELD SYSTEM IMPROVED ORGAN ALLOCATION FOR PEDIATRIC LIVER TRANSPLANT RECIPIENTS WITH CHRONIC LIVER DISEASE? Paolo Salvalaggio,[1] Katie Neighbors,[2] Susan Kelly,[2] Karan M. Emerick,[2] Kishore Iyer,[1] Riccardo A. Superina,[1] Peter F. Whitington,[2] Estella M. Alonso.[2] *[1]Surgery, Children's Memorial Hospital, Chicago, IL; [2]Hepatology, Children's Memorial Hospital, Chicago, IL.*

Background: The MELD/PELD score was adopted to improve liver allocation by establishing an objective, verifiable system that reduces subjectivity in listing practices and advantages patients with a higher probability of waiting list mortality. The PELD score was validated to predict mortality and transfer to an intensive care unit (ICU) in children with chronic liver disease. **Aim:** To determine if the PELD system has improved liver allocation for children as measured by changes in recipient characteristics and regional variation in listing practices. **Methods:** Data reported to the UNOS registry for children (0-18years) receiving primary liver or liver/kidney transplantation between two time periods, January 1, 2000 to December 31, 2001 (era 1, n =788) and March 1, 2002 to July 31, 2003 (era 2, n=532) were included. Patients coded as acute hepatic necrosis of any etiology were excluded to focus the analysis on patients with chronic liver disease. **Results:** Waiting-list time was similiar (221 days in era 1 vs. 231 days in era 2, NS). The new system has not reduced the percentage of children transplanted while in an ICU (28% in era 1 vs. 27% in era 2, NS) or as status 1 (31% in era 1 vs 28% in era 2, NS). Active exception letters were used for allocation in 15% of patients. Therefore, a calculated PELD score was used for allocation in only 57% of children with chronic liver disease. The mean PELD score at transplant was 16±14.33. Regional means were compared to national means by t test to examine variability in patient status and listing practices. Significant regional variation was found for the rate of children transplanted as status 1 in 3 regions, the mean PELD score at transplant in one region, the rate of exception letters in 3 regions and the percentage of children in the ICU at transplant in 3 regions. Patient and graft survival computed by Kaplan Meier at 3, 6 and 12-months were not significantly different with the new system. **Conclusions:** The new allocation system appears to serve only 57% of children with chronic liver disease, with 15% of these bypassing the scoring system with exception letters. The new system has not reduced the percentage of children with decompensated chronic liver disease who require ICU support at the time of transplant. Regional variation in listing practices suggests that the system may not have reduced subjectivity in listing practices in pediatric liver transplantation.

Abstract# 1020

REGIONAL REVIEW BOARDS DO NOT INCREASE MORTALITY BY DENYING ACCELERATED LISTING TO PATIENTS. Michael D. Voigt,[1] Bridget Zimmerman,[2] Daniel A. Katz,[3] Stephen C. Rayhill.[3] *[1]Internal Medicine, University of Iowa, Iowa City, IA; [2]Biostatistics, University of Iowa, Iowa City, IA; [3]Surgery, University of Iowa, Iowa City, IA.*

Experienced transplant professionals may predict mortality in their sickest patients better than the (Pediatric) Model for End-stage Liver Disease score (MPS). This score has never been tested in the highly selected cirrhotic patients referred to regional review boards for accelerated listing. Physician requests for accelerated listing are frequently denied. Denied patients may thus have a greater risk of dying before receiving a transplant. This study was done to establish if: 1) such denials increased mortality; 2) experienced physicians could predict mortality better than the MPS.

Methods: We analyzed 1965 requests made between Feb and Nov 2002. Data was obtained form the UNOS scientific registry of nationwide referrals, excluding Status 1 patients. We compared mortality in patients denied accelerated listing to those approved by RRBs, using Kaplan-Meier survival analysis, and did Cox proportional Hazards analysis to establish if the referring physician risk assessment was more predictive of mortality than the MPS.

Results: Review boards denied more requests for cirrhotic patients (45%) than for all other disease categories (23%) (P<0.0001). Fewer patients denied (46.6% vs 63.8% p<0.0001) accelerated listing had a transplant and their time till transplant was longer [47 days in denied (SE 3, CI 40-54) vs 33 days in approved group (SE 2, CI 30-36 days) p=0.001]. Despite this, they had better survival to transplantation, [mortality 20 out of 244 (8.1%) in denied vs 39 of 250 (15.6%) in approved], indicating RRBs were denying low-risk, while approving high-risk patients. Referred patients approved for accelerated listing had higher mortality risk, than patients on the national liver transplant list (15.6% vs 10.4% p<0.03). The requesting physicians assessment of mortality risk had no significant (p=0.23) effect on predicting mortality, in the Cox proportional hazards model whereas the MELD had a highly significant effect (p=0.0003).

Conclusion: Regional review boards are able to accurately distinguish patients at low from those at high risk of death, indicating that the process is fair. The (Pediatric) Model for End Stage Liver Disease remains the most significant predictor of mortality, but the RRB process adds to its predictive ability, providing additional safeguards to sick patients. Referring physicans risk assessment was not predicitve of patient mortality.

Abstract# 1021

SPLIT LIVER TRANSPLANTATION: STATEWIDE USAGE OF THE RIGHT TRISEGMENTAL GRAFT. Kenneth Washburn,[1] Glenn Halff,[1] Pat Wood,[2] Luis Mieles,[4] Robert Goldstein,[3] John Goss.[5] *[1]Univ Texas HSC, San Antonio, TX; [2]St. Lukes Hospital, Houston, TX; [3]Baylor Medical Center, Dallas, TX; [4]Univ Texas HSC, Houston, TX; [5]Baylor College of Medicine, Houston, TX.*

Background: Split liver transplantation (SLT) has been used to effectively expand the cadaveric donor pool and provide size-appropriate left lateral segment(LLS) grafts for children. To optimize use of a limited resource, the remaining right trisegmental (RTS) graft can be transplanted into adolescents or adults. Little data exists addressing the outcomes of RTS allografts and no US report describes a multi-center, multi-OPO cooperative SLT sharing alliance.

Methods: Recipients from the 5 participating adult liver transplant programs that received a split RTS liver allograft over a 5 yr period were identified. Prospective donor and recipient (table 1)information collected from the individual transplant programs.

Table 1

BMI	24.6
MELD	20.9
Re-Tx	3/63
Status 1	2/63fs

Results: 63 RTS grafts were generated with the implementation of SLT at 5 Texas centers. Donors were generally young, healthy, and stable with 70% traumatic deaths. 83% of livers were allocated to pediatric recipients. Splitting occurred via the *in-situ*(64%) and *ex-vivo*(36%) techniques. The celiac axis was often maintained with the LLS(52%) requiring vascular reconstruction in 48% of RTS grafts. HCV was the most common indication for OLTx. 33% of RTS grafts were shared among different centers. Mean cold ischemic time was 7:22 ± 3:00 hours. 1 yr patient and graft survival is comparable to UNOS results for primary OLTx(table 2).

Table 2

	Patient	Graft
Texas	87.9%	86.2%
UNOS	87.1%	81.7%

Both RTS allografts transplanted into status 1 patients were lost: one due to hepatic artery thrombosis with subsequent successful re-transplantation with a second RTS graft; in the second a RTS allograft was a second graft due to PNF of a whole organ and the patient expired. MELD score tended to be higher in RTS grafts lost(23.4 vs 20.7, p=.23). There were no cases of primary non-function in the RTS allografts. Complications included; HAT(6), portal vein thrombosis(0), and biliary complications requiring reoperation(7).

Conclusions: SLT consistently generates 2 functional allografts from one cadaveric donor thus expanding the donor pool. 1 yr patient and graft survival with the RTS grafts compare favorably with UNOS averages. Non-function of the RTS graft is rare. Use of RTS allografts in status 1 patients, or those deemed to be high risk should be avoided when possible. The Texas alliance shows that broader application of SLT with inter-OPO and inter-programatic sharing successfully expands the donor pool for adults and children.

Abstract# 1022

ADDITION OF SERUM SODIUM INTO THE MELD SCORE PREDICTS WAITING LIST MORTALITY BETTER THAN MELD ALONE. A SINGLE-CENTER EXPERIENCE. Andres E. Ruf,[1] Silvina E. Yantorno,[1] Valeria I. Descalzi,[1] Oscar C. Andriani,[1] Luis G. Podesta,[1] Federico G. Villamil.[1] *[1]Liver Unit, Fundacion Favaloro, Buenos Aires, Argentina.*

Patients with severe portal hypertension and refractory ascites may have normal bilirubin, PT and creatinine and thus a low MELD score. It has been suggested that MELD may not serve well this patient population. Elevation of creatinine has a major impact on MELD. However, renal failure is a late event in patients with ascites and may have an accelerated course leading to death before a donor becomes available. Dilutional hyponatremia reflects an impairment of renal perfusion and has been found to be a predictor of hepatorenal syndrome and death in cirrhotics with ascites. In this study we investigated the prognostic value of serum sodium (Na) and hyponatremia (Na ≤130) in patients listed for liver transplantation (OLT) and whether the addition of Na may increase the accuracy of MELD to estimate waitlist mortality. **Patients and Methods:** From 06/95 to 01/03, 262 adult cirrhotics without HCC were listed for OLT in our center. After 3 months of follow-up, 175 patients (67%) remained alive (A), 19 (7%) died (D) and 68 (26%) underwent OLT. The prognostic value of Na, hyponatremia and MELD was analyzed in the 194 patients of the A and D groups. All patients with hyponatremia had ascites. **Results:** Hyponatremia was present in 22/175 A (12%) and 10/19 D (52.6%, p<0.001) and elevated creatinine (≥1.2 mg/dL) in 14/175 (8%) and 3/19 (16%, p=NS) respectively. Twenty patients (11.4%) of the A group and 8 of the D group (42%) had hyponatremia with normal serum creatinine (p<0.001). The prevalence of hyponatremia according to MELD scores were: ≤12 (n=54): 7.4%; 13-18 (n=86): 11.6%; 19-24 (n=33): 33.3%; 25-30 (n=15): 33.3% and ≥31 (n=6): 33.3%. Mortality rates for the same MELD categories were: 1.8%, 2.3%, 15.1%, 46.6% and 66.6% respectively. The c-statistic scores were 0.753 for hyponatremia, 0.784 for Na and 0.894 for MELD. The MELD and Na together yield a score of 0.908. This increase was statistically significant (p=0.026) and represented a net improvement because it allowed a better identification of patients who survived or died during the study period. **Conclusions:** In this study hyponatremia and Na were significant predictors of 3-month waitlist mortality. More importantly, addition of Na to the MELD significantly increased the accuracy of the score. Na appears as an attractive variable for a numerical score because it is objective, quantitative and easily available. The benefits of incorporating Na into the MELD score requires further confirmation

Abstract# 1023

IMPACT OF LIVER TRANSPLANTATION OF PATIENTS WITH HIGH DISEASE SEVERITY ON HEALTH CARE RESOURCES: UNIVERSITY HEALTHSYSTEM CONSORTIUM (UHC) BENCHMARKING PROJECT. Rafik M. Ghobrial,[1] C. Wright Pinson,[2] Jeffrey D. Punch,[3] Jackie Dostal,[4] Danielle Carrier.[4] *[1]Hepatobiliary & Liver Transplant Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Hepatobiliary & Liver Transplant Surgery, Vanderbilt University Medical Center, Nashville, TN; [3]Dept of Surgery, University of Michigan Health System, Ann Arbor, MI; [4]University HealthSystem Consortium, Chicago, IL.*

Objective: The impact of liver transplantation (LT) of patients with high disease severity based on the model for end stage liver disease (MELD) on the health care system is undetermined. This project analyzed the effect of high MELD scores with clinical outcomes and health resource utilization.

Patients and methods: Retrospective review of 682 adult single organ LT recipients (ICD-9, 50.51, 50.59) in the 15-month period ending March 2003, were included in the study. Data was voluntarily provided by 34-member institution and analyzed independently by UHC. Recipients were classified into 4 groups (<10, 10-20, 20-30, >30) based on their MELD scores.

Results: MELD scores of recipients at LT were <10 in 100, 11-20 in 322, 21-30 in 176, and >30 in 84 patients. At 3 months, patient survival was similar between all groups (96.5% - 98%, *P* NS). However, graft survival was significantly lower in patients with MELD >30 when compared to patients with MELD <10 (85.7 and 96%, *P* <0.05). Posttransplant complication rates exhibited a stepwise progression from 38% to 60.7% and were highest in recipients with MELD scores >30. Average ICU lengths of stay (LOS) were 4.3, 5.1, 7.2, and 9.8 days for patients with MELD <10, 10-20, 20-30, and >30, respectively. Average hospital LOS post-LT also steadily increased from 11.6 to 13.4, 16.7 and 21.1 days. More importantly, only 50% of patients with MELD>30, compared to 85% with MELD <10, were discharged to home. Cost analysis for the initial transplant period averaged 96,943/99,658/118,581, and 160,182 for recipients with MELD <10, 10-20, 20-30, and >30, respectively. Most significant cost increases were reflected in blood product utilization (5,215 to 16,534), pharmacy (11,425 to 21,598) and dialysis (4,541 to 8,512) when MELD increase from <10 to >30. In contrast, cost of surgical and medical services was not significantly different amongst all MELD groups (15,025 to 18,011, *P* NS).

Conclusions: LT in recipients with high severity of illness is performed with good survival outcomes in the short-term. Recipients with higher MELD scores exhibit extended ICU stays, prolonged hospital LOS, increased hospital costs and requirement for extended care following hospital discharges. This study argues for adjusted compensation based on the severity of disease. Additionally, the impact of cost should be considered by policymakers when determining allocation priority systems.

Abstract# 1024

EASTERN CO-OPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS IS ASSOCIATED WITH POST LIVER TRANSPLANTATION MORTALITY. ON BEHALF OF THE UK & IRELAND LIVER TRANSPLANT AUDIT. Mathew Jacob,[1] Lynn P. Copley,[1] James D. Lewsey,[1] Giles J. Toogood,[2] Mohamed Rela,[3] Alex Gimson,[4] Jan van der Meulen.[1] *[1]Clinical Effectiveness Unit, Royal College of Surgeons of England, London, United Kingdom; [2]Transplant & Hepatobiliary Unit, St James' University Hospital, Leeds, United Kingdom; [3]Institute of Liver Studies, King's College Hospital, London, United Kingdom; [4]Liver Transplant Unit, Addenbrookes NHS Trust, Cambridge, United Kingdom.*

Background: The Eastern Co operative Oncology Group (ECOG) Performance status is a simple tool to assess a patient's disease progression, assess the impact of disease on the daily living abilities of the patient, and to determine appropriate treatment and prognosis for cancer patients. It is measured on a scale from 0 to 4 with 0 being patients who are fully active and able to carry on normal activity without restriction and 4 for completely disabled, unable to carry on any selfcare, totally confined to bed or chair. It is evident that the ECOG score reflects the physiological status of a patient. The objective of this study is to investigate the possible association between ECOG performance status and post liver transplantation mortality in patients receiving a first liver transplantation in the UK & Ireland.

Methods: We included all 4112 adult patients who underwent a non-urgent first liver transplantation between 1 March 1994 and 31 March 2003. As a first step the impact of ECOG performance score on 90-day patient mortality was assessed by univariate analysis. Multivariate logistic regression modelling was then employed to adjust the ECOG score for other risk factors using bootstrap sampling techniques.

Results: The overall 90-day patient mortality was 9.7% (95% confidence interval 8.8% to 10.6%). When compared with patients with a score of 0 the unadjusted odds ratios for 90-day mortality for scores of 1, 2, 3 and 4 were 1.2, 1.8, 2.3 and 6.8, respectively (p<0.001). After adjustment for other risk factors the ECOG score remained a significant predictor of 90-day mortality (p<0.001). Recipient female sex, serum creatinine, cold ischaemia time, abnormal donor organ appearance and use of partial organs were also significantly associated with mortality (p<0.05).

Conclusions: The ECOG performance status indicator is a simple score, which can be easily measured. Patients with higher ECOG scores have a worse outcome after liver transplantation compared with those with lower scores. The results of this study suggest that performance status of the patient should be taken into consideration when selecting patients for liver transplantation.

Abstract# 1025

ARE ROUTINE CHEST COMPUTED TOMOGRAPHY AND BONE SCAN REQUIRED IN PATIENTS WITH HEPATOMA AND CIRRHOSIS UNDERGOING LIVER TRANSPLANT EVALUATION? A COOPERATIVE STUDY BY THE HEPATOMA AND LIVER TRANSPLANTATION (HALT). B. Koneru,[1] L. Teperman,[2] C. Manzarbeitia,[3] M. Facciuto,[4] K. Cho,[1] D. Reich,[3] D. Campbell,[2] P. Scheiner,[4] A. Fisher,[1] M. Korogodsky,[1] K. Noto.[3] *[1]Surgery, New Jersey Medical School, Newark, NJ; [2]Surgery, New York University Medical center, New York, NY; [3]Surgery, Albert Einstein Medical Center, Philadelphia, PA; [4]Surgery, New York Medical College, Valhalla, NY.*

UNOS policy 3.6.4.4 requiring chest CT (CCT) and bone scan (BS) in liver transplant candidates with HCC is consensus rather than evidence based. We hypothesized that such policy does not improve patient selection and is not cost effective.

This retrospective study included all patients with HCC evaluated for transplant between Jan 1999 to Dec 2002 at 4 centers and excluded HCC diagnosed after transplant. Majority of CCT/BS were performed at transplant centers. Scans with an indeterminate result were repeated a few weeks later. Evaluation outcomes were Accepted for listing, Not accepted-advanced HCC, Not accepted-other reasons. Outcomes following listing were Transplantation, Waiting for transplant, Delisted- HCC progression or Delisted-other reasons. The charges were $1,608/CCT and 1,689/BS. Physician fee and personnel costs were not included.

187 patients with HCC were evaluated at 4 centers. Number of scans performed and interpretations are shown.

	# initial scans	Negative	Indeterminate	Additional scans	Invasive Procedures
CT/MRI Chest	173	132	41	78	5*
Bone scan	172	142	30	65	1

*One patient died from biopsy of mediastinal mass (later proved benign) seen on CCT but not on chest x-ray; Only one patient was declined for listing based on the results of the initial scans, after 3 indeterminate BS followed by biopsy of a metastatic lesion in hip.158 patients were listed. 18 and 11 were declined for listing due to HCC and other reasons, respectively. After listing 120 patients were transplanted, 13 are waiting and 16 and 9 were delisted due to HCC and other reasons, respectively.

Excluding invasive procedures, CCT/BS incurred $ 803,901 charges. After transplantation 5/120 patients had recurrence. All 5 had negative CCT before transplant and 1 BS was indeterminate.

Conclusions: 1. Routine CCT and BS in candidates with HCC have low positive yield, do not improve selection and incur substantial charges. 2. Scan findings sometimes lead to additional invasive (unnecessary) procedures with potentially serious complications. 3). We propose revision of policy 3.6.4.4; Routine chest x-ray should replace CCT/BS except in patients with suspicious findings on chest radiography or symptoms of local bone pain.

Lymphocyte Activation: Costimulatory Molecules and Costimulatory Blockade

Abstract# 1026

PROLONGED IMMUNOLOGICAL SYNAPSE FORMATION IS CRITICAL FOR B7 COSTIMULATORY ACTIVITY. Robert S. Liwski,[1] Jennifer Chase,[1] Geoff Rowden,[1] Kenneth A. West.[1] *[1]Departments of Medicine, Pathology and Immunology, Dalhousie University, Halifax, NS, Canada.*

We have previously demonstrated that dendritic cells (DC) actively rearrange their cytoskeleton to form the immunological synapse (IS) with allogeneic CD4+ T cells. We have now examined the localization and function of DC costimulatory molecules during IS formation. We used a TCR transgenic model that recognizes a specific OVA peptide in the context of MHC Class II. Mature DC were pulsed with different doses of agonist or control peptide (10nM-10000nM) or media alone and conjugates were formed at 1:3 to 1:10 ratios with naive $CD4^+$ DO11.10 transgenic T cells by low speed centrifugation. The time course of DC-T cell conjugate formation was determine by labeling DC with CFMDA and T cells with CM-Dil dyes and examining conjugates at different timepoints using an inverted fluorescence microscope. We performed time-lapse video microscopy to determine conjugate stability. To determine the functional role and duration of costimulation during IS formation T cells were loaded with CFSE dye and then anti-CD80, anti-CD86 or both were added to DC-T cell conjugates 0, 0.5, 1, 2, 6, 12 or 24 hours after conjugate formation. T cells were isolated after 48, 60 or 72 hours and evaluated for a first cell division by flow cytometry. DC-T cell conjugates were stained using antibodies against CD80 and CD86. Conjugates were examined by confocal microscopy and scored blindly. DC-T cell conjugation occurred rapidly reaching a plateau by 30 minutes. The majority of conjugates were stable for >12 hours. Blockade of CD80 inhibited progression of naive T cells through the first cell division by 26% while inhibition of CD86 did not significantly affect cell division. Blockade of both costimulatory molecules inhibited first cell division by 43% at 300nM peptide and 74% at 30nM peptide. Inhibition of cell division and IL-2 production by intracellular staining occurred when antibodies were added for up to 12 hours following conjugate formation indicating that T cell activation requires prolonged costimulation. Interestingly, there was only modest polarization of either CD80 or CD86 into the IS in the presence of peptide which was not significantly different than control. These results demonstrate that prolonged synapse formation is critical for delivery of an appropriate costimulatory signal to naive T cells. The relatively low level of CD80/CD86 polarization suggests that sufficient molecules are present in the IS for lattice formation with CD28 resulting in optimal signaling.

Abstract# 1027

TARGETING CD40 VERSUS CD40 LIGAND YIELDS DIFFERENTIAL OUTCOMES FOLLOWING CARDIAC TRANSPLANTION. Theodore Welling,[1] Sherri Chan Wood,[1] Keri Csencsits,[1] Guanyi Lu,[1] D. Keith Bishop.[1] *[1]General Surgery, University of Michigan, Ann Arbor, MI.*

Background: Inductive anti-CD40L mAb is highly effective as a monotherapy in preventing allograft rejection, indicating that the CD40-CD40L pathway is critical to the rejection process. Most studies have employed anti-CD40L mAb to investigate the role of this pathway. While this approach is often referred to as a "blockade" of CD40-CD40L interactions, the precise mechanism of action of anti-CD40L mAb remains to be established. Very little is known regarding the role of CD40 in the rejection process. This study was designed to test the hypothesis that disrupting CD40-CD40L interactions by targeting CD40 as opposed to CD40L will yield distinct outcomes.
Methods: Wild type (WT) C57BL/6 (B6) or BALB/c cardiac allograft recipients were injected i.p. with 1 mg anti-CD40L mAb (MR1) on days 0, 1, & 2 relative to transplantation. Experimental results were compared to CD40-/- B6 or BALB/c recipients of CD40-/- allografts. Primed and precursor donor-reactive Th1 and Th2 responses were monitored by ELISPOT assays, and the impact of donor-derived dendritic cells (DC) on outcome was assessed.
Results: Inductive anti-CD40L therapy was remarkably effective at promoting graft survival (>60 days) and inhibiting T cell priming in both B6 and BALB/c recipients. Similarly, graft survival was prolonged and T cell priming was prevented in CD40-/- B6 recipients. In contrast, CD40-/- BALB/c recipients acutely rejected their allografts and mounted both Th1 and Th2 responses. CD40-/- B6 mice could be "forced" to acutely reject their allografts by injection of CD40-/- donor-derived DC, which induced Th1 and Th2 responses. However, donor-derived DC failed to induce rejection in WT B6 mice treated with anti-CD40L mAb. To assess the impact of donor-derived DC on "accepted" allograft function, B6 CD40-/- or anti-CD40L treated WT recipients were injected with donor DC on day 30 post-transplant. DC induced rejection in the majority of CD40-/- recipients, but not in anti-CD40L treated mice. However, DC failed to induce Th1 or Th2 in either setting, suggesting the induction of Treg.
Conclusions: The observation that anti-CD40L mAb prevents rejection in BALB/c recipients, while CD40 is not required in this strain supports the notion that CD40 and CD40L play roles independent of their interaction with one another. The observation that DC induce rejection of "accepted" grafts in B6 CD40-/- recipients but not following anti-CD40L therapy further points to differences in targeting CD40 vs. CD40L to prevent rejection.

Abstract# 1028

***IN VIVO* FUNCTIONS OF ALLOREACTIVE MEMORY CD4 T CELLS REMAIN INTACT DESPITE DONOR SPECIFIC TRANSFUSION AND ANTI-CD154 THERAPY.** Anna Valujskikh,[1] Yifa Chen,[1] Peter S. Heeger.[1] *[1]Immunology, The Cleveland Clinic Foundation, Cleveland, OH.*

Memory T cells have properties that are beneficial for host protection, but may be deleterious for the transplanted organ. It has been shown that the presence of donor-reactive memory CD4 T cells prevented the beneficial effect of donor-specific cell transfusion plus anti-CD154 mAb MR1 (DST/MR1) on heart allograft survival in mice. The goal of this study was to understand the mechanisms of resistance to costimulatory blockade mediated by memory CD4 T cells.

We first transferred B6 mice with either naïve or C3H-reactive memory CD4 T cells followed by C3H DST/MR1 treatment and C3H heart allografts. As anticipated, recipients of naïve CD4 T cells had prolonged graft survival (>60 d, n=3) compared to non-treated mice (8 d, n=6), while recipients of memory CD4 T cells rejected the grafts by d. 13 (n=5). We next evaluated the effect of CD4 memory cells on the endogenous donor reactive CD8 T cells. CD8 T cells from animals that received naïve CD4 T cells plus DST/MR1 (prolonged graft survival) responded weakly to donor stimulator cells (200 IFNg producers/million by ELISPOT and 18% lysis by in vivo CTL assay) similar to naïve non-transplanted mice. In contrast, anti-donor CD8 T cell response was strong in the animals transferred with memory CD4 T cells (1050 IFNg spots/million and 98% lysis), comparable to non-treated rejecting recipients. These findings suggest that endogenous CD8 T cells participate in graft destruction under these conditions. To test this, we transferred memory CD4 T cells into B6 recipients of C3H heart grafts treated with anti-CD8 depleting Ab. Depletion of CD8 T cells resulted in significant prolongation of heart graft survival (MST of 21.6±0.7 days) although all grafts were ultimately rejected suggesting that cells other than CD8 mediated graft destruction under these circumstances. Consistent with this, the rejecting grafts in the depleted recipients were heavily infiltrated with CD4 but not CD8 T cells. Furthermore, serum obtained from both anti-CD8 depleted and non-depleted recipients contained anti-donor alloantibodies.

These data clearly indicate that DST/MR1 treatment did not affect the ability of memory CD4 T cells to provide help for activation of anti-donor CD8 T cells and B cells and that the induced alloimmunity functioned in multiple ways to mediate graft destruction. As memory T cells comprise a significant portion of alloreactive T cell repertoire in humans, our results may translate into improved therapies for human transplant recipients.

Abstract# 1029

BLOCKING CD134 COSTIMULATION IS REQUIRED TO PREVENT CD8+ MEDIATED REJECTION. Minh Diem Vu,[1] Terry B. Strom,[1] Mohamed H. Sayegh,[1] Xian C. Li.[1] [1]*Department of Medicine Harvard Medical School, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA.*

Introduction: Costimulatory signals play a key role in the activation of naïve T cells. However, activation of CD4+ and CD8+T cells in certain transplant models appears to be differentially dependent on CD28/CD154 costimulation. In contrast to the CD4+ T cells, CD8+ T cells are often resistant to conventional CD28/CD154 costimulatory blockade. In this study, we critically examined the role of CD8+ T cells in mediating skin allograft rejection and their activation requirement to execute such effector function. The objective of this study is to evaluate the role of novel unconventional costimulatory molecules in the CD8+ mediated skin allograft rejection.

Materials and methods: Fully MHC-mismatched skin transplantation was performed using donor DBA/2 (H-2^d) and recipient B6.CD4$^{-/-}$ knockout (CD4KO) (H-2^b) mice. CTLA-4Ig and MR1 (0.5 mg/each, i.p.) were administered on days 0, 1, 3 to block conventional CD28 and CD154 costimulation. Also, the role of CD134/CD134L costimulatory pathways in supporting CD8+ mediated rejection was examined using a blocking anti-CD134L mAb (0.5 mg, i.p., administered on days 0, 2, 6, 8) given alone or in combination with CD28/CD154 blockade.

Results: CD4KO mice in which rejection is mediated solely by the activation of CD8+ T cells promptly rejected the skin allograft. Treatment with CTLA-4Ig, MR1, anti-CD134L or either two combinations all failed to prevent rejection. In stark contrast, transient blockade of CD28/CD154/CD134 costimulatory pathways using CTLA-4Ig, MR1 and anti-CD134L induced long-term skin allograft survival in CD4KO mice.

Treatment	Survival (days)	MST (days)
No treatment	11, 11, 11, 12, 12, 13, 14, 15	12
CTLA-4Ig/MR1	12, 12, 15, 16, 22	15
Anti-CD134L	9, 12, 12, 13, 13, 16	13
Anti-CD134L/MR1	9, 21, 24, 28, 33	24
CTLA-4Ig/MR1/anti-CD134L	82, 98, 98, >100, >100	>98

Discussion: In contrast to the common belief that CD8+ T cells are resistant to costimulatory blockade, we have shown that the costimulatory signals are critically important in CD8+ mediated rejection. Besides the conventional CD28/CD154 costimulatory blockade, concurrent blockade of the novel CD134/CD134L costimulatory pathway is essential in preventing the CD8+ mediated rejection. Our study also suggests that activation of a subset of CD8+ T cells is dependent on CD134 costimulation.

Conclusion: These data suggest that activation of CD8+ T cells during the allograft response requires engagement of multiple costimulatory pathways, and CD28/CD154/CD134 costimulatory blockade can prevent the CD8+ mediated rejection.

Abstract# 1030

THE B7 HOMOLOG, B7-H3, PROMOTES ACUTE AND CHRONIC ALLOGRAFT REJECTION. Liqing Wang,[1] Christopher C. Fraser,[2] Andrew D. Wells,[1] Anthony J. Coyle,[2] Lieping Chen,[3] Wayne W. Hancock.[1] [1]*Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA;* [2]*Inflammation, Millennium Pharmaceuticals, Cambridge, MA;* [3]*Immunology, Mayo Clinic, Rochester, MN.*

Though the field seems to be awash with new costimulation molecules, in many cases, their regulation, distribution and function in vivo are unknown. One such example, B7-H3, was reported to costimulate T cell proliferation through binding to an unknown receptor on T cells and enhance IFN-g production, but recent data suggested this ligand dampens host immune responses. We report the first clinical and experimental data concerning expression and function of B7-H3 in alloresponses. Immunohistologic studies showed florid B7-H3 expression by infiltrating mononuclear cells during human renal allograft rejection. In addition, immunohistology and real-time RT-PCR showed a 4-5-fold upregulation in murine cardiac allografts vs. isografts, and flow cytometry showed induction of surface B7-H3 expression by activated CD4 and CD8 T cells (CD3/CD28), as well as activated macrophages and mature dendritic cells. To analyze the significance of B7-H3 in rejecting allografts we generated B7-H3-/- mice by homologous recombination and used them, along with wild-type mice (B6/129), as recipients of cardiac BALB/c allografts. Though cardiac allografts in knockout or control mice were rejected with comparable speed (7-8 d) in this fully MHC-mismatched model, synergistic effects of targeting B7-H3 in conjunction with limited immunosuppression were observed. Thus, a subtherapeutic course of CsA led to rejection by 10 days in control mice, whereas CsA use in B7-H3-/- mice led to a mean survival of 35 d ($p<0.01$). Moreover, a regimen of RPM which gave 12-14 days of survival in controls, led to permanent engraftment (>100 d, 80%, $p<0.001$) in B7-H3-/- mice. Studies by real-time RT-PCR showed that allografts in B7-H3-/- mice had decreased production of cytokine (IL-2, IFN-g), chemokine (IP-10, MCP-1) and chemokine receptor (CXCR3) mRNA compared to wild-type controls, and in each case the decreases in mRNA expression were enhanced in stepwise manner by immunosuppression (RPM>CsA). Consistent with this, analysis of CFSE-labeled T cell responses following adoptive transfer in vivo (parent to F1) showed B7-H3-dependent production of IL-2 and IFN-g by CD4 and CD8 T cells, respectively. Lastly, in contrast to controls B7-H3-/- mice treated with CD40L mAb showed an absence of chronic rejection. We conclude that the B7 homolog, B7-H3, promotes Th1-mediated immune responses and the development of acute and chronic allograft rejection in small animal models and, likely, clinical allograft rejection.

Abstract# 1031

THE CD134/CD134L PATHWAY IS CRITICAL IN CD8-MEDIATED COSTIMULATION BLOCKADE RESISTANT REJECTION. Andrew B. Adams,[1] Thomas R. Jones,[1] Erik A. Heiss,[1] Nozomu Shirasugi,[1] Phyllis A. Rees,[1] Hideo Yagita,[2] Thomas C. Pearson,[1] Christian P. Larsen.[1] [1]*Surgery, Emory University School of Medicine, Atlanta, GA;* [2]*Immunology and Obstetrics and Gynecology, Juntendo University School of Medicine, Tokyo, Japan.*

Two of the best characterized costimulatory pathways include CD28/B7 and CD40/CD154. Blockade of these pathways simultaneously, using fusion protein constructs (eg. CTLA4-Ig) and/or mAbs, can promote prolonged allograft survival. Interestingly there are some models in which costimulation blockade is less effective, primarily due to the ability of CD8 T cells to act in a CD28/CD154 independent manner. In recent years numerous novel costimulatory pathways have been described. In the current study we tested whether signaling through any of these alternative costimulatory pathways could be critical in "CD28/CD154 blockade resistant rejection." For the initial evaluation we employed a well-described GvHD model. Irradiated BALB/c mice received B6 T cells which had previously been labeled with CFSE to track cellular division in-vivo. Experimental groups included animals which received no treatment, costimulation blockade (CoB) alone (CTLA4-Ig/anti-CD154) or CoB in combination with an additional agent. Additional agents tested included anti-ICOSL, anti-CD70, anti-41BBL, or anti-CD134L. The combinations of CoB + anti-ICOSL ($p<0.02$) and CoB + anti-CD134L ($p<0.03$) significantly inhibited both CD4 and CD8 T cell proliferation when compared to CoB alone. In the following experiments we evaluated these agents in a fully allogeneic mouse skin graft model.

Untreated mice rejected their skin graft promptly (MST 11 days). Animals treated with CB alone demonstrated a slight prolongation (MST 20 days). Despite inhibiting T cell proliferation in the GvHD model the addition of anti-ICOSL only modestly prolonged skin graft survival when compared to CB alone (MST 30 days). The addition of either anti-CD70 or anti-41BBL did not significantly prolong survival (MST 22 and 23 days respectively). In contrast the combination of anti-CD134L and CoB dramatically prolonged fully disparate skin allografts (MST 130 days). Further experiments defined a critical role for IFNγ. The addition of anti-IFNγ mAb or the use of IFNγ-/- mice abrogated the beneficial effects of the combined CoB/anti-CD134L treatment. Treatment of animals reconstituted with CD8 T cells from IFNγR-/- mice results in early rejection (MST =28 days vs >54 days in wt animals w/o reconstitution) suggesting that one of the effects of IFNγ is directly on CD8 T cells. Further studies aim to identify the role of CD134/CD134L interactions in CD8 T cell activation.

Abstract# 1032

TARGETING THE NOVEL CD28 HOMOLOG, BTLA, PROLONGS ALLOGRAFT SURVIVAL. Ran Tao,[1] Qunrui Ye,[1] Theresa L. Murphy,[2] Kenneth M. Murphy,[2] Wayne W. Hancock.[1] [1]*Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA;* [2]*Pathology & Immunology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO.*

Mice deficient in expression of a newly described CD28 homolog, B and T lymphocyte attenuator (BTLA), show increased antibody production and enhanced sensitivity to EAE, suggesting that BTLA, like CTLA-4 and PD-1, is an inhibitory receptor on T cells. We report the rationale for studying BTLA expression post-transplant, and the first and unexpected data concerning BTLA function during alloresponses.

Flow cytometric studies using an anti-murine BTLA Ab showed weak expression by resting CD4 (36%) and CD8 (25%) T cells, plus >90% of B cells. T cell expression was markedly upregulated upon activation in vitro using CD3/CD28 mAbs. BTLA mRNA was also upregulated within the spleen and allograft of unmodified recipients; e.g. 17-fold upregulation of BTLA mRNA expression in murine cardiac allografts (BALB/c to B6.129) vs. native hearts, and BTLA protein was localized to infiltrating lymphocytes. These data indicated upregulation of BTLA by naive T cells undergoing priming, as well as by graft-infiltrating effector T cells, leading us to analyze the function of BTLA in this context using BTLA-/- vs. wild-type mice.

Studies of CFSE-labeled T cells undergoing in vivo activation (parent to F1 adoptive transfer) showed delayed entry into the cell cycle of alloreactive T cells of BTLA-/- mice, and decreased production of IL-2 and IFN-g. Comparable effects on activation and cytokine production were seen upon in vitro stimulation of BTLA-/- vs. control T cells using CD3/CD28 mAb. Hence, studies using CFSE-labeled cells indicated that T cells mount an attenuated Th1 response in the absence of BTLA costimulation. Survival of cardiac allografts was significantly prolonged in BTLA-/- mice (mean 15 d vs. 8 d in controls, $p<0.05$), with decreased intragraft mRNA expression of multiple cytokines (e.g. IL-2, IFN-g), chemokines and chemokine receptors. A single dose of CTLA4.Ig (200 ug) significantly prolonged graft survival in BTLA-/- mice (mean 39 d vs. 12 d in

CTLA4.Ig-treated controls, p<0.001), indicating the cooperative interaction of these 2 pathways in T cell activation, and the relevance of dual targeting to prevent acute rejection. Lastly, a sub-therapeutic course of rapamycin significantly prolonged allograft survival in BTLA-/- (mean >40 d vs. 10 d in RPM-treated controls, p<0.001).
Our data indicate that in the context of alloresponses, BTLA promotes both CD28-dependent and CD28-independent acute rejection, and its targeting can contribute to long-term allograft survival.

Abstract# 1033

SYNERGISTIC EFFECT OF ICOS/B7RP-1 AND CD40/CD40L COSTIMULATION BLOCKADE IN THE INDUCTION OF TOLERANCE IN A RAT HEART TRANSPLANTATION MODEL. Carole Guillonneau,[1] Venceslas Aubry,[1] Karine Renaudin,[2] Katsunari Tezuka,[3] Ignacio Anegon.[1] *[1]U437, INSERM, Nantes, France; [2]IFR26, Service d'Anatomie Pathologique, Nantes, France; [3]Biological/ Pharmacological Research Laboratories, Osaka, Japan.*

Blockade of the CD40-CD40L costimulatory pathway results in long-term allograft survival but does not prevent chronic rejection and therefore new immunotherapies are needed to obtain tolerance. ICOS and its receptor, B7RP-1, are the recently described members of the CD28-B7 families which play an important role in T cell activation and survival.A previous study of the blockade of CD40-CD40L interactions in a rat heart allograft transplantation model using an adenovirus coding for CD40Ig resulted in long-term (>300 days) survival concomitant to the development of chronic rejection.We analyzed the effect of co-treatment with an anti-ICOS MAb (JTT1, 1mg/week) and CD40Ig on chronic rejection.CD40Ig and anti-ICOS treatment also led to indefinite graft survival in all recipients (>120 days, n=7).Treatment with anti-ICOS alone resulted in a modest but significant prolongation of allograft survival (17±1.5 vs 7.6±1.6, p=0.002, n=4).Analysis of chronic rejection lesions at day 120 after transplantation showed in the CD40Ig+anti-ICOS group (n=7) vs. CD40Ig alone (n=17) that the percentage of vessels occluded (15.2±6.6 vs. 33.7±4.4, p=0.017), degree of vessel occlusion (1.1±0.5 vs. 2.4±0.2, p=0.007) and lesions of the vascular wall (1±0.3 vs. 2.3±0.1, p<0.001) but not fibrosis were significantly lower.Importantly, 4 of the 7 CD40Ig+anti-ICOS-treated recipients showed complete absence of chronic rejection lesions whereas all CD40Ig-treated recipients showed signs of chronic rejection.The CD40Ig+anti-ICOS group showed decreased graft infiltration by TCRαβ+, CD4+ and CD8α+ cells as well as macrophages and mast cells.Recipients in the CD40Ig+anti-ICOS group displayed significant inhibition of anti-donor CTL activity.Alloantigenic proliferative responses of splenocytes in the CD40Ig+anti-ICOS group were strongly inhibited and were reversed by IL2.CD40Ig treatment strongly but incompletely inhibited total IgG, IgG1 (Th2), IgG2a (Th2) and IgG2b (Th1) alloantibody production vs. untreated rejecting recipients.The residual total IgG and IgG2b but not IgG1 and IgG2a were significantly reduced in the sera of recipients of CD40Ig+anti-ICOS treated allografts at day 120, suggesting a preferential inhibition of Th1 responses.These data indicate that the chronic rejection mechanisms that are CD40-CD40L-independent are ICOS/B7RP-1-dependent and that operational tolerance can be obtained by simultaneous blockade of these two costimulatory pathways.

Abstract# 1034

EFFECT OF INFLAMMATION ON COSTIMULATION BLOCKADE RESISTANT ALLOGRAFT REJECTION. Katsuyoshi Habiro,[1] Motoko Kotani,[1] Kazuya Omoto,[2] Kazunari Tanabe,[2] Hiroaki Shimmura,[2] Hiroshi Toma,[2] Ryo Abe.[1] *[1]Division of Immunobiology, Reseach Institute for Biological Sciences, Tokyo University of Science, Noda City, Chiba, Japan; [2]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan.*

It was shown that simultaneous blockade of CD28- and CD40-mediated costimulatory signals significantly prolonged allograft survival. Although these results led to an expectation of the establishment of specific immuno-tolerant therapy for organ transplantation, it became evident that these treatments rarely resulted in indefinite allograft survival. In order to uncover the mechanisms underlying these costimulation blockade-resistant allograft rejections, first, we have studied the process of allogenic skin graft rejection, by the use of CD28 and CD40L double deficient mice (dKO). It was found that skin grafts from MHC-missmatched donors were rapidly rejected by dKO mice and these rejection were mediated by CD8+ T cells that were primed with donor antigens via direct antigen presentation. These data indicated that some elements beside CD28- and CD40-mediated costimulatory signals would provide stimulatory signals for the activation of donor-specific CD8+ T cells. We postulated that nonspecific inflammation associated with transplantation resulted in the production of various inflammatory cytokines, which facilitates immune response against graft. Thus, second, we investigated the role of non-specific inflammation in the graft rejection. RAG -/- mice were transplanted with BALB/c skin graft and adoptively transferred with B6 CD8+ T cells in the presence or absence of blockade of CD28 and CD40L mediated costimulatory signals. If CD8+ T cells were transferred at the time of transplantation, grafts were acutely rejected (MST: 12.3 days). Costimulation blockade had only a limited effect on prolongation of graft survival in this setting (MST: 28.6 days). In contrast, if CD8+ T cells were transferred at 50 days after transplantation at the time when transplanted grafts were well-healed, costimulation blockade effectively prolonged skingraft survival (MST: 81.4 days) compared to no treatment group (MST: 15.7 days). These results indicated that in the absence of CD28- / CD40- costimulation, factors associated with inflammation play an important role for the priming of donor specific CD8+ T cells, and suggested that control of inflammation at the site of grafts may be the key element for the successful treatment of graft rejection by the costimulation blockade.

New Agents in Immunosuppression

Abstract# 1035

CO-STIMULATION BLOCKADE WITH LEA29Y IN RENAL TRANSPLANT: IMPROVED RENAL FUNCTION AND CV/ METABOLIC PROFILE AT 6 MONTHS COMPARED WITH CYCLOSPORINE. B. Nashan,[1] J. Grinyo,[2] F. Vincenti,[3] P. Halloran,[4] D. Hagerty,[5] W. Zhou,[5] B. Charpentier,[6] LEA29Y Study Group. *[1]Klinik fur Viszeral-und Transplantations-Chirurgie, Germany; [2]Hospital de Bellvitge, Spain; [3]University of California San Francisco; [4]University of Alberta, Canada; [5]Bristol-Myers Squibb; [6]Bicetre Nephrologie, France.*

Introduction: Preserving renal function and managing CV/metabolic risk factors are important for improving long-term outcomes in renal transplantation. Strategies aimed at avoiding calcineurin inhibitor (CNI)-related toxicities have therefore gained momentum. LEA29Y is a modified version of CTLA4Ig, designed to provide immunosuppression in transplantation via blockade of the B7/CD28 co-stimulatory pathway. This multicenter study compared renal function and CV/metabolic profile in a cyclosporine A (CsA) vs. CNI-free maintenance regimen with LEA29Y in renal transplant recipients. **Methods:** Renal allograft patients received mycophenolate mofetil, corticosteroids and basiliximab and were randomized 1:1:1 to maintenance treatment with CsA (N=73) or LEA29Y more or less intensive dosing regimen. The CsA and LEA29Y treatment arms were open-label. Assignment to lower- and higher-intensity LEA29Y groups remained blinded and groups were combined for analysis (N=148). Renal function, hypertension, hyperlipidemia, and post-transplant diabetes (PTD) were assessed. **Results:** Baseline recipient and donor characteristics were comparable and biopsy-proven acute rejection (AR) rates were similar. Median measured GFR was 14ml/min/1.73m² higher in LEA29Y treated patients (p<0.05). Systolic blood pressure (BP) and mean arterial BP were lower in LEA29Y treated patients (129 vs. 136 mmHg and 95 vs. 99 mmHg for LEA29Y and CsA, respectively, p<0.05). There was less post-transplant hypertension among LEA29Y-treated patients (85% vs. 93% for CsA). Use of anti-hypertensive medication was reduced to pre-transplant level in the LEA29Y group (84%), unlike the CsA group (91%). In addition, total cholesterol was significantly lower with LEA29Y treatment (203 vs. 225 mg/dL for CsA), as was non-HDL-cholesterol (146 vs. 166 mg/dl for CsA), (p<0.05). HDL levels were also proportionally lower in LEA29Y treated patients. There were two cases of new onset of PTDM (3%) with CsA and none with LEA29Y. **Conclusions:** LEA29Y, a potent co-stimulation blocker, demonstrated similar efficacy at preventing AR, better renal function, and reduced hypertension, hyperlipidemia, and PTD compared with CsA at 6 months in renal transplant recipients. These data suggest that co-stimulation blockade with LEA29Y may offer a new paradigm for improving long-term outcomes in maintenance immunosuppression following renal transplant.

Abstract# 1036

TREATMENT OF KIDNEY TRANSPLANT PATIENTS WITH THE NOVEL CO-STIMULATORY BLOCKER LEA29Y (BMS-224818) AND ANTI-IL2 RECEPTOR ANTIBODY DOES NOT IMPEDE THE DEVELOPMENT OF REGULATORY T CELLS. Kenzo Hirose,[1] Andrew M. Posselt,[1] Peter G. Stock,[1] Ryutaro Hirose,[1] Flavio Vincenti.[2] *[1]Department of Surgery, University of California-San Francisco, San Francisco, CA; [2]Department of Medicine, University of California-San Francisco, San Francisco, CA.*

BACKGROUND: The effect of immunosuppression with co-stimulatory blockade and anti-IL2 receptor antibody on regulatory T cells is not known. This study examines regulatory T-cells in the peripheral blood of kidney transplant patients who participated in a Phase II multicenter trial that involved induction therapy with anti-IL2Rα (CD25) antibody and maintenance with a calcineurin inhibitor free regimen using LEA29Y. LEA29Y is a modified version of CTLA4Ig specifically designed to provide potent immunosuppression via blockade of the B7/CD28 co-stimulatory pathway.

METHODS: Peripheral blood was collected between 6-30 months post-transplant from 21 of 33 kidney transplant recipients at our site enrolled in a prospective, randomized trial studying the use of LEA29Y. All patients received induction therapy with anti-IL2Rα antibody (basiliximab) as well as standard maintenance with MMF and steroids. 18 of these patients also received LEA29Y, while the other 3 received cyclosporine A (CsA). Peripheral blood was also collected from 3 healthy donors. Flow cytometry was used to determine the number of CD4+CD25hi cells as a proportion of all CD4+ lymphocytes. Using magnetic bead separation, CD4+ lymphocytes were then isolated, followed by separation into CD4+CD25hi and CD4+CD25lo/CD25- populations. Proliferation in culture was measured by ^{3}H-thymidine uptake in response to platebound anti-CD3. Suppressive activity of the CD4+CD25hi cells was measured by titration into the CD4+CD25lo/CD25- population of cells.

RESULTS: The numbers of CD4+CD25hi lymphocytes as a percentage of CD4+ lymphocytes were 4.5%, 4.3%, and 4.2% for healthy donors, patients receiving LEA29Y, and patients receiving CsA respectively. The CD4+CD25hi T-lymphocytes exhibited properties characteristic of regulatory T cells, namely reduced proliferation in response to nonspecific stimulation by anti-CD3, and dose-dependent suppression of proliferation of CD4+CD25lo/CD25- lymphocytes. The overall efficacy of patients treated with LEA29Y was comparable to CsA treated patients.

CONCLUSIONS: CD4+CD25hi T-cells are present in the peripheral blood of kidney transplant patients who had induction therapy with monoclonal anti-IL-2Rα antibody and maintenance with either CsA or the novel co-stimulation blocker, LEA29Y. These cells appear to possess characteristics of regulatory T cells, similar to those found in healthy donors.

Abstract# 1037

CO-STIMULATION BLOCKADE WITH LEA29Y IN A CALCINEURIN INHIBITOR FREE MAINTENANCE REGIMEN IN RENAL TRANSPLANT: 6-MONTH EFFICACY AND SAFETY. F. Vincenti,[1] F. Muehlbacher,[2] B. Nashan,[3] C. Larsen,[4] E. Atillasoy,[5] K. Natarajan,[5] B. Charpentier,[6] LEA29Y Study Group. *[1]University of California San Francisco; [2]General Hospital Vienna, Austria; [3]Klinik fur Viszeral-und Transplantations-Chirurgie, Germany; [4]Emory University; [5]Bristol-Myers Squibb; [6]Bicetre Nephrologie, France.*

Introduction: Development of modern immunosuppressive regimens in transplantation has focused on maintaining efficacy while improving long-term outcomes by preserving renal function and managing CV risk factors. Minimizing or avoiding calcineurin inhibitor (CNI)-related toxicities has gained recognition as a means for achieving these goals. This multicenter Phase II non-inferiority design study compares the safety and efficacy of the potent co-stimulation blocker LEA29Y vs. cyclosporine A (CsA) in a quadruple immunosuppressive drug regimen in renal transplant. LEA29Y is a modified version of CTLA4Ig, designed to provide immunosuppression in transplantation via blockade of the B7/CD28 co-stimulatory pathway. **Methods:** Renal allograft patients received mycophenolate mofetil, corticosteroids and basiliximab and were randomized 1:1:1 to maintenance treatment with CsA (N=73) or LEA29Y, more or less intensive dosing regimen. CsA and LEA29Y treatment arms were open-label. Assignment to lower- and higher-intensity LEA29Y groups remained blinded and groups were combined for analysis (N=148). The primary 6-month endpoint was incidence of biopsy-proven acute rejection (AR). **Results:** Baseline recipient and donor characteristics were comparable among groups. Biopsy-proven AR rates were similar (19% for LEA29Y vs. 18% for CsA). Incidence of Grade I or Grade II AR was also comparable (4% vs. 6% and 15% vs. 12%, respectively, for LEA29Y vs. CsA). 6-month renal function with LEA29Y treatment was significantly better compared with CsA. Median measured GFR was 14ml/min/1.73m^2 higher in LEA29Y treated patients ($p<0.05$). Graft loss was 3% (LEA29Y) vs. 4% (CsA) and death rate was lower with LEA29Y (1% vs. 5% for CsA). Discontinuation and adverse events, including infection and malignancies, were similar across groups. LEA29Y infusions were well tolerated. LEA29Y was less often associated with typical CNI-related toxicities such as hyperlipidemia, hypertension, and diabetes. **Conclusions:** Compared with CsA, potent co-stimulation blockade with LEA29Y as part of a CNI-free maintenance regimen provided similar efficacy, was generally safe and well tolerated, showed less decline in renal function, and reduced the CV/metabolic risk. These data suggest that co-stimulation blockade with LEA29Y may offer a new paradigm for improving long-term outcomes in maintenance immunosuppression following renal transplant

Abstract# 1038

SYNGERGY OF IMMUNOSUPPRESSION BY PG490-88 AND FK506 IS THROUGH DOWNREGULATION OF NF-kB AND IL-2. Hongtao Sun,[1] Gang Chen,[2] Ximo Wang,[2] Iram Siddiqui,[1] Weihua Liu,[1] Kouichi R. Tamura,[3] Yuji Sudo,[3] Robert Zhong,[1,2,4] Bertha Garcia.[1] *[1]Pathology, University of Western Ontario, London, ON, Canada; [2]Surgery, London Health Sciences Centre, London, ON, Canada; [3]Fujisawa Pharmaceutical Co., Ltd, Osaka, Japan; [4]Transplantation Group, Robarts Research Institute, London, ON, Canada.*

PG490-88 is a semisynthetic derivative of a novel compound PG490 (triptolide) purified from a Chinese herb (*Tripterygium wilfordii Hook F*). It has been demonstrated that PG490-88 combined with FK506 or cyclosporin A prolongs allograft survival in rodent models. The present study was undertaken to determine whether PG490-88 and FK506 would prolong renal allograft survival in a non-human primate model. Extensive pathological studies were conducted to explore the possible mechanisms of this novel immunosuppressive agent. **Materials and Methods**: 17 cynomolgus monkeys were divided into five groups: A. control; B. FK506 (1mg/kg/day); C. PG490-88 (0.03mg/kg/day); D. PG490-88 (0.03mg/kg/, for 30days) plus FK506 (1mg/kg/day); E. PG490-88 (0.01mg/kg/, for 30days) plus FK506 (1mg/kg/day). Following bilateral nephrectomies, a single kidney was transplanted. The grafts and other organs were collected and stained with H&E, PAS, MSB and trichrome methods. Immunohistochemistry was used to detect IL-2, NF-kB, CD3, CD4, CD8, CD20, CD68, IgG, IgM, C3, C4d, C5b-9 and Fibrin. IL-2 and NF-kB expression were quantified by Western blotting. Degrees of rejection were evaluated with Banff 97 type.

Results: PG490-88 combined with FK506 significantly prolonged the renal allograft graft survival (MST: 77 ± 22 days). Three out of 8 monkeys in this group have been alive for more than 150 days. Pathological studies showed that lymphocyte infiltration was markedly decreased in the groups treated with PG490-88 plus FK506 compared to the control and FK groups treated alone. PG490-88 plus FK506 treatment also significantly inhibited IL-2 and NE-kB expression in infiltrated lymphocytes and reduced the tubulitis and necrosis. Immunohistochemical staining showed that IgG, IgM and C3 deposition were much less in the Groups A, B and C than in the Groups D and E.

Conclusion: PG490-88 combined with FK506 synergistically prolonged renal allograft survival and attenuated cellular infiltration. The inhibition of NF-kB and IL-2 expression in infiltrated lymphocytes with a combined therapy of PG 490-88 and FK 506 may contribute to the efficacy of this novel therapy.

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Abstract# 1039

NEW IMMUNOSUPPRESSANT FK778 SHOWS EFFICACY IN RENAL TRANSPLANTATION. Johannes P. van Hooff,[1] Yves Vanrenterghem,[2] Marian Klinger,[3] Zbigniew Wlodarczyk,[4] Jean-Paul Squifflet,[5] the European FK778 Kidney Transplant Study Group. *[1]Interne Geneeskunde, Academisch Ziekenhuis Maastricht, Maastricht, Netherlands; [2]Inwendige Geneeskunde-Nefrologie, Universitaire Ziekenhuizen Gasthuisberg, Leuven, Belgium; [3]Department of Nephrology, Wroclaw Medical University, Wroclaw, Poland; [4]Samodzielny Publiczny Szpital Kliniczny, Bydgoszcz, Poland; [5]Département de Transplantation Rénale, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium.*

Purpose: To provide the first efficacy and safety data of the malononitrilamide FK778 in transplant patients to date. This new agent is structurally unrelated to known and currently used immunosuppressants. In animal and in vitro models, it inhibits acute rejection, modifies vasculopathy and shows anti-viral activity. In this European, multicentre, phase II study, FK778 was administered to kidney transplant recipients at two concentration-controlled ranges.

Methods: In a double-blind manner, 149 patients were randomized to a 12-week treatment with FK778 in combination with tacrolimus (Tac) and corticosteroids (S). 49 patients of the high-level group (H) received 2x600 mg/day FK778 and continued on 150 mg/day, 54 patients of the low-level group (L) got 1x600 mg/day followed by 75 mg/day and 46 patients received placebo (P). Subsequent FK778 doses were adjusted to trough levels of 100-200 µg/mL (H) and 10-100 µg/mL (L). An independent unblinded pharmacokinetic panel advised the necessary dose adjustments. The primary endpoint was the incidence of biopsy proven acute rejection (AR).

Results: Graft survival at week 16 was 89.7%, 88.8% and 91.3%, the incidences of AR were 26.5%, 25.9% and 39.1% for group H, L and P. For the subgroup of patients in which target levels were reached by week 2, incidences were 7.7% (2/26), 27.1% (13/48) and 39.1% (18/46), respectively. Anaemia, the most frequently reported adverse event especially in group H, was reversible. Renal function as assessed by median serum creatinine values was similar between groups. Mean total cholesterol and LDL-cholesterol levels were reduced during FK778 treatment compared to group P.

Conclusions: FK778 is pharmacologically active, well tolerated and safe. To fully benefit from this promising new drug FK778 dosing will be optimized in subsequent studies.

Abstract# 1040

FTY720, A SPHINGOSINE 1-PHOSPHATE RECEPTOR AGONIST, DOES NOT ELICIT ADVERSE EFFECTS ON CARDIAC RHYTHM IN RENAL TRANSPLANT PATIENTS ON MAINTENANCE THERAPY. A. Jardine,[1] J. M. Grinyo,[2] S. Mulgaonkar,[3] R. M. Ferguson,[4] M. Cremer,[5] R. Preiss.[5] *[1]Western Infirmary, Glasgow, United Kingdom; [2]L'Hospitalet de Llobregat, Barcelona, Spain; [3]Saint Barnabas Medical Center, Livingston, NJ; [4]Ohio State University Medical Center, Columbus, OH; [5]Novartis Pharma AG, Basle, Switzerland.*

INTRODUCTION: In phase I and II clinical trials a transient, reversible, first-dose effect of FTY720 on heart rate (HR) was evident, causing bradycardia in some patients but no difference in cardiac morbidity. This study investigated cardiac effects in renal transplant recipients treated with FTY720 or MMF for a minimum of 12 months. **METHODS**: 421 recipients (FTY720, n=94; MMF, n=327) pooled from phase II studies underwent ECG and 24-hour Holter monitoring. Results were compared between pooled FTY720, MMF and the FTY720 dosing groups (2.5 and 5mg). **RESULTS:** Demographic characteristics were comparable except for a higher prevalence of patients >60 yrs in the MMF group vs FTY720 (18% vs 9%, respectively). Analysis of mean hourly HR yielded no significant difference between groups. Analyses based on discrimination by day/night, concomitant use of β-blocker, gender or age revealed no differences. The incidence of bradycardia (HR <50bpm); either sustained (for >1min), severe (<35bpm) and sustained and severe (HR <35bpm for >1min) were observed more frequently with MMF. Among patients maintained on β-blockers the incidence of bradycardia was comparable between FTY720 and MMF groups. Serious Holter findings were observed only in the MMF group; ventricular tachycardia (n=1), Torsade des Pointes (n=1) and second degree atrioventricular block (n=2). Autonomic cardiovascular responsiveness (measured by laying and standing systolic and diastolic BP, and HR) was comparable between groups. ECG derived intervals did not differ significantly between FTY720 and MMF groups for mean PR (155.1 vs 158.0msec) and QTc interval (Bazett 409.7 vs 404.1msec; Frederica 401.9 vs 395.8msec), respectively. The lack of significant differences for these parameters supports the absence of a dose-dependent effect. **CONCLUSIONS:** This study demonstrates the absence of any clinically significant effect of FTY720 on cardiac rhythm in patients on chronic therapy, thus confirming that the transient reduction in HR after the first dose of FTY720 does not persist in the maintenance phase.

	FTY720 5 mg	FTY720 2.5 mg	FTY720 pooled	MMF
Patients (n)	37	57	94	327
Min HR (bpm)	42	37	37	30
Median HR + β-blocker	62.5	64.0	64.0	69.0
HR 35-50bpm	12 (32%)	23 (40%)	35 (37%)	172 (53%)
HR <35bpm	0	0	0	6 (2%)
Sinus pause >2 (>3) sec	0 (0)	1 (0)	1 (0)	0 (0)

Abstract# 1041

CYCLOSPORIN A (CSA) MAY BE USED INTERCHANGEABLY WITH TACROLIMUS (TAC) IN MAINTENANCE MINIMIZATION PROTOCOLS FOLLOWING ALEMITUZUMAB (CAMPATH-1H) INDUCTION IN RENAL TRANSPLANT RECIPIENTS. Richard Lewis,[1] Tracy Van Ness,[1] Lisa Etter,[1] David Bekofsky,[1] Michael Ko,[1] Carla Stark,[1] Wayne Waltzer.[1] *[1]Renal Transplantation, State University of New York at Stony Brook, Stony Brook, NY.*

Campath-1H (C1H) induction in renal transplant (RTx) recipients (recips) has been associated with a state of 'near tolerance' reflected in good immunologic outcomes coupled with use of less than normative levels of maintenance immunosuppressive therapy (MIRx). Recently, no acute rejection (AR) or graft loss was reported in 23 recips receiving C1H (30 mg); 3 doses of solumedrol (500 mg intraop, 250 mg POD #1, 125 mg POD #2); and dual drug, Pred-free MIRx with Mycophenolate mofetil (MMF) (1 gm bid) and low dose Tac (Am J Transplant 2002; 2(Suppl3)(A):397). In view of non-overlapping toxicities associated with CsA vs Tac, the former may be preferable in selected recips receiving such dual drug, maintenance minimization protocols. Some controversy exists, however, viz. the relative immunologic efficacy of CsA vs Tac. To determine if CsA can be utilized as effectively and safely as Tac in the dual drug MIRx regimen described above, we have undertaken a comparative study alternating use of the two calcineurin inhibitors in our RTx recips. Results from the first 17 of these patients are reported herein. Mean recip age is 47 yrs (21-73). Recip race: Cauc (n=9), Hisp (n=4), AA (n=3), other (n=2). Donors: deceased (n=6) and living (n=11). Target Co from 0-3 months were 8-10 ng/ml for Tac (n=8) and 150-200 ng/ml for CsA (n=9). Mean follow-up is 2.3 ± 1.4 mo. No AR has occurred in either cohort. There has been one infectious complication (cellulitis surrounding a drain site [resolved]) and one lymphocele (drained via a peritoneal window). No CMV or other opportunistic infections have occurred. To date, two recips have been converted from MMF to Pred due to leucopenia. Both were from the Tac cohort. Creatinine (Cr) levels did not differ significantly between the CsA and Tac recips. Combining the cohorts, Cr at 1 mo, 2 mo, and 3 mo are 1.7 ± 0.6 mg% (n=12), 1.4 ± 0.4 mg% (n=9), and 1.1 ± 0.3 mg% (n=6), respectively (mean ± SD). The data suggest that CsA and Tac may be used interchangeably in maintenance minimization protocols following C1H induction without compromising either immunologic efficacy or safety. Further modifications of the present protocol will focus on reducing target Co for both CsA and Tac and the possibility that drug interactions may predispose Tac-MMF recips to more severe leucopenia.

Abstract# 1042

CONTROLLED STUDY TO REDUCE THE IMMUNOSUPPRESSIVE LOAD AFTER KIDNEY TRANSPLANTATION GUIDED BY DONOR SPECIFIC CTLp MONITORING. Jacqueline van de Wetering,[1] Barbara J. van der Mast,[1] Petronella de Kuiper,[1] Nicolle M. van Besouw,[1] Jacqueline Richen-Vos,[1] Jan N. M. IJzermans,[2] Willem Weimar.[1] *[1]Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]General Surgery, Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

Background

Tapering the immunosuppressive medication is indicated to prevent long term side effects. Recently we have shown that renal transplant recipients can safely be converted from calcineurine-inhibitors to MMF or AZA when their donor specific cytotoxic T lymphocyte precursor frequencies (CTLpf) are below $10/10^6$ PBMC. We wondered whether the CTLpf also have predictive value when immunosuppression was reduced in patients only on MMF or AZA and steroids.

Methods

Renal transplant recipients with stable renal function, without proteinuria and at least two years after transplantation were included. If their CTLpf was low ($<10/10^6$ PBMC), the MMF or AZA dose was reduced to 75% at 4 months and to 50 % at 8 months after inclusion. If their CTLpf was high ($\geq 10/10^6$ PBMC), they were randomized in two groups. In one group immunosuppressive medication was tapered, the other group served as control.

Results

Sixty-eight patients have reached the one year follow up end point. Their median time after transplantation was 4.2 years (range 2.0-15.5 years). CTLpf were low in 41 (60%) and high in 27 (40%) patients. All patients had detectible cytotoxicity against third party. In all patients with low and in 15 patients with high CTLpf the MMF or AZA dose was reduced, while 12 patients served as controls. During tapering the immunosuppression, reversible acute rejection was observed in 1 of 41 (2%) patients with low and 2 of 15 (13%) patients with high CTLpf. Biopsy proven chronic allograft nephropathy was diagnosed in 1 patient of each group.

Conclusion

The majority of patients on MMF or AZA long after kidney transplantation have undetectable donor specific CTLpf. In this group a 50% reduction of immunosuppression is save and further decreasing their immunosuppressive load is the obvious next step. Patients with high CTLpf seem to have a higher risk to develop acute rejection, but in most of them (87%) reducing the immunosuppression is possible.

Abstract# 1043

ASSOCIATION OF AREA UNDER THE CURVE OF MYCOPHENOLATE MOFETIL AND SUBCLINICAL ALLOGRAFT REJECTION EPISODES. Charles F. Shield III,[1] Joan S. Kramer,[2] John L. Smith.[1] [1]*Kidney Transplant Program, Via Christi Regional Medical Center, Wichita, KS;* [2]*Clinical Research, Pharmacy Department, Wesley Medical Center, Wichita, KS.*

Introduction: There is little data published that addresses mycophenolate area under the curve (AUC) and allograft rejection episodes. Protocol allograft biopsies have allowed us to study this question. In this study, AUCs were obtained in 35 kidney transplant recipients who underwent protocol biopsy. Mycophenolic acid (MPA) AUC values were calculated using the trapezoidal AUC method. **Methods:** Fasting MPA troughs were obtained in 35 consecutive kidney transplant recipients (57% male) from March 2002 through August 2003. The average dose of mycophenolate mofetil (MMF) was 2 grams daily (maximum 3.5 grams/day; minimum 0.25 grams/day). The standard immunosuppression regimen was prednisone, tacrolimus, and MMF in > 90% of patients. The majority of patients also received phosphorus 250mg 4 tabs 4 times daily and calcium 650 mg 4 tabs 4 times daily for electrolyte abnormalities, which are known to interfere with MMF absorption, even though patients were instructed to take the doses at least 1 hour prior to or 2 hours after MMF doses. **Results:** Three levels of AUC were determined: low (AUC < 20 mcg.hr/mL; N=16), mid (AUC 20.1 to 40 mcg.hr/mL; N=15), and high (AUC > 40.1 mcg.hr/mL; N=4). Subclinical rejection episodes occurred in 75% of patients with an AUC low, 47% in AUC mid, and 25% in AUC high group.

Biopsy Proven Rejections and Therapy

	Low (AUC < 20)	Mid (AUC 20.1-40)	High (AUC > 40.1)
Rejection Therapy	N = 16	N = 15	N = 4
0	4	8	3
Steroids x 1	8	4	1
Steroids x 2	3	2	0
OKT3	1	1	0

Conclusion: The early AUC appears to determine the frequency of subclinical allograft rejection. The standard one gram twice daily MMF dose resulted in 60% of AUCs in the low group. Late chronic allograft nephropathy may be due to inappropriately low early AUCs resulting in subclinical rejection. AUCs should therefore be used to get an appropriate level early and maximal benefits of MMF to thereby decrease these rejections.

TRANSLATIONAL RESEARCH IN LIVER TRANSPLANTATION

Abstract# 1044

GENE EXPRESSION PATTERNS IN CIRRHOSIS AND HEPATOCELLULAR CARCINOMA (HCC) IN HCV-INFECTED PATIENTS AWAITING LIVER TRANSPLANTATION. Valeria R. Mas,[1] Daniel G. Maluf,[1] Richard K. Sterling,[1] Bradly Clark,[1] Cheryl Rodgers,[1] Andrea Ferriera-Gonzalez,[1] Robert A. Fisher.[1] [1]*Virginia Commonwealth University, Medical College of Virginia Hospitals, Richmond, VA.*

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death in the world. We investigated the genes involved in viral carcinogenesis and tumor progression in patients awaiting liver transplantation with Hepatitis C Virus (HCV) and HCC. We performed the analysis of hepatic gene expression in HCV-infected liver recipient patients with different stages of disease with and without HCC.

Methods: Gene expression profiling using microarray technology was performed in different RNA pools from liver tissue including: early HCV-cirrhosis, late-HCV cirrhosis, early-HCV-HCC, HCV-HCC (T3-T4), and normal liver tissues. In addition, we compared the gene expression profile from HCV-cirrhotic livers with alcoholic cirrhotic livers. Expression summaries for every probe set were calculated using three different algorithms.

Results: Transcripts more highly expressed in HCV-cirrhosis than in alcoholic-cirrhosis included IFN-inducible genes and those associated with antigen presentation and processing. The down-regulated genes from the comparison between HCV-cirrhosis and alcoholic-cirrhosis were those related to extracellular matrix production and deposition. The transcript *DLK1* was up regulated 86-fold in the advanced HCC pool vs. early HCC. The expression of alpha-fetoprotein in advanced HCC pool was more than 50-fold higher than in early HCC pool. Glypican 3 was highly expressed in T3-T4 pool. Five probe sets showed up-regulation of the gene expression for *CD24* from the comparison between HCC pools with the normal liver pool. In addition, *IGF-2* transcript was elevated 5-fold in HCV-HCC samples. The expression of *CYP3A4* and *CYP3A7* was higher in HCV-HCC pool, whereas it was significantly lower in normal liver tissues.

Conclusions: We found consistent differences between the gene expression patterns in HCV-HCC compared with early HCV-cirrhosis and late HCV-cirrhosis, and normal control livers. The expression patterns in HCC were also readily distinguished between early and advanced HCC tumor stages. We found different gene expression patterns between early cirrhosis and late cirrhosis. These findings confirm the presence of multiple molecular alterations during HCV-HCC hepatocarcinogenesis and provide a practical and testable method to identify prognostic factors associated with HCV-HCC progression and recurrence.

Abstract# 1045

ELUCIDATING THE PATHOGENESIS OF HEPATIC ISCHEMIA/REPERFUSION INJURY IN CLINICAL LIVER TRANSPLANTATION USING MICROARRAY. Ian D. McGilvray,[1] Limin Chen,[2] Ivan Borozan,[2] Jing Sun,[2] Maha Guindi,[3] Mark S. Cattral,[1] Paul Greig,[1] Aled M. Edwards,[2] David R. Grant.[1] [1]*Surgery, University Health Network, Toronto, ON, Canada;* [2]*Institute of Medical Genetics, University of Toronto, Toronto, ON, Canada;* [3]*Pathology, University Health Network, Toronto, ON, Canada.*

The clinical consequences of hepatic ischemia-reperfusion injury (HIRI) in liver allografts can be profound. However, little is known about the pathogenesis of this process. **Methods:** We performed sequential liver biopsies over the course of 17 living donor liver transplant operations and defined hepatic gene expression profiles at initial laparotomy (OPENING), after liver transection but before clamping of vascular inflow (PRECLAMP), and 2 hours following arterial reperfusion of the liver (post hepatic artery, PHA). Total cellular RNA was extracted from each biopsy and interrogated using either a 19K human microarray or a 2K ImmuneArray specific to human inflammatory genes. **Results:** Histological analysis confirmed that livers at OPENING were entirely normal; PHA biopsies had evidence of inflammatory change and early hepatocyte apoptosis. Tissue glutathione (GSH) levels decreased from the PRECLAMP to the PHA phase, confirming the underlying ischemic insult (relative to OPENING, GSH 1.30±3 PRECLAMP vs 0.70±2 PHA, $p<0.03$ ANOVA). Microarray gene expression data was normalized using GeneTraffic (Iobion) and in-house software and fold changes calculated in relation to baseline OPENING data. Nine annotated genes were increased by ≥2 fold with a p value ≤0.01 in the PRECLAMP phase (43 ≥1.5 fold), and 13 in the PHA phase (39 ≥1.5 fold)

Gene Expression Changes vs OPENING

PRECLAMP		PHA	
Gene Symbol	Fold Increase	Gene Symbol	Fold Increase
MT2A	3.6	SIAT1	5.2
SIAT1	3.5	MT2A	3.3
NNMT	2.9	SYJ2	2.8
MT1G	2.7	TM4SF3	2.7
CD7	2.7	NNMT	2.6
CAD	2.5	PTPRB	2.5
RAD52	2.0	MT1G	2.4
SYJ2	2.0	CAD	2.2
S100B	2.0	HLA-B	2.2
		DAP	2.2
		FGG	2.2
		ZNF183	2.1

RT-PCR was used to verify the microarray data. In order to suggest the roles of these genes we performed a functional cluster analysis guided by PathwayAssist software (Iobion). Genes were organized into discrete groups involved in apoptosis/survival (MT2A, GSK3, DAP, CRLF, ID2, CRLF1), cell proliferation (CD7, LTB, SDC4), and immune/inflammatory responses (GSK3, MT2A, HLA-B, TM4SF, SIAT1, S100B). **Conclusions:** Microarray analysis of in vivo HIRI results in a novel gene expression profile made up of genes with inflammatory and apoptotic functions. This finding correlates well with the inflammatory, pro-apototic microenvironment we noted histologically, and therefore suggests targets for future therapeutic interventions.

Abstract# 1046

EVIDENCE THAT TH1/TH2 IMMUNE DEVIATION IMPACTS ON EARLY GRAFT ACCEPTANCE AFTER PEDIATRIC LIVER TRANSPLANTATION: RESULTS OF IMMUNOLOGICAL MONITORING IN 40 CHILDREN. Jérémie Gras,[1] Anne Cornet,[2] Dominique Latinne,[2] Raymond Reding.[1] [1]*Dept of Surgery, Université Catholique de Louvain, Brussels, Belgium;* [2]*Transplantation Immunology Laboratory, Saint-Luc University Clinics, Brussels, Belgium.*

The clinical relevance of Th1/Th2 immune deviation is still a matter of debate in organ transplantation, and particularly in liver transplantation (LT). To address this question, immunological monitoring was performed in pediatric LT recipients, using the incidence of acute rejection (AR) as a marker of graft acceptance. Patients and Methods: 40 pediatric recipients (median age: 1.8y) of a primary living (n=22) or post-mortem (n=18) donor LT, transplanted between 11/99 and 02/02 under tacrolimus-steroids (group TS, n=20) or tacrolimus-basiliximab (group TB, n=20), were submitted to an immunological monitoring focused on a possible Th1/Th2 immune deviation. Cytokines gene polymorphisms (IFNγ, TNFα, TGFβ, IL6, IL10) were determined before LT, and cytokines blood levels (IFNγ, TNFα, IL2, IL4, IL5, IL10) were prospectively monitored pre-LT, at +1 and +2 hours following portal unclamping, and subsequently on days 1, 4, 7, 14, 28, 90, 180, and 365 post-LT. AR was diagnosed according to conventional clinical and biochemical criteria, with mandatory positive histology. Results: All patients were alive with their first graft at a median (range) follow-up of 24.8 months (12.7-40.1),

except for one child lost on day 159 from tumor recurrence. Incidence of early (<28 days post-LT) AR was 7/20 (35%) in group TS, versus 1/20 (5%) in group TB (p=0.044). Gene polymorphism for IFNγ, TNFα, and IL10 could be correlated neither with clinical outcome nor with pre- and post-LT levels of the corresponding cytokines. The immunological monitoring showed: (1) a peri-operative IL10 peak (as assessed by variations from baseline), which was significantly more sustained in the 32 children without early AR (+1h: p<0.001; +2h: p<0.001; day 1: p=0.004), when compared with the 8 children with AR (+1h: NS; +2h: p=0.018; day 1: NS); (2) a statistically significant decrement in IL2, IL4, IFNγ, and TNFα blood levels between day 1 and day 28 in the children without AR, whereas the corresponding levels remained stable in children with AR. Conclusions: Early AR was associated with lower peri-operative peak of the Th2-type cytokine IL10, and with subsequent lack of post-LT decrease of Th1-type cytokines. This study suggests the clinical relevance of Th1/Th2 immune deviation as a predictor/indicator of early AR in pediatric liver transplantation, to be confirmed with the currently ongoing intra-graft analysis of cytokines mRNA precursors using quantitative PCR technique.

Abstract# 1047

T-SUPPRESSORS MAY EXPLAIN REDUCED IMMUNOSUPPRESSION IN PEDIATRIC LIVER-INTESTINE (LTx-SBTx) RECIPIENTS RECEIVING STEROID-FREE, THYMOGLOBULIN (rATG) PRETREATMENT. Rakesh Sindhi,[1] Sanil Manavalan,[2] Amy Magill,[1] Jorge Reyes,[1] Adrianna Zeevi.[1] *[1]Transplantation, University of Pittsburgh, Pittsburgh, PA; [2]Columbia-Presbyterian Medical Center, New York, NY.*

Background/Purpose: rATG pretreatment may reduce immunosuppression (IM) needs via recipient T-suppressor (T-sup, CD8+28-)-mediated inhibition of donor antigen presentation. *Methods*: Twelve pediatric LTx (n=7), liver-kidney (LKTx-1), and SBTx (n=4) recipients, median age 6.5 years (1-21), who received rATG and steroid-free Tacrolimus/Sirolimus (TAC/SRL) were screened for T-sup. Four control LTx recipients, median age 15 years (5-20), in whom conventional IM without rATG was successfully discontinued for at least one year (no-IM) were also screened as a reference population. Magnetic-bead sorted, >90% pure, B-cells (CD19+), which present antigen, were stimulated by T-helper cell line (D1.1), transfected to overexpress CD40 ligand costimulatory receptor. The resulting frequency (%) of B-cells expressing CD86 (B7.2), a costimulatory/activation marker, represented baseline expression. T-sup function was present if this was decreased to <90% of baseline value after addition of recipient CD8+28- cells. No change or CD86 upregulation indicated absence of T-sup. *Results*: Median time to T-sup analysis was 16 months (2-24) in rATG subjects, and 168 months (16-228) in no-IM subjects. Nine of 16 subjects demonstrated T-sup (%CD19+86+ reduced to 69±28 of baseline) while 7 subjects did not (%CD19+86+ increased to 114±16 of baseline). T-sup were present in 4/4 children with no IM, and absent from 3 of 4 subjects with rejection (REJ), all of whom experienced > 1 REJ episode. An 8-year old subject with one REJ episode previously, demonstrated T-sup cells at admission for diarrhea, 6 months after SBTx. TAC trough levels (C_0) were reduced to 5-7 ng/ml for adenoviral enteritis, in the absence of biopsy-proven REJ.No rejection occurred. In the reduced IM group (n=8), 4 children demonstrated T-sup, and 4 did not. In this group, a 6-year old LKTx demonstrated T-sup on annual followup, during clinically-indicated reduction of TAC C_0 to 5-8 ng/ml, to protect declining renal allograft function. Conclusions: Donor-specific T-suppressor cells may characterize transplant recipients, in whom graft function can be maintained with minimal or no immunosuppression. Their absence from some subjects who are on reduced immunosuppression suggests that alternative adaptive mechanisms such as negative selection, or novel homeostasis, must be entertained. Such assays may also permit safe evaluation of prospective immunosuppression withdrawal strategies.

Abstract# 1048

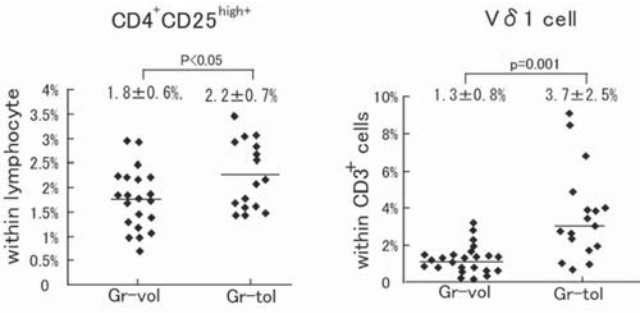
ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN OPERATIONAL TOLERANCE AFTER LIVING-DONOR LIVER TRANSPLANTATION (LDLT). Ying Li,[1] Takaaki Koshiba,[1] Atsushi Yoshizawa,[1] Atsushi Ito,[1] Hiroto Egawa,[1] Shimon Sakaguchi,[2] Nagahiro Minato,[3] Kathryn J. Wood,[4] Koichi Tanaka.[1] *[1]Department of Transplantation Immunology, Kyoto University, Faculty of Medicine, Kyoto, Japan; [2]Department of Experimental Pathology, Frontier Medical Sciences, Kyoto University, Kyoto, Japan; [3]Laboratory of Immunology and Cell Biology, Graduate School of Biostudies, Kyoto University, Kyoto, Japan; [4]Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington, Oxford, United Kingdom.*

Operational tolerance {graft acceptance in an immunosuppression (IS)—free environment} after LDLT occurs in a substantial number (approximately 40%) of the patients by our elective protocol (Transplantation; 72,449,2001). There is, nevertheless, no reliable parameter to monitor and select patients who may discontinue IS without a risk of rejection. To identify such parameters, we analyzed systemically liver transplant (Tx) patients exhibiting operational tolerance for the profiles of peripheral blood lymphocytes.

Methods. Seventeen liver Tx patients (Gr-tol) regarded as tolerant were enrolled in this study. FACS analysis was performed to determine the phenotype of peripheral blood lymphocytes. As controls, twenty-four age-matched volunteers with normal liver function (Gr-vol) were analyzed.

Results. An increase was observed in the percentages of B cells, $CD8^+$ T cells, $CD4^+CD25^{high+}$ cells and $V\delta1\gamma\delta T$ cells in peripheral blood lymphocytes from Gr-tol, compared with those from Gr-vol ($p<0.01$, $p=0.14$, $p<0.05$, $p<0.01$, respectively). On the other hand, the percentages of $CD4^+$ T cells, NK cells, NKT cells, and $V\delta2\gamma\delta T$ cells were decreased in Gr-tol, compared with those in Gr-vol ($p=0.17$, $p<0.05$, $p<0.05$, $p<0.01$, respectively). There was no significant difference between the two groups with respect to the percentages of pan-T cells, total $CD4^+CD25^+$ cells and αβ T cells and γδ T cells (NS, respectively). The similar tendency was found in the absolute number of each lymphocyte phenotype.

Conclusion. Present results revealed several intriguing features of peripheral blood lymphocyte subsets in patients exhibiting operational tolerance after LDLT. Although the contribution of those subsets to the tolerant state remains elusive, the results may provide clues for reliable indicators of tolerance after human liver Tx.



Abstract# 1049

ALLOGENIC CELL THERAPY FOR TREATMENT OF LIVER FAILURE. John W. Ludlow,[1] Andrew T. Bruce,[1] Michael J. Kulik,[1] Darell W. McCoy,[1] Sonya O. Meheux,[1] Thomas M. Asfeldt.[1] *[1]Vesta Therapeutics, Inc., Durham, NC.*

A. The purpose of this study was to develop a method whereby livers rejected for whole-organ transplantation could be processed in a regulatory compliant manner suitable for the manufacture of human cell therapy products.

B. Donated livers, not suitable for orthotopic liver transplantation, were obtained from federally designated organ procurement organizations. Cells were isolated utilizing a two-step collagenase digestion protocol. Separation of live from dead cells was facilitated by using a density gradient within the closed system of a commercially available cell washer. Cryopreservation methodologies were used to preserve the live cells. Commercially available primary antibodies and fluorochrome-conjugated secondary antibodies were employed for determination of cell phenotypes.

C. Processing donated livers yields a suspension containing billions of viable cells. These liver cell isolates can be reliably cryopreserved and thawed with a high degree of cell viability and recovery. The majority of the recovered cells are hepatocytes, based on expression of albumin. The remaining cells are a mixture of Kupffer cells, biliary cells, T-cells, and progenitor cells, based on their expression of a panel of specific cell surface and cytoplasmic markers. *In vitro* expansion of mature hepatocytes has been achieved using hormonally-defined medium.

D. We have successfully developed a process to manufacture a cell therapy product containing predominantly mature hepatocytes, and have received an allowance by the FDA to begin Phase I clinical trials of this product. It is anticipated that several patients can be treated using the mature hepatocytes obtained from just one donated organ. Expansion of these mature cells should enable treatment of tens to hundreds of patients.

Abstract# 1050

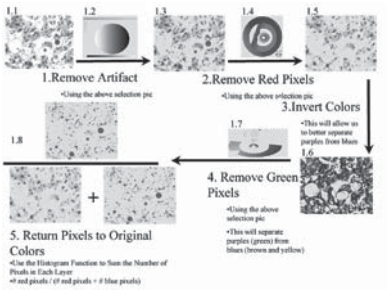
DEVELOPMENT OF AN OBJECTIVE METHOD FOR THE ESTIMATION OF LIVER STEATOSIS. Ryan N. Fiorini,[1] Jeremy Kirtz,[1] Basker Periyasamy,[1] Zachary Evans,[1] David Lewin,[1] Michael Schmidt,[1] Kenneth Chavin.[1] *[1]Department of Surgery, Division of Transplant, Medical University of South Carolina, Charleston, SC.*

Steatotic livers have a greater risk of primary non-function (PNF). Consequently, approximately 25% of livers are discarded secondary to steatosis. Evaluation of H&E stained biopsies by visual interpretation is subjective. We hypothesized that H&E staining of frozen sections fails to estimate the degree of steatosis from a liver biopsy of a potential donor organ. We developed a computer program to objectively assess fat content based upon differential quantification of color pixels in Oil Red O (ORO) stained liver biopsies. This was then compared to standard H&E and ORO, pathologist-read biopsies.

H&E and ORO stains were performed on 25 consecutive frozen sections of donor liver biopsies to determine fat content. Slides were read by a pathologist, a transplant surgeon, and evaluated by the computer program. Adobe PhotoShop 5.5' was used as base software in conjunction with ORO stained slides to objectively measure fat (fig 1). Results from the 3 methods were grouped based on computer ORO fat content into 3 groups. The human estimated ORO slides were compared to human estimated H&E slides and to the computer evaluated slides.

Samples with a fat content >20% showed marked variation between human interpretation and computer analysis. There was also a significant difference in the human interpretation of fat based upon staining method. This difference ranged from 3% to 37% with H&E. When compared to computer assessment, estimations of fat content for human read H&E sections were underestimated by at least 5% for 40% of the samples.

Use of ORO resulted in more consistent estimation of steatosis compared to H&E. Human interpretations failed to correlate with computer measurements. Such differences in fat-content estimations may result in rejection of a transplantable organ or acceptance of a marginal one. Ideally, our methodology can be applied clinically for the purpose of increasing transplantable organs.

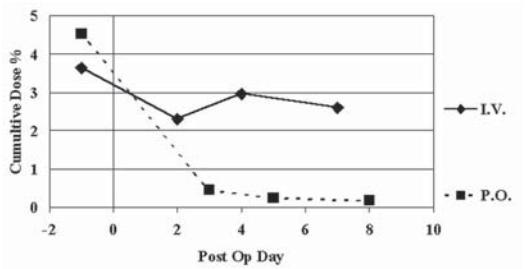


Abstract# 1051

HEPATIC OXIDATION OF ORALLY DELIVERED AMINO ACIDS REMAINS IMPAIRED FOR WEEKS AFTER LIVING LIVER DONATION. Richard B. Freeman,[1] Michelle Dixon,[1] Beth Horth,[1] Mary Beth Palladino,[1] Ann Marie Melanson,[1] Jeffery T. Cooper,[1] Richard J. Rohrer,[1] Anil Modak.[2] *[1]Division of Transplant Surgery, Tufts-New England Medical Center, Boston, MA; [2]Clinical Development, Cambridge Isotope Laboratories, Andover, MA.*

Although metabolic response after partial hepatectomy has been well studied in animal models, there are few studies examining restoration of metabolic capacity after right hepatectomy in humans. We used 13 C-labeled phenylalanine (13 C-P) administered orally or IV in 7 living liver donors and measured exhaled 13 C-P labeled CO2 to determine the extent of metabolic impairment and time course of its return. For orally administered 13 C-P, all subjects had dramatic drops in 13 C-P metabolism 2-4 days after surgery. Two of the 7 patients had restoration of the 13 C-P metabolic capacity 4 and 7 days after right lobectomy. The remaining 5 did not achieve pre-op levels of 13 C-P oxidation by as long as 58 days after surgery for orally administered substrate. Those recovering 13 C-P metabolism had significantly higher dose recovery 60 minutes after ingestion by day 4 (0.97 vs. 3.06, P=0.033) and day 7 (1.50 vs. 5.02, P=0.031). One patient given intravenous 13 C-P exhibited only a 43% reduction of 1 C-P metabolism on day 2, 25% on day 4 and 35% on day 7 compared to an 86% reduction on day 3, 92% on day 5 and 94% on day 8 for orally administered 13 C-P (figure 1). We conclude that orally administered amino acids may are not well absorbed and/or metabolized in some subjects for weeks after partial hepatectomy whereas intravenously delivered substrates are much better oxidized by the regenerating liver. These findings may be due to impaired gut motility or portal venous flow that reduces delivery of oral agents after liver surgery. These preliminary findings have wide implications for nutrition and drug delivery in the early recovery phase for living liver donors.

Figure 1.Metabolic Capacity for IV or Orally administered 13 C-P 30 minutes after dosing



Abstract# 1052

PHARMACODYNAMIC (PD) CORRELATES OF REJECTION WITH THYMOGLOBULIN (rATG) AND TACROLIMUS MONOTHERAPY (TAC) IN PEDIATRIC LIVER RECIPIENTS. Rakesh Sindhi,[1] Raman Venkataramanan,[1] Amy Magill,[1] Jorge Reyes,[1] Adrianna Zeevi,[1] Art Wetzel,[2] Silvester Czanner.[2] *[1]Transplantation, University of Pittsburgh, Pittsburgh, PA; [2]Pittsburgh Supercomputing Center, Pittsburgh, PA.*

Purpose: To characterize PD correlates of rejection in pediatric liver recipients. Methods: Forty one subjects, median age 4 years (0.45-19) receiving liver allografts alone (n=38) or with kidney (n=3) were evaluated serially for TAC whole blood concentrations, peripheral lymphocyte/leukocyte subset (PLS) distribution and function, and mixed lymphocyte response (MLR) to donor and non-HLA-identical donor (third-party). T- and B-cells served as surrogates for effector and antigen presenting cell (APC) types, respectively. Hill (PD) equations were used for effect: concentration analysis. Support Vector Machine (SVM) analysis, a computational classification algorithm of iterative relative weight assignments, was used to classify rejectors and non-rejectors using B-cell responses. Results: Median followup was 13 months (1-28), time to histopathological rejection was 15 days (4-104) in 17/41 children. Steroid-resistance led to OKT3 use in 3/17. CD3, CD4 and CD8 subsets demonstrated early decline, and gradual reconstitution. Frequencies of APCs such as B-cells, and monocytes, and progenitor dendritic cells (pDC1 and pDC2) were unchanged in rejectors and non-rejectors. Mitogen-stimulated T-cell cytokine production was minimally inhibited by increasing TAC concentrations. Compared with non-rejectors, rejectors demonstrated: 1. Similar TAC trough levels (14 vs 11 ng/ml, p=NS), 2. Numerically greater amounts of TAC required for half-maximal inhibition of B-cell receptors CD54, CD95, CD86, CD25, CD69, and CD71 (p=NS), 3. Significantly enhanced MLR to donor antigen (Mean Donor stimulation Index 59±42 vs 16±11, p=0.05), and 4. No differences in third-party stimulation index (41±34 vs 40±40, p=NS). SVM analysis of B-cell receptor expression in a test subset of patients (n=9) yielded a classification algorithm, which identified rejectors and non-rejectors with 100% accuracy in a validation subset (n=6). Conclusions: In children treated with rATG, rejection despite T-cell hyporesposiveness was associated with enhanced donor-specific alloreactivity, and not with decreased tacrolimus levels. A relative sparing of APCs from the depleting effects of rATG, and the ability of B-cell responses to distinguish rejectors from non-rejectors suggests that antigen-presenting mechanisms and their modulation are pivotal determinants of the balance between rejection and graft adaptation in pediatric liver recipients pretreated with rATG.

TRANSPLANT RELATED MALIGNANCIES

Abstract# 1053

A MULTI-CENTER RANDOMIZED TRIAL OF GANCICLOVIR VS. GANCICLOVIR PLUS IMMUNE GLOBULIN FOR PROPHYLAXIS AGAINST EBV RELATED PTLD IN HIGH RISK SOLID ORGAN TRANSPLANT RECIPIENTS: ONE-YEAR RESULTS. Atul Humar,[1] Diane Hebert,[2] Dele Davies,[3] Abhi Humar,[4] Derek Stephens,[5] Brenda O'Doherty,[6] Upton Allen.[6] *[1]Infectious Diseases, University of Toronto, Toronto, ON, Canada; [2]Pediatric Academic Multiorgan Transplant Programme, Hospital for Sick Children, Toronto, ON, Canada; [3]Pediatrics, University of Alberta, Calgary, AB, Canada; [4]Transplant, University of Minnesota, Minneapolis, MN; [5]Research Institute, Hospital for Sick Children, Toronto, ON, Canada; [6]Infectious Diseases, Hospital for Sick Children, Toronto, ON, Canada.*

Background: Pediatric and adult transplant recipients who are EBV seronegative and receive an organ from a seropositive donor (EBV D+/R-) are at increased risk for EBV and may benefit from specific antiviral prophylaxis. Viral load testing has become common in this setting and can be used as a surrogate outcome for clinical trials assessing prophylaxis. We performed a multi-center RCT assessing two different antiviral regimens and their effect on EBV replication.

Methods: Pediatric and adult EBV D+/R- transplant recipients were enrolled at 4 North American centers. Patients were randomized to receive either ganciclovir + placebo/no product or ganciclovir + immune globulin (IG) for 3 months. Following this, patients were unblinded and IG patients received additional IG therapy up to 6 months. All patients received oral antiviral therapy until 1 year post-transplant. EBV viral loads were done once monthly until 12 months post-transplant.
Results: A total of 34 patients completed the study protocol (16 in placebo arm and 18 in IG arm). 25 patients were pediatric and 9 patients were adults (22 male,12 female). Transplant types included liver (n=13), kidney (n=12), lung (n=8) and pancreas (n=1). Both groups were comparable at baseline in terms of age, sex, and donor/recipient CMV status. All patients had an undetectable EBV viral load at baseline. The incidence of detectable viremia within the first year post transplant was 13/16 (81.3%) in the ganciclovir arm vs. 13/18 (72.2%) in the ganciclovir+IG arm (p=0.8). Time to first detectable viremia, and time to high level viremia (viral load 3 $\log_{10}$ copies or higher) were not significantly different between the two arms. By a repeated measures ANOVA analysis, and by estimation of viral load AUC, no significant effect of randomization group was observed on EBV viral loads. Viral loads in adults were lower than pediatric patients in the first 6 months but no difference was observed by 12 months. PTLD developed in 3 (8.8%) patients (all 3 in IG arm; p=0.23) within the first 12 months.
Conclusions: No significant difference in EBV viral load suppression was observed when ganciclovir was compared with ganciclovir + IG in high risk EBV D+/R- patients. Viremia was common in both arms despite anti-viral prophylaxis.

Abstract# 1054

ANTIVIRAL USE AND ITS ASSOCIATION WITH POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) IN RENAL TRANSPLANT PATIENTS. Mark D. Pescovitz,[1] Donnie P. Funch,[2] Gary Schneider,[2] Alexander M. Walker.[2] *[1]Surgery/ Microbiology/Immunology, Indiana Univ, Indianapolis, IN; [2]Epidemiology, Ingenix Pharmaceutical Services, Auburndale, MA.*
The objective of this multi-center study was to assess the impact of antiviral exposure on risk of PTLD. **Methods.** All patients received a renal-only transplant on or after July 1, 1995 and were treated at one of 20 U.S. transplant centers. PTLD cases were confirmed by external review; pathologic information was available for 92%. Up to four controls were identified by UNOS for each case and matched on center, date of transplant and age. Center personnel abstracted data from medical records; data included initial and maintenance immunosuppression and rejection therapies, demographics, pre-transplant viral EBV status, rejection, risk factors (black race, cadaver donor, prior transplant, HLA mismatches, dialysis time) and antiviral exposure. Antiviral exposure was coded as days on acyclovir and ganciclovir separately (discounting the 30 days prior to PTLD diagnosis and a comparable time for controls to avoid including therapy that was given in response to PTLD symptoms). Multivariate conditional logistic regression models (stratified by first-year PTLD (early) and post first-year PTLD (late)) were used to determine the influences of cumulative time on acyclovir and ganciclovir, adjusted for each other as well as other abstracted data. Indication for the antiviral therapy was not taken into account although the majority of the recorded indications were for prophylaxis. **Results.** Data were collected for a total of 108 PTLD cases (67 early and 41 late) and 404 controls. For early PTLD, cumulative time on ganciclovir was significantly associated with lower risk of PTLD compared to patients with no antiviral exposure. For every 30 days of ganciclovir, risk of PTLD was lowered by 41% (Odds Ratio [OR]=.71; 95% CI=0.51-.99). There was no such effect for patients on acyclovir. Negative EBV serostatus of the recipient, and history of rejection or prior non-lymphoma malignancies were also associated with early PTLD with ORs of 24.56 (6.21-97.18), 4.83 (1.91-12.20), and 10.19 (2.50-41.58), respectively. Only rejection history was associated with late PTLD after adjusting for cumulative antiviral exposure and other abstracted information (OR=2.58; 95% CI=1.05-6.36)**. Conclusion.** These results provide support for the role of ganciclovir in reducing PTLD risk during the first year posttransplant. Ganciclovir use was independent of the other predictors of PTLD, suggesting that these risk factors could be useful in identifying patients most likely to benefit from extended treatment with ganciclovir.

Abstract# 1055

QUANTITATIVE EBV TITERS AND IMMUNOSUPPRESSIVE ALTERATIONS TO DECREASE THE INCIDENCE OF PTLD IN PEDIATRIC LIVER TRANSPLANTION. T. C. Lee,[1] N. R. Barshes,[1] B. Savoldo,[2] C. M. Rooney,[2] H. E. Heslop,[2] L. Nguyen,[1] L. J. Bristow,[1] J. D. Scott,[1] S. J. Karpen,[3] R. E. Quiroz-Tejeira,[3] J. A. Goss.[1] *[1]Dept of Surgery, Baylor College of Medicine, Houston, TX; [2]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX; [3]Dept of Pediatrics, Baylor College of Medicine, Houston, TX.*
Post-transplant lymphoproliferative disorder (PTLD) is the leading cause of late morbidity and mortality in the pediatric orthotopic liver transplant (OLT) recipient. This is due to the convergence of multiple factors including: primary EBV infection, EBV donor/recipient mismatches, and long-term immunosuppression.
Purpose: To determine if prospectively collected and analyzed EBV titers and titer-driven immunosuppressive modifications could reduce the incidence of PTLD in our OLT population.
Methods: Between 4/2001 and 10/2003, 34 pts (14 females, 20 males all <18 yrs old [median age 5.8 yrs]), 21 of which were EBV seronegative underwent OLT (25 EBV seropositive and 9 EBV seronegative donors) and were enrolled into this prospective study. Maintenance immunosuppression consisted of tacrolimus and prednisone. Tacrolimus troughs were maintained at 10-12 ng/ml, 8-10 ng/ml, and 5-8 ng/ml for post-OLT mos 1-3, 4-6, and after 6, respectively. Prednisone was tapered off by 6 mos following OLT. All pts were followed for EBV infection via serial clinical examination and monthly serum EBV titers utilizing real-time EBV PCR. EBV titers were considered elevated at >4000 copies/ug DNA. Upon the development of 2 EBV titers >4000 copies/ug DNA, tacrolimus was reduced to a trough of 3-5 ng/ml and if not already completed, prednisone was discontinued. There was no concurrent use of ganciclovir or other treatment modalities. Immunosuppression was left at this level as long as liver function remained normal irrespective of lower EBV titers. Follow-up for these pts is from 8-922 days.
Results: Prior to 4/2001, 30 pts underwent OLT and 5 pts (16%) developed biopsy-proven PTLD (Table 1). Since 4/2001, 34 children have entered the protocol and have had their immunosuppressive management assisted by serial serum EBV titers. 21 EBV seronegative recipients underwent OLT with 17 EBV seropositive and 4 EBV seronegative allografts. Since the initiation of this protocol no patient has developed PTLD. This is statistically significant (p-value<0.05, chi-square test) when compared to pre-protocol PTLD incidence.
Conclusion: The use of EBV titers as a tool to monitor EBV viral loads and assist in immunosuppressive management has an important role in the care of pediatric liver transplant recipients and leads to a decreased incidence of PTLD.
Table 1

	PTLD	Non-PTLD
Pre-protocol	5	25
Post-protocol	0	34

Abstract# 1056

IFN-γ GENOTYPE AND TGF-β INTERACTIONS CONTRIBUTE TO EBV-ASSOCIATED LYMPHOPROLIFERATIVE DISORDER (EBV-LPD) DEVELOPMENT. Anne M. VanBuskirk,[1] Tyler C. Hoppes,[1] Amy K. Ferketich,[3] Sameek Rowchowdhury,[2] Robert Baiocchi.[2] *[1]Surgery, The Ohio State University, Columbus, OH; [2]Internal Medicine, The Ohio State University, Columbus, OH; [3]Division of Epidemiology and Biometrics, The Ohio State University, Columbus, OH.*
In post-transplant lymphoproliferative disorder (PTLD), EBV-reactive T cell memory responses are unable to control EBV-driven lymphoproliferation and transformation. The mechanisms leading to the memory immune response failure in PTLD are not well delineated, although a reduction in EBV-reactive CTL activity is important. IFN-γ contributes to T cell and macrophage activation. In contrast, TGF-β antagonizes the effects of IFN-γ, modulating responses by T cells and antigen presenting cells, and contributes to EBV reactivation. Previous data from a small study indicated an association of a specific IFN-γ polymorphism (A/A at base 847) with both PTLD and TGF-β mediated inhibition of EBV-reactive CTL in vitro. To more completely test the association of cytokine genotype and EBV LPD, we performed a prospective study comparing cytokine genotype and LPD development by engrafting peripheral blood leukocytes (PBL) from 49 EBV-reactive, IFN-γ genotyped donors into SCID mice (hu PBL-SCID mice). The A/A genotype for IFN-γ was significantly more prevalent (p=. 014) in rapid LPD producers compared to intermediate/late LPD producers or donors whose PBL did not produce LPD. All rapid LPD donors exhibited the A/A or T/A genotype, indicating that the A allele is significantly more prevalent in rapid LPD donors (p=. 025). Thus, LPD in hu PBL-SCID mice is associated with the same IFN-γ polymorphisms as PTLD and inhibition of CTL restimulation. Because TGF-β has been shown to counteract the effects of IFN-γ, we went on to assess the contribution of TGF-β to LPD in vivo. Hu PBL-SCID mice were randomized to receive therapy with anti-TGF-β antibody or vehicle. ELISA demonstrated significant reductions in serum TGF-β (p=. 02) in treated mice, compared to controls. Preliminary data indicate that TGF-β neutralization results in expansion of human CD8+ cells in hu PBL-SCID mice. Importantly, this pilot study also shows that fewer treated mice (25%) develop LPD compared to controls (90%). Thus, TGF-β appears to contribute to LPD development at least in part by inhibiting CD8+ cell expansion. Given our previous in vitro data demonstrating the ability of TGF-β to inhibit EBV-reactive CTL restimulation in A/A and T/A genotype PBL donors, and the association of these genotypes with PTLD and LPD development, we suggest that TGF-β can inhibit CTL restimulation and expansion in people with the IFN-γ A/A or T/A genotype, thus rendering them more susceptible to PTLD.

Abstract# 1057

PROGRESSION OF CANCER CELLS CARRIED FROM THE GRAFTED LIVER. Takashi Murakami,[1] Mitsunobu Matsumoto,[1] Eiji Kobayashi.[1] *[1]Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, Kawachi-gun, Tochigi, Japan.*

The conventional immunosuppressive drug, cyclosporine A (CsA), affects cancer growth and metastasis, but it is still unclear how cancer is progressed under general immunosuppressive therapy after organ transplantation. Here we addressed how CyA promotes the dissemination of cancer cells that were carried to the grafted liver. The metastatic cell line of rat colon cancer, RCH-H4, was stably transduced with firefly luciferase, and then syngeneically inoculated (7 x 106 cells) into the liver of Fisher rats through the portal vein. The tumor-burden liver was then transplanted into the Lewis rat. The animals were followed daily by 15 mg/Kg of CyA (p.o.). Tumor progression was chased and evaluated by in vivo luciferase imaging system. Substantial luciferase expression was observed in the grafted liver of the CyA-treated animals at day 15, but not in the graft of mock-treated animals. In the un-transplanted Fisher rats, this immunosuppressive regimen showed that tumor cells metastasized to the liver (nearly 100%) and mesenteric lymph nodes (~50%) at day 15. Luciferase expression was not observed in the lung, bone, and brain. This CyA regimen did not alter the number of natural killer cells and their killing function. Mock treatment with olive oil showed low metastatic frequency in the liver (17%). Because a limited repertoire of chemokine receptors has recently been implicated with organ-tropic metastasis and progression of tumors, we also examined their expression by RT-PCR. Interestingly, this tumor cell line expressed both CXCR4 and CCR7 chemokine receptors. The liver was rich in the CXCR4 ligand (CXCL12), and lymph nodes were also rich in the CCR7 ligand (CCL21) and CXCL12. Thus, these results suggest that the immunosuppressive regimen with CyA allows the tumor cells in the donor graft to survive in the recipient, and that their tumor dissemination may be mediated by chemokine receptor CXCR4 and/or CCR7.

Abstract# 1058

DIFFERENCES IN SURVIVAL PATTERNS BETWEEN TRANSPLANT RECIPIENTS AND GENERAL POPULATION PATIENTS DEVELOPING DE NOVO COLORECTAL CANCER. J. F. Buell,[1] H. T. Papaconstantinou,[1] B. Sklow,[1] T. M. Beebe,[1] T. G. Gross,[1] M. J. Hanaway,[1] R. R. Alloway,[1] J. Trofe,[1] E. S. Woodle.[1] *[1]Israel Penn International Transplant Tumor Registry/Div. of Transplantation, University of Cincinnati, Cincinnati, OH.*

Despite recent data indicating that the incidence of colorectal cancer (CRC) is higher in transplant (TXP) recipients compared to the general population, the effects of immunosuppression on CRC are not well defined.

Methods: We examined all transplant recipients with de novo CRC. Analysis of patient demographics, tumor stage, tumor location, cadaveric vs living related (LR) renal TXP grafts, and immunosuppression was performed. Diagnosis age and survival rate were compared to CRC patients in the SEER National Cancer Institute (NCI) database.

Results: 150 TXP recipients with de novo CRC were identified, among which were 93 (62%) kidney, 29 (19.3%) heart, 27 (18%) liver, and 1 (0.7%) lung recipient. Median age of TXP was 54 years, compared to a median age of 72 years for patients in the SEER NCI database; median age at diagnosis of CRC was 59 years. Time from transplant to diagnosis of CRC relative to sex, race, tumor location, or tumor stage was not significant. Recipients of cadaveric renal TXP grafts were compared to those who received LR renal grafts. The results indicated a significantly shorter time from TXP to diagnosis of CRC (63.4±7.3 months *vs.* 103.5±19.2; p=0.023 respectively), with no significant difference in survival. However, compared to patients from the SEER NCI database, recipients with Duke's A through C stage disease were noted to experience a significant decrease in 5-year survival (Table 1).

Table 1

Stage	5 Yr. Survival-Txp Recipient	5-yr Survival-SEER/NCI	p-value
Duke's A & B	74%	90%	<0.001
Duke's C	20%	65%	<0.0001
Duke's D	0%	9%	NS

Conclusions: The early age at presentation of CRC in transplant recipients suggests that the development of de novo CRC may be effected by the degree of immunosuppression. Decreased 5-year survival rates, occurring at younger (median) diagnosis ages in TXP recipients with CRC, suggest that chronic immunosuppression may result in more aggressive tumor biology. Further study may indicate a post transplant CRC screening program with closer interval followup may be beneficial to these patients, as well as more aggressive reduction in immunosuppression.

Abstract# 1059

DONOR TRANSMITTED RENAL CELL CARCINOMA - WHAT TREATMENTS ARE EFFECTIVE. T. Merchen, M. Gupta, R. Boardman, T. G. Gross, T. Beebe, J. Trofe, R. R. Alloway, M. J. Hanaway, E. S. Woodle, J. F. Buell. *Division of Transplantation, University of Cincinnati, Cincinnati, OH.*

Renal cell carcinoma (RCC) is one of the most common donor transmitted malignancies encountered. Still, as little information is available defining the most effective treatment modalities, donor derived malignancies present a difficult therapeutic dilemma. **Purpose:** To examine the outcomes and therapeutic options of transplant patients with donor derived renal cell carcinoma. **Methods:** Treatment and mortality information on all solid organ transplant recipients with potential donor derived renal cell carcinoma was reviewed from our database. **Results:** 72 patients were identified to have received organs from donors with RCC. 47 recipients (65%) had tumor transmission of RCC. In 14 of these patients (30%) incidental tumors of the renal allograft were noted prior to implantation and local excision was performed. No tumor recurrences were noted and there was a 93% patient and graft survival rate. In 3 (6%) patients incidental RCC of the allograft was recognized within 6 months of transplantation. Each patient underwent partial nephrectomy with no recurrence and 100% patient and graft survival. The remaining 30 recipients (64%) were diagnosed with extensive allograft involvement and/ or diffuse metastatic disease. Of these patients, 17 (57%) had isolated allograft involvement. The mean time to diagnosis was 2.9 months. All of these patients underwent explantation with an 89% survival (neither of the 2 deaths were due to malignancy). 9 patients (30%) had allograft involvement and metastatic disease. The mean time to diagnosis was 9.5months. 2 patients survived (22%) with combined therapy of explantation, immunosuppression discontinuation (ISD) and immunotherapy (ITX). The final 4 patients (13%) had metastatic disease. The mean time to diagnosis was 21.6 months. 1 patient survived (25%) with explantation, ISC and ITX. **Conclusions:** The presentation of donor transmitted RCC can differ greatly. As a small allograft confined lesion the tumor can be excised safely while preserving renal function. In cases of extensive allograft involvement explantation and ISD is essential to salvage the patient. Finally, in patients with metastatic disease survival is poor but is associated with explantation, ISD and ITX.

Abstract# 1060

IL-2 RECEPTOR ANTIBODIES AND MALIGNANCY: AN ANALYSIS OF EARLY PEDIATRIC RENAL TRANSPLANT REGISTRY DATA. Vikas R. Dharnidharka,[1] Donald M. Stablein.[2] *[1]University of Florida, Gainesville, FL; [2]Emmes Corporation, Rockville, MD.*

Introduction: Malignancy such as PTLD is a major complication of solid organ transplantation. Others have reported an increased risk of PTLD associated with certain immunosuppressive agents, such as OKT3 and tacrolimus. Our analysis of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) registry was the first to show that tacrolimus, when used appropriately, was not a risk factor for PTLD (Pediatric Transplantation 2002;6:396-9). In all cases, the higher risk was not noted with early prospective clinical trials with short primary endpoints, but became apparent with longer follow up through retrospective analyses, either single-center based or registry-based. Dacluzimab and basiliximab are newer anti-IL2R blocking agents that are used as induction therapy in solid organ transplants. With longer follow up, the risks for malignancy due to these agents has not been reported.

Methods: In this study, we queried the NAPRTCS registry for the rate of malignancy development in patients who received either dacluzimab, basiliximab, OKT3, or other (polyclonal) induction. These agents have been listed separately in the database since 1997 onwards.

Results: In a previous analysis (Transplantation 2001;71:1065-8), we had reported no increased risk for PTLD with OKT3 use in pediatric renal transplant recipients. A re-review of the pre-1997 data showed a malignancy rate of 3.32% for OKT3 induction recipients versus 2.81% for those who did not receive OKT3 (P = NS). In the post 1997 era, the rate of malignancy in patients receiving OKT3 was higher at 3.9%, but this was not significant versus other strategies by Fisher's exact test (P = 0.23). OKT3 use has declined significantly in recent years, with only 7 recipients after the year 2000. The malignancy rates with dacluzimab and basiliximab are not higher than in those receiving other induction antibody (Table), nor are they different from each other.

Conclusions: In this early analysis, the first to look at malignancy rates with longer follow up times, dacluzimab and basiliximab do not appear to be associated with higher malignancy rates than other induction therapies or versus each other.

Frequency of maligancy with different induction agents

Agent	Transplants	Malignancies	Percent
Dacluzimab	503	11	2.2
Basiliximab	482	11	2.3
OKT3	202	8	3.9
None	1535	40	2.6

Abstract# 1061

DE NOVO BREAST CANCER POST SOLID ORGAN TRANSPLANTATION. R. E. Boardman,[1] E. S. Woodle,[1] T. G. Gross,[1] M. J. Hanaway,[1] R. R. Alloway,[1] J. Trofe,[1] M. R. First,[1] T. Beebe,[1] J. F. Buell. *[1]Israel Penn International Transplant Tumor Registry, University of Cincinnati, Cincinnati, OH.*

Breast cancer (CA) is the most common malignancy in women and the second most common of cancer related deaths. However, little data exists regarding the outcomes of de novo breast CA in solid organ transplantation. The purpose of this study was to define the course of de novo breast CA in transplant recipients. We examined 151 pts diagnosed (Dx) with de novo breast CA post transplant. **Results:** 97% (147) females and 2.6% (4) males at a median age of 52 ± 10.3 yrs were Dx with breast CA at a median of 40.3 (3-300) months post transplant. 56% (85) were Caucasian, 11% (17) African American, and 32% (48) unknown. Transplanted organs consisted of 119 kidneys, 17 hearts, 10 livers, 3 lungs, 1 pancreas, and 1 intestine.Tumor histology, staging, and lymph node involvement was similar to the general population (GP) and consisted of 66% intraductal, 15% lobular, 51% stage I, 34% stage II, 9% stage III, 5% stage IV, and 19% (28) lymph node (+). Secondary site of tumor involvement included liver (10), bone (7), lung (5), and skin (2). IS consisted of 46% antibody induction, 83% calcineurin inhibitor, 90% antimetabolite, and 96% prednisone. Surgery was performed on 97% of pts, 38% had chemotherapy, and 23% had radiation. Fewer pts had breast conservation therapy (lumpectomy 28%) and more pts had aggressive surgical management (mastectomy 23%, modified mastectomy 40%). Median f/u post Dx was 27 (0-235) mos. Overall survival rates at 1, 3, 5, 7 and 10 yrs were 92%, 79%, 74%, 72% and 70%. Results by stage are shown below.

		Stage I (N=77)	Stage II (N=52)	Stage III (n=14)	StageIV (N=8)
Age (mean)		52 ± 11	53 ± 10	50 ± 8	54 ± 8
Time from TXP to Diagnosis (mos)		53 (1-300)	30 (6-216)	24 (93-163)	56 (9-181)
Histology	Intraductal (%)	65	75	36	63
	Lobular (%)	13	13	29	25
Surgery	Lumpectomy (%)	35	21	21	13
	Mastectomy (%)	23	29	14	0
	Modified Mastectomy (%)	34	42	57	50
Chemotherapy (%)		8	71	79	50
Radiation (%)		21	25	36	13
Recurrence (%)		9	8	0	13
Median Time to Recurrence (months)		17 (2-49)	22 (17-86)	N/A	17(---)
Metastasis (%)		3	12	36	100
Lymph Node + (%)		0	31	57	50
Survival 1,3, 5, 7 & 10 yr (%)		100, 94, 90, 87% & 86	94, 79, 69, 65% & 63	64, 36, 36, 36% & ---	62, 25, ---, ---, & ---
Cancer Specific Mortality (%)		8	19	64	75

Conclusion: Tumor histology, stage and survival appear equivalent to that observed in the general population. Despite this, a more aggressive surgical approach is utilized in TX pts than the GP. We thus believe breast conservation should be encouraged in appropriate transplant patients with breast cancer.

Abstract# 1062

ADOPTIVE TRANSFER OF SPECIFIC SUBSETS OF DENDRITIC CELLS CHANGES THE XENOGRAFT SURVIVAL AND PATTERN OF REJECTION IN A RAT-TO-MOUSE CARDIAC TRANSPLANTATION MODEL. Hao Wang,[1,2] Jacqueline Arp,[2] Nobuyuki Kanai,[2] Xuyan Huang,[2] Bertha Garcia,[3] Weiping Min,[2] Robert Zhong.[1,2,3] *[1]Department of Surgery, London Health Sciences Centre, London, ON, Canada; [2]Transplantation Group, Robarts Research Institute, London, ON, Canada; [3]Department of Pathology, The University of Western Ontario, London, ON, Canada.*

Dendritic cells (DCs) are major regulators of both T cell and B cell immune responses. While we have previously demonstrated that Th2 cytokines are detrimental and Th1 cytokines are beneficial to xenograft survival (*Nature Medicine* 2000), the immunological factors controlling cytokine profiles of anti-xeno responses remain unknown. We previously reported that $CD11c^+CD8\alpha^+$ cells (DC1-like) were predominant in C57BL/6 recipients, in which Lewis rat heart grafts were slowly rejected with typical cell-mediated rejection (CMR) in 21 days. In contrast, $CD11c^+CD8\alpha^-$ (DC2-like) were predominant in BALB/c mice, in which the Lewis heart grafts were rapidly rejected with typical acute vascular rejection (AVR) in 6 days. These data suggest that DCs may play an important role in navigating xenograft immune responses. The present study was undertaken to elucidate the role of distinct subsets of DCs in controlling xenograft rejection. Purified $CD8\alpha^+$ or $CD8\alpha^-$ DCs isolated from wild type BALB/c mice were adoptively transferred into naive BALB/c mice respectively, and they subsequently received Lewis heart grafts. Adoptive transfer of $CD8\alpha^+$ DCs into BALB/c mice shifted the response to Th1, thereby altering the pattern of rejection from AVR to CMR and significantly prolonging xenograft survival to 14.2 ± 0.8 days, while transfer of $CD8\alpha^-$ DCs did not change the pattern of rejection and the grafts were rejected at 6 days. In addition, transfer of exogenous $CD8\alpha^+$ DCs rather than $CD8\alpha^-$ DCs from wild-type C57BL/6 mice into IL-$12^{-/-}$C57BL/6 mice prevented AVR and prolonged xenograft survival to 16.4 ± 0.9 days, while IL-$12^{-/-}$ C57BL/6 mice without transfer rapidly rejected the xenograft with AVR in 6 days. Furthermore, adoptive transfer of $CD8\alpha^-$ DCs from IL-$12^{-/-}$ mice into wild-type C57BL/6 recipients shifted the immune response to Th2, thereby altering the pattern of rejection from CMR to AVR and the grafts were rapidly rejected in 9.5 ± 0.6 days. We conclude that transfer of $CD8\alpha^+$ DCs (DC1-like) induces Th1-mediated CMR and prolongs xenograft survival, whereas administration of $CD8\alpha^-$ DCs (DC2-like) facilitates Th2-mediated AVR and accelerates xenograft rejection. These data suggest that distinct DC subclasses differentially affect the mechanism of xenograft rejection and ultimately affect graft survival.

Abstract# 1063

PORCINE CTLA4-Ig AS A SPECIES-SPECIFIC REAGENT TO PREVENT DIRECT PATHWAY XENOSPECIFIC T CELL SENSITIZATION. Vincenzo Mirenda,[1] Joseph Read,[1] Ivan Berton,[1] Anthony Warrens,[1] Robert Lechler.[1] *[1]Immunology, Imperial College London, London, London, United Kingdom.*

Although species incompatibility underlies most of the hurdles to xenotransplantation, it can also be exploited to achieve donor-specific immunosuppression. With this in mind we cloned the pig homologue of CTLA4 (pCTLA4) and constructed a fusion protein consisting of the extracellular regions of pCTLA4 and the constant regions of human IgG1 (pCTLA4-Ig). This failed to inhibit costimulation provided by human B7. The aim of this study was to confirm species specificity of PCTLA4-IG in a model of pig-to-mouse islet transplantation. We demonstrated that pCTLA4-Ig bound poorly to murine $CD80^+$ and CD86 expressed on transfectants, and gave minimal staining of the $CD80^+$ murine cell line DAP.3 and to mature mouse dendritic cells, as revealed by cytofluorometric analysis. In a T cell proliferation assay, pCTLA4-Ig blocked mouse T cell responses to pig but not mouse stimulator cells, whereas control murine CTLA4-Ig inhibited responses to both. In vivo, a single injection of 200ug CTLA4-Ig was incapable of inhibiting the delayed hypersensitivity response to oxazolone in mice. We therefore tested the efficacy of pCTLA4-Ig at preventing pancreatic islet rejection. Pig islets were transplanted under the kidney capsule of streptozotocin-induced diabetic mice. Mice injected with pCTLA4-Ig exhibited prolonged islet survival (36.5±9.4 days, n=6) compared to controls treated with isotype matched antibody (9.8±2.9 days, n=6). When combined with two subsequent injections of murine CTLA4-Ig to inhibit the indirect T cell xenoresponse, indefinite survival was achieved. Our results indicate that pCTLA4-Ig is a relatively specific inhibitor of the direct mouse T cell response to porcine tissues and therefore, is a potential therapeutic reagent to use in clinical xenotransplantation.

Abstract# 1064

COMPLETE INHIBITION OF ACUTE HUMORAL XENOGRAFT REJECTION BY REGULATED ENDOTHELIAL CELL EXPRESSION OF NOVEL ANTICOAGULANT FUSION PROTEINS. Anthony Dorling,[1] Daxin Chen,[1] Robert I. Lechler.[1] *[1]Dept. of Immunology, Imperial College London, London, United Kingdom.*

Acute humoral xenograft rejection (AHXR) remains a significant immunological problem after xenotransplantation. Histologically, organs rejected by AHXR show prominent widespread microvascular thrombosis, often accompanied by systemic coagulation disturbances in the recipient. This has led to the hypothesis that abnormal activation of clotting might be of primary importance to the pathophysiology of AHXR. We have tested the effect of inhibiting coagulation in a mouse heart-to-rat model of AHXR. Control C57BL/6 hearts, transplanted heterotopically without immunosuppression were rejected after a mean of 2.8 (+/-0.4) days. Histology showed widespread intravascular fibrin deposition and features typical of AHXR. Hearts were harvested from one of two novel strains of transgenic mice expressing membrane-tethered human tissue factor pathway inhibitor or hirudin fusion proteins under the control of a modified CD31 promoter for expression on activated endothelial cells (EC). These hearts survived for a mean of 6.6 (+/-0.49) days and 6.4 (+/-1.02) days respectively. Histology on the day of rejection showed no evidence of thrombosis but the grafts were infiltrated with CD3+ cells. Experiments were repeated under cover of daily cyclosporin. Control hearts were still rejected at 2-3 days by AHXR. In contrast, hearts from both transgenic donors are still beating more than 28 days post-transplantation. These experiments are ongoing. These data show that efficient inhibition of intravascular coagulation by expression of anticoagulants on EC completely inhibits AHXR in this small animal model, implying that activation of coagulation factors is an important element in the pathophysiology of humoral rejection.

Abstract# 1065

POTENTIAL VALUE OF CLONED PIGS EXPRESSING ENDO-β-GALACTOSIDASE C FOR XENOTRANSPLANTATION. Takaaki Kobayashi,[2] Akira Onishi,[1] Masaki Iwamoto,[3] Daiichiro Fuchimoto,[1] Shunichi Suzuki,[1] Satoshi Watanabe,[1] Satoko Arikawa,[3] Yuko Miwa,[2] Takaharu Nagasaka,[2] DaGe Liu,[2] Kenji Kadomatsu,[4] Takashi Muramatsu,[4] Kunio Morozumi,[5] Kazuharu Uchida,[5] Akimasa Nakao.[2] *[1]Department of Developmental Biology, National Institute of Agrobiological Sciences, Tsukuba, Japan; [2]Department of Surgery II, Nagoya University School of Medicine, Nagoya, Japan; [3]Prime Tech LTD, Tsukuba, Japan; [4]Department of Biochemistry, Nagoya University School of Medicine, Nagoya, Japan; [5]Kidney Center, Nagoya Daini Red Cross Hospital, Nagoya, Japan.*

INTRODUCTION Suppression of αGal antigen-antibody reaction is important for successful pig-to-human xenotransplantation. Genetic modification of donor pigs has been actively attempted in the world, and consequently, homozygous knockout cloned pigs for α1,3 galactosyltransferase (GT) were successfully produced. However, there is a possibility that such cloned pigs might express low levels of αGal epitopes by the function of a second GT gene such as iGb3. We have focused our attention on production of cloned pigs expressing endo-β-galactosidase C (EndoGalC) which can effectively digest αGal antigens on pig cells. The purpose of this study was to analyze the cells isolated from cloned piglets expressing EndoGalC and to assess its potential value for xenotransplantation.

METHODS The coding sequence of EndoGalC derived from Clostridium perfringens was ligated into pCAGGS expression vector. The sequences of cytoplasmic tail, transmembrane domain and stem region of GT were fused upstream of EndoGalC gene to localize EndoGalC to the trans-Golgi network effectively. EndoGalC gene was inserted into primary cultured cells isolated from Meishan or Landrace neonate pigs. Two pig cell lines in which αGal expression was reduced to below 2% were obtained. These cells were used for nuclear transfer. The normal cleavage and development was observed in the nuclear transferred embryos after two days of in vitro culture. 1282 embryos developed to 2 cell - 16 cell stage were transferred to 15 recipient pigs.

RESULTS Two cloned piglets were born by natural birth. Flow cytometric analysis revealed that more than 98% of αGal epitopes were removed in fibroblasts and red blood cells from cloned piglets. EndoGalC gene expression was detected on various organs. Immunohistochemical staining with GS-IB4 demonstrated that the level of αGal expression in the heart, kidney and liver was reduced to an undetectable level.

CONCLUSIONS The introduction of EndoGalC gene could eliminate αGal antigens almost completely. Its efficacy for αGal elimination was comparable to the targeted disruption of GT gene. The project on producing cloned pigs for xenotransplantation is now being implemented, but EndoGalC gene expression appeared to be useful as an alternative method to GT knockout.

Abstract# 1066

USE OF GALT-KO DONORS AVOIDS BOTH HYPERACUTE AND ACCELERATED HUMORAL XENOGRAFT REJECTION FOLLOWING MINIATURE SWINE-TO-BABOON RENAL TRANSPLANTATION. K. Yazawa,[1] T. Iwanaga,[1] A. Shimizu,[1] M. Nuhn,[1] S. Moran,[1] S. Nobori,[1] P. A. Vagefi,[1] D. K. C. Cooper,[1] R. Hawley,[2] H.-J. Schuurman,[2] J. Greenstein,[2] D. H. Sachs,[1] K. Yamada.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA; [2]Immerge BioTherapeutics, Cambridge, MA.*

Survival times following miniature swine-to-nonhuman primate renal xenotransplantation (XTx) have remained limited despite removal of natural antibodies (nAb) directed toward the α-1,3-gal (Gal) epitope and the use of T cell tolerance induction regimens aimed at preventing elicitation of new, T cell dependent antibodies. The inexorable recurrence of anti-Gal nAb leads to accelerated humoral xenograft rejection (AHXR) and coagulation disorders within 2 weeks following XTx. We now demonstrate that neither hyperacute, nor AHXR occur using Gal knock-out (GalT-KO) inbred miniature swine kidneys, even without treatment to remove nAb and/or complement. **Methods:** Renal xenograft function in the early postoperative period of baboon recipients of GalT-KO grafts (n=9) was compared to historical control recipients of Gal+ grafts from miniature swine. GalT-KO graft recipients received donor vascularized thymic grafts, splenectomy, and completed a full immunosuppressive (IS) regimen designed to induce T cell tolerance (including T cell depletion, MMF, steroids and a human anti-human anti-CD154 mAb). Gal+ grafts recipients received extracorporeal immunoadsorption of anti-Gal nAb and complement inhibition (CI) with a full IS regimen. GalT-KO graft recipients did not receive EIA, and 2 of the 9 received no CI. **Results:** Recipients of Gal+ kidneys rejected their grafts within the first 2 weeks, showing evidence of humoral rejection. Life-supporting GalT-KO donor renal xenografts showed no evidence of humoral rejection (with the possible exception of mild thrombotic microangiopathy), and graft survivals were extended to a mean of ≥31 days, with the longest survival at 81 days with a functioning xenograft (death was secondary to pneumonia). A slightly higher creatinine (Cr) level was noted in the two recipients of GalT-KO xenografts that did not receive CI in the first week (1.12 mg/dl without CI vs. 0.67 mg/dl with CI on POD 7). However, by POD 14 the Cr levels were similar (1.51 vs 1.27). **Conclusions:** GalT-KO donor kidneys appear resistant to hyperacute and AHXR, without the need for removal of nAb or CI, although the latter may protect the grafts from non-specific injury during the early post-transplant period. Tolerance induction strategies may prevent subsequent T-cell dependent antibody responses to other determinants on the GalT-KO kidneys.

Abstract# 1067

PIG-TO-BABOON LIFE-SUPPORTING KIDNEY PLUS THYMUS TRANSPLANTATION USING GALT-KO DONORS. K. Yamada,[1] K. Yazawa,[1] A. Shimizu,[1] M. Nuhn,[1] S. Moran,[1] T. Iwanaga,[1] P. O'Malley,[1] P. A. Vagefi,[1] J. Fishman,[1] D. K. C. Cooper,[1] C. Patience,[2] R. Hawley,[2] J. Greenstein,[2] H.-J. Schuurman,[2] M. Awwad,[2] M. Sykes,[1] D. H. Sachs.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA; [2]Immerge BioTherapeutics, Cambridge, MA.*

We have previously reported that vascularized thymic grafts, transplanted either as composite thymokidneys (TK) or vascularized thymic lobe (VTL) grafts, induce tolerance across allogeneic barriers in inbred miniature swine, and survive up to 30 days in pig-to-baboon xenotransplants using normal pig donors. We have now extended the latter studies using α-1,3-gal knockout (GalT-KO) pig donors. **Methods:** Baboon recipients of either TK (n=5) or VTL plus kidney (n=6) xenografts, from GalT-KO pigs, were treated with a protocol directed at tolerance induction (thymectomy, splenectomy, and T-cell depletion, followed post-transplant by a human anti-human anti-CD154 mAb, low dose steroids, and MMF). Control baboons received a GalT-KO kidney alone (n=3); one was similarly treated and 2 received chronic immunosuppression (CIS). Nine of 11 experimental recipients, and all controls, received CVF for the first 2 weeks. **Results:** Currently, 2 recipients of kidney plus VTL are POD 16. Immunosuppression was discontinued due to infection on PODs 5 and 20 in 2 recipients, and they rejected their kidneys on PODs 13 and 33, respectively. The other 7 recipients did not show evidence of rejection; two survived > 50 days (plasma creatinine < 1.5 mg/dl), and five expired from other causes (PODs 4, 16, 18, 26, and 31), but with normal renal function (although some proteinuria). Patchy thrombotic microangiopathy was seen histologically. One long-term survivor demonstrated excellent renal function for 68 days before expiring during surgery to replace an infected IV catheter. Another experienced an apparent rejection crisis starting on POD 53, which reversed with anti-T cell rejection therapy; however, the recipient expired on POD 81 from pneumonia. Control baboons receiving CIS rejected their grafts on PODs 20 and 33, and the remaining control rejected its kidney on POD 34. Neither hyperacute nor accelerated humoral rejection was observed in any animal. **Conclusion:** Use of GalT-KO donors has extended the maximum survival of vascularized thymus plus renal xenografts in baboons from 30 to over 80 days. Although the induction regimen still needs to be modified to reduce complications, these initial results are encouraging. Co-transplanting vascularized thymic tissue with xenografts holds the potential of achieving long-term tolerance across pig-to-primate barriers.

Abstract# 1068

TRANSPLANTATION (Tx) OF HEARTS FROM α1,3-GALACTOSYL-TRANSFERASE GENE-KNOCKOUT (GalT-KO) PIGS INTO BABOONS. Yau-Lin Tseng,[1] Kenji Kuwaki,[1] Frank J. M. F. Dor,[1] Akira Shimizu,[2] Stuart L. Houser,[3] Todd M. Sanderson,[2] Courtney J. Lancos,[1] Derek D. Prabharasuth,[1] Kazuhiko Yamada,[1] Robert Hawley,[2] Clive Patience,[2] Michel Awwad,[2] Jay A. Fishman,[4] Simon C. Robson,[5] David H. Sachs,[1] Henk-Jan Schuurman,[2] David K. C. Cooper.[1] *[1]TBRC, MGH/Harvard Medical School, Boston, MA; [2]Immerge Biotherapeuthics, Cambridge, MA; [3]Department of Pathology, MGH/Harvard Medical School, Boston, MA; [4]Infectious Diseases Division, MGH/Harvard Medical School, Boston, MA; [5]Center for Immunobiology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: We have to date performed 8 GalT-KO heart transplants in baboons.

Methods: Baboons received induction therapy with anti-thymocyte globulin, thymic irradiation (700cGy, n=7), cobra venom factor (CVF) from days -1 to 14 (n=2) or days -1 to 3 (n=3), and maintenance therapy with a human anti-human CD154 mAb, mycophenolate mofetil, and methylprednisolone. Heparin was administered to all baboons, anti-thrombin from days 1-12 to 3 baboons, and aspirin to 4 baboons. Two hearts from Gal compound heterozygous pigs expressing low but detectable levels of Gal were transplanted into baboons receiving the same regimen (without CVF) as controls.

Results: Both control hearts were rejected hyperacutely within 20 minutes. Hyperacute rejection was not seen in any GalT-KO heart, even in the absence of CVF therapy. Two grafts failed from a thrombotic microangiopathy (TM) on days 59 and 67, respectively, one of which showed focal interstitial hemorrhage and edema. One baboon died (day 56) and two were euthanized (days 23 and 16) for unrelated causes, all with functioning

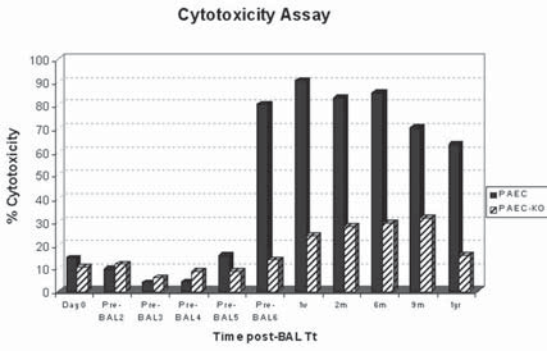
grafts, two of which showed mild TM (at 56 and 23 days). Three grafts continue to function well at 8, 19 and 105 days, respectively. The 105-day surviving heart shows some TM on biopsies. Mixed lymphocyte reaction to pig cells has been unresponsive and elicited antibody has been detected in only one baboon while receiving immunosuppressive therapy. Cellular infiltration of the grafts has been minimal or absent, and immunoglobulin and C4d deposition has been variable.
Conclusions: GalT-KO hearts are not susceptible to hyperacute rejection, but TM develops that can lead to graft failure. Its cause is uncertain. Anticoagulation strategies are being tested to inhibit TM.

Abstract# 1069

THROMBOTIC MICROANGIOPATHY IN HDAF AND GalT-KNOCKOUT PIG HEARTS FOLLOWING TRANSPLANTATION INTO BABOONS. Stuart L. Houser,[1] Akira Shimizu,[2,3] Kenji Kuwaki,[2] Frank J. Dor,[2] Yau-Lin Tseng,[2] Christoph Knosalla,[2] Jane Cheng,[3] Henk-Jan Schuurman,[3] David H. Sachs,[2] David K. C. Cooper.[2] *[1]Pathology, Massachusetts General Hospital, Boston, MA; [2]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA; [3]Immerge Biotherapeutics, Cambridge, MA.*
Background: Acute humoral xenograft rejection (AHXR) is an immunologic barrier in pig-to-baboon organ transplantation (Tx). We report histopathology of 3 groups (**A, B**, and **C**) of xenograft hearts which failed with microvascular thrombosis and myocardial necrosis. **Methods:** Eight baboons underwent heterotopic heart Tx from pigs transgenic for human decay accelerating factor; 4 (**A**) received heparin from day 2 after Tx, and 4 others (**B**), from the day of Tx (day 0). Six (**C**) received hearts from GalT-knockout pigs and were heparinized from day 0. All recipients received thymic irradiation, anti-thymocyte globulin, cobra venom factor (only 2 in **C**), mycophenolate mofetil, and methylprednisolone. **A and B** underwent anti-Gal antibody depletion with synthetic Gal oligosaccharide conjugates. Grafts were removed when palpable contractions stopped. Stained tissue sections from harvested grafts were studied by light and fluorescence microscopy. **Results:** Grafts survived 12-36 (m 23; med 14), 25-139 (m 68; med 54), 56-67 (m 63; med 63) days in **A, B**, and **C**, respectively. In **C**, two grafts failed, 3 recipients with beating grafts died of unrelated causes (m32; med 23), and one graft is ongoing at >104days. In all grafts, multiple platelet-rich fibrin thrombi occluded myocardial vessels. Interstitial neutrophils were focal in **A and C**; mononuclear cells were present but rare in all groups. Hemorrhage and edema were diffuse or regional in **A** and focal in **B and C**. Ischemic injury included myocytolysis, contraction band necrosis, coagulative necrosis, and myocyte dropout. Marked intimal thickening like that of allograft vasculopathy was seen in the longest surviving graft (139 days). Vascular IgG deposition occurred in 3 **A** grafts and 1 **B** graft; IgM, in 2 **C** and 2 **A** grafts. Patchy, sparse C4d staining of capillaries was seen in all groups. Notable C3 staining was absent. **Conclusions:** At varying times after Tx, AHXR and/or a non-immunologic coagulopathy caused microvascular thrombosis and myocardial infarction. Cardiac xenograft vasculopathy (chronic rejection) can occur with prolonged graft survival. On-going studies are directed toward determining the immunological or non-immunological basis of the observed findings.

Abstract# 1070

XENOANTIBODY RESPONSE TO α-Gal KNOCK-OUT PIG ENDOTHELIAL CELLS FROM LIVER FAILURE PATIENTS EXPOSED TO PIG HEPATOCYTES FOLLOWING BIOARTIFICIAL LIVER TREATMENT. Angeles Baquerizo,[1] Veneta Kirilova,[1] Ian Williamson,[1] Ova Oakley,[1] Henk-Jan Schuurman,[3] Clive Patience,[3] Achilles A. Demetriou,[1] Ronald W. Busuttil,[2] Christopher Shackleton.[1] *[1]Center for Liver Diseases and Transplantation, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Dumont-UCLA Transplant Center, UCLA, Los Angeles, CA; [3]Immerge Biotherapeutics, Cambridge, MA.*
Binding of human xenoantibodies (XAb) to the α-Gal antigens in pig endothelial cells (PEC) is considered one of the initial steps in the hyperacute rejection of pig to human solid organ xenotransplants. The recent development of transgenic pigs lacking the α-Gal epitope (GalT-KO) holds exciting prospects. We analyzed the long-term anti-pig XAb response developed by acute liver failure patients exposed to pig hepatocytes following bioartificial liver treatment (BAL). **Methods**: Patients' plasma from 5 patients was collected before and at different intervals after BAL. Flow-cytometric and ELISA assays (1:10 plasma dilution) were used to assess patient's IgM and IgG XAb cytotoxicity and binding to PEC from wild-type (wt) and GalT-KO miniature swine. **Results**: The binding of patients' IgG and IgM XAb to PEC from GalT-KO pigs were 30-57% lower than those from wt pigs. The natural anti-pig XAb levels fell during multiple BAL treatments (25-50%), the reduction being more pronounce for cells from GalT-KO pigs. After the BAL treatment was ended, the XAb titers rebounded by 35-50% in the 1st week, and remained stable afterwards. The XAb cytotoxicity results of one patient are depicted in Figure. The increase in cytotoxicity post-BAL treatments was markedly attenuated using cells from GalT-KO pigs. **Conclusions**: Human XAb recognize and bind to non-αGal epitopes on PEC from GalT-KO miniature swine. The anti-pig XAb response to GalT-KO pigs is reduced by 30-50% compare to wt pigs. Despite rebound in the XAb titers post-BAL, there was a marked reduction of cytotoxicity toward cells from GalT-KO.



CARDIAC TRANSPLANTATION: ALLOGRAFT VASCULOPATHY AND ACUTE REJECTION

Abstract# 1071 Poster Board #-Session: P1-III

LEFT VENTRICULAR ASSIST DEVICES IN THE DEVELOPMENT OF POST TRANSPLANT VASCULOPATHY. Gonzalo V. Gonzalez-Stawinski,[1] Patrick M. McCarthy,[1] Daniel J. Cook,[1] Fernando Atik,[1] Patrick E. Parrino,[1] Katheryn Hoercher,[1] David O. Taylor,[2] Randall A. Starling,[2] Mohamed H. Yamani,[2] James B. Young.[2] *[1]Thoracic and Cardiovascular Surgery, The Cleveland Clinic Foundation, Cleveland, OH; [2]Cardiovascular Medicine, The Cleveland Clinic, Cleveland, OH.*
Purpose: A common consequence of left ventricular assist device (LVAD) implantation is the development of anti-HLA alloantibodies. While alloantibodies have been shown to be associated to vascular rejection the ultimate impact on vasculopathy is still ill defined. Previously observed the presence of Class I anti-HLA donor reactive antibodies was associated with significant early post-transplant vasculopathy. To examine this we obtained coronary angiograms from 196 patients bridged with LVAD and compared their post transplant coronary angiograms to a non-LVAD cohort (n=112) examined in a previous study. **Methods:** Adult patients undergoing orthotopic heart transplant between 1999-2000 were retrospectively studied and compared to our LVAD population. Coronary angiograms were retrospectively reviewed and severity of coronary vasculopathy was categorized as either normal, mild, moderate or severe. Other variables studied included cytotoxic panel reactive antibodies (PRA) against T-cell targets and flow cytometric crossmatching against donor t lymphocytes. **Results:** As anticipated there was an increase sensitization in those patients receiving a LVAD. Twenty-one percent of LVAD patients had a T-cell PRA greater than 10% at the time of transplant compared to 3.6% of the controls ($p<0.0001$, Fisher's exact). Likewise, 25% of the LVAD patients had positive T-cell flow crossmatches compared to 5.4% of the controls ($p<0.0001$). Despite this coronary angiograms revealed no significant difference in the degree of coronary vasculopathy between the groups at follow-up. Normal coronary anatomy was detected in 72.5 % of LVAD patients and 64.6% of non-LVAD patients ($p=0.79$). These results were nearly identical at 2- and 3- year follow-up (71.8% vs. 64.6% and 58.3% vs.59.7%) **Conclusion:** While preoperative LVAD use is associated with a risk of recipient sensitization these patients develop transplant vasculopathy at the same rate as those not receiving a LVAD.

Abstract# 1072 Poster Board #-Session: P2-III

INFLUENCE OF DONOR GENDER ON ALLOGRAFT CORONARY VASCULOPATHY: EVIDENCE FROM INTRAVASCULAR ULTRASOUND. Sabri K. Erinc,[1] Mohamad H. Yamani,[1] Randall C. Starling,[1] James B. Young,[1] Tim Crowe,[1] Daniel J. Cook,[2] Robert Hobbs,[1] Corinne Bott-Silverman,[1] Gustavo Rincon,[1] Patrick M. McCarthy,[3] E. Murat Tuzcu.[1] *[1]Cardiovascular Medicine; [2]Allogen Laboratory; [3]Cardiothoracic Surgery, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Allograft coronary vasculopathy is a major risk factor for mortality following cardiac transplantation. Several factors including recipient-donor characteristics, immunosuppressive agents, and non-immune mechanisms have been evaluated as risk factors.

Objective: We evaluated the influence of donor gender on the progression of coronary vasculopathy in heart transplant recipients.

Methods: Eighty-nine heart transplant recipients (67 male, 22 female, mean age: 56±12 yr) underwent serial intravascular ultrasound analysis (IVUS) at baseline (within one month) and at one year after transplantation. Patients were divided into 2 groups: Forty-five recipients of female allografts (Group A) and forty-four recipients of male allografts (Group B). Ultrasound images were recorded on a super VHS tape during a distal to proximal automated pullback. Side branches were used to match the sites at baseline and 1 year of transplant. Volumetric analysis of the matched sites was performed and equal number of slices (Average=22) were evaluated. The following IVUS indices for the left anterior descending artery were measured for each patient: Change in maximal intimal thickness (MIT, mm), total plaque volume (Total PV, mm3), average intimal area (Av IA, mm2) and intimal index (%).

Results: Patients were similar in baseline characteristics including age, gender, diabetes mellitus, hyperlipidemia, etiology of heart failure, ischemia time, use of assist device, cytomegalovirus disease, rejection episodes, and immunosuppressive therapy. At 1 year of transplantation, measurements of allograft vasculopathy were found to be significantly higher in recipients of female allografts (Table). The recipient gender had no influence on coronary vasculopathy progression.

Conclusion: Female donor allograft is associated with increased risk of coronary vasculopathy independent of recipient gender.

Progression of coronary vasculopathy in recipients of female donors

	Group A (Donor-Female)	Group B (Donor-Male)	P-value
N	45	44	
ΔMIT (mm)	0.54 ±0.43	0.24± 0.26	< 0.001
ΔTotal PV (mm3)	25.59±43.39	10.21±24.15	0.04
ΔAv IA (mm2)	0.99± 1.72	0.29± 0.74	0.02
ΔIntimal index (%)	6.4±10.2 %	2.5 ±4.3 %	0.02

(Abbreviations: ΔMIT: Change in maximal intimal thickness, Δ Total PV: Change in total plaque volume, Δ Av IA: Change in average intimal area)

Abstract# 1073 Poster Board #-Session: P3-III

SIMPLE NON-INVASIVE SCREENING TO SELECT PATIENTS FOR LATE FOLLOW-UP INVASIVE EXAMINATIONS AFTER HEART TRANSPLANTATION. Michael Dandel,[1] Hans Lehmkuhl,[1] Manfred Hummel,[1] Roland Hetzer.[1] *[1]Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, Berlin, Berlin, Germany.*

Routine endomyocardial biopsies (EMBs) and coronary angiographies (CAs) later after heart transplantation (HTx) are distressing to the patients and not unfailing for early detection of acute rejections (ARs) or transplant coronary arteriopathy (TCA). Routine annual CAs often fail to detect coronary stenoses prior to a clinical event. The need of routine EMBs for late AR surveillance is also controversial. We showed that left ventricular (LV) wall motion assessment by pulsed-wave tissue Doppler (PW-TD) is easy to perform and useful for detection of LV dysfunction linked to AR and TCA. Now we assessed the value of PW-TD for timing of EMBs and CAs later after HTx.

Methods: In 165 patients (post-HTx time >2 years) monitored routinely by PW-TD we compared the diagnostic efficiency of routine EMBs and CAs (performed annually, unrelated to PW-TD results) with that of diagnostic EMBs and CAs (timed by PW-TD). Diagnostic CAs and/or EMBs were carried out whenever the systolic velocity (Sm) at the LV basal posterior wall showed a drop of >15% from previous values.

Results The 107 routine CAs revealed in 28 patients (26.2%) a new appearance or aggravation of TCA associated also with a Sm drop of >15%. The 58 diagnostic CAs revealed in 31 patients (53.4%) a new appearance or aggravation of TCA. Of the other 27 patients without angiographic changes, despite of Sm reduction, 26 (96.3%) showed relevant ARs in their EMBs. Among the 102 routine EMBs, 84 (82.4%) were ISHLT grade 0 and other 15 (14.7%) were ISHLT grade 1A or 1B. Sm reduction was detected in 12 of these patients, all with evidence of TCA. In 3 patients (2.9%) routine EMBs revealed relevant ARs (3A), which were also associated with Sm reduction. Among the 48 diagnostic EMBs performed because of relevant Sm reduction only 9 (18.8%) were ISHLT grade 0, but in 7 of these patients subsequent CAs showed either new appearance or aggravation of TCA. The other 39 diagnostic EMBs showed cellular ARs of different degrees (56.4% ≤ grade 2; 43.9% grade 3A or 3B) and 28 (71.8%) of these patients also showed intense vascular reaction (relevant vascular rejection in 8 patients).

Conclusion: Routine CAs and EMBs detect only a fraction of relevant coronary lesions and late ARs, respectively. Both late ARs and TCA are associated with Sm reduction of >15%. Serial PW-TD screenings followed by CAs and/or EMBs whenever Sm reduction of >15% is detected increase the efficiency of invasive follow-up examinations and provide an efficient strategy for TCA and late AR surveillance.

Abstract# 1074 Poster Board #-Session: P4-III

NONINVASIVE ASSESSMENT OF CORONARY FLOW RESERVE IN HEART TRANSPLANTATION RECIPIENTS PREDICTS CARDIAC ALLOGRAFT VASCULOPATHY. Francesco Tona,[1] Alida L. P. Caforio,[1] Roberta Montisci,[1] Antonio Gambino,[2] Giuseppe Feltrin,[2] Giuseppe Toscano,[2] Cristiano Sarais,[1] Riccardo Carta,[1] Annalisa Vinci,[1] Annalisa Angelini,[3] Gino Gerosa,[2] Sabino Iliceto.[1] *[1]Cardiology; [2]Cardiac Surgery; [3]Cardiac Pathology, University of Padova, Padova, Italy.*

Cardiac allograft vasculopathy (CAV) is the major factor limiting long-term survival in heart transplantation (HT). The evaluation of coronary flow reserve (CFR) provides valuable information on the status of coronary vessels. Intracoronary Doppler flow analysis suggested progressive CFR impairment in HT, although its correlation with CAV is controversial. We assessed the potential role of noninvasive CFR evaluation as predictor of CAV.

Methods: To evaluate the CFR in the left anterior descending coronary artery (LAD) of cardiac allograft we examined 26 HT recipients (13 male, aged 46 ± 12 years at HT) without wall motion abnormalities and hypertrophy by transthoracic echocardiography. Coronary blood flow velocity in the LAD was noninvasively detected at rest and during intravenous infusion of adenosine (0.14 mg/kg/min) at 7 ± 5.5 years after HT. CFR was obtained as the ratio of hyperaemic diastolic mean velocity (DMV) to resting DMV. Noninvasive assessment of CFR was achieved blindly from coronary angiography results and within 24 hours from catheterization. A CFR >2 was regarded as normal. Comparison of means was made by Student's t test. A p value <0.05 was considered to be significant.

Results: CAV was diagnosed in 12 out of 26 patients (pts) (46%) (group A) while 14 pts had normal coronary angiograms (group B). Septal and posterior left ventricular wall thickness were similar in the two groups (9.6 ± 0.9 vs 9.8 ± 1.16 mm; 8.9 ± 0.6 vs 9.4 ± 0.8 mm respectively, p= NS). Blood haemoglobin, heart rate, systolic and diastolic blood pressure were also similar. CFR was 2.7 ± 0.7 in all pts and reduced in group A vs group B (2.19 ± 0.46 vs 3.13 ± 0.64, p=0.0001).

Conclusions: Noninvasive assessment by transthoracic color Doppler echocardiography reveals that impaired CFR is associated with angiographically detectable CAV. CFR impairment seems related to dysfunction of the coronary microcirculation rather than enhanced myocardial oxygen consumption. Contrast-enhanced transthoracic echocardiography may represent a promising noninvasive diagnostic tool in CAV.

Abstract# 1075 Poster Board #-Session: P5-III

THE DEVELOPMENT OF LEFT VENTRICULAR HYPERTROPHY AFTER HEART TRANSPLANTATION: IS THIS A MARKER FOR POOR OUTCOME? Jignesh K. Patel,[1] Angela Marquez,[1] Eric Sue,[1] Erin Kobashigawa,[1] Hillel Laks,[1] Jon A. Kobashigawa.[1] *[1]University of California at Los Angeles, Los Angeles, CA.*

The development of left ventricular hypertrophy in patients after heart transplantation is usually associated with rejection, both cellular or humoral. The long-term outcome of these patients has not been established. Therefore, we reviewed 160 heart transplant patients with normal baseline echocardiograms one month after transplant surgery. 11 patients were subsequently found to develop posterior wall thickness 15 mm within the next 6 months. These patients constitute the Hyptrophy Group and the remaining 149 patients are the Control Group. There was no difference between groups for 5-year survival, freedom from cardiac allograft vasculopathy and freedom from any treated rejection. In addition, the mean arterial pressure (MAP) at 6 and 12 months and echocardiographic left ventricular ejection fraction (LVEF) at 6 months post transplant between the two groups were not different (see table). The Hypertrophy Group was 90% male, had a mean age of 53 years, and donor mean age of 38 years which were all similar to the Control Group. 6/11 patients of the Hypertrophy Group normalized their increased wall thickness within 2 years. The remaining 5 patients had persistent hypertrophy.

Hypertrophy vs. Control Patients

	N	5-Yr Survival	5-Yr Freedom from TCAD	5-Yr Freedom from any Treated Rejection	Avg. MAP for 6 Mos.	Avg. MAP for 12 Mos.	Avg. LVEF for 6 Mos.
Hypertrophy	11	82%	82%	73%	100.0 mmHg	97.6 mmHg	54.3%
Control	149	84%	87%	87%	100.0 mmHg	98.7 mmHg	55.2%

Conclusion: The development of left ventricular hypertrophy after heart transplantation appears transient for most patients and does not appear to be a marker for poor outcome. Systemic hypertension does not appear to be the cause of the left ventricular hypertrophy as blood pressures were similar/normal in both groups. The exact cause of left ventricular hypertrophy remains unclear but may be related to a self-limiting inflammatory state.

Abstract# 1076 **Poster Board #-Session: P6-III**

HEART TRANSPLANTATION FOR PATIENTS GREATER THAN 65 YEARS: ARE WE EXPECTING TOO MUCH? Jignesh K. Patel,[1] Sheryllene Go,[1] Gregg Fonarow,[1] Brandy T. Oeser,[1] Hillel Laks,[1] Jon A. Kobashigawa.[1] *[1]University of California at Los Angeles, Los Angeles, CA.*

The field of heart transplantation continues to progress with improved outcomes. As more of the large baby boomer generation reach older age and develop end-stage heart disease, we are finding that an increasing number of older patients are undergoing heart transplant surgery. However, data from several registries cite older patients as a risk factor for having less optimal outcome as co-morbidities of older age may compromise outcome. We reviewed 251 adult heart transplant patients between 1/90 and 9/97 to determine if older age 65 years is a true risk factor for poor outcome. Patients were divided into groups≥65 and < 65 years of age. The mean age of the older group was 67±2 years with 15% female while the younger group had a mean age of 51±11 years with 31% female. The 5-year outcome for the older group vs the younger group was comparable in survival and freedom from angiographic cardiac allograft vasculopathy (see table). Although not significant, the older group had a higher incidence of cancer (33% vs 17%), which expected. The older group vs the younger group had a significantly lower first year average biopsy score (ISHLT grade 0=0, grade 1A=1, grade 1B=2, grade 2=3, grade 3A=4, grade 3B=5, grade 4=6) and less infection (see table). This is consistent with immunoscenence in the older age group with regard to less rejection and subsequently less infection (due to reduced rejection therapy).

Older vs. Younger Heart Transplant Outcome

	N	5-Year Survival	5-Year Freedom from Angio CAV	Avg 1st Year Bx Score	5-Year Infection Incidence	5-Year Cancer Incidence
< 65 Years	217	81%	63%	0.77	35%	17%
≥ 65 Years	34	77%	60%	0.52	13%	33%
p-value		0.573	0.539	0.007	0.013	0.988

Conclusion: Heart transplantation in older patients appears comparable to younger patients. However the usual age-related co-morbidities such as cancer may limit longer-term survival.

Abstract# 1077 **Poster Board #-Session: P7-III**

AMIODARONE UTILIZATION PRIOR TO CARDIAC TRANSPLANTATION DOES NOT INCREASE RISK OF RE-TRANSPLANTATION: AN ANALYSIS OF THE UNITED NETWORK FOR ORGAN SHARING THORACIC REGISTRY. Rajan Krishnamani,[1] Andrew D. Feingold,[1] Wenjun Li,[1] Richard D. Patten,[1] David DeNofrio.[1] *[1]Division of Cardiology, Tufts-New England Medical Center, Boston, MA.*

Purpose

Earlier studies have shown that the pre-transplant use of amiodarone may increase the risk of both mortality and re-transplantation following cardiac transplant. We therefore, explored whether amiodarone use was independently associated with the risk of cardiac re-transplantation.

Methods

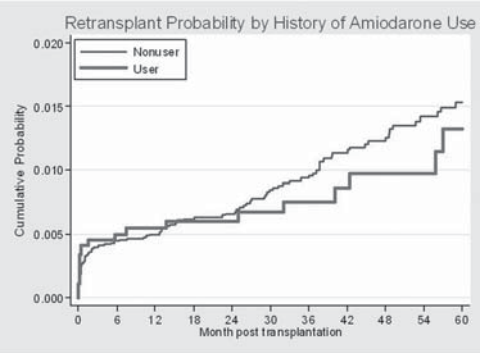
A retrospective analysis was conducted using the Organ Procurement and Transplantation Network data as of 5/31/2002 on the United Network for Organ Sharing (UNOS) Thoracic Registry. The analysis was restricted to first-time single organ cardiac transplant recipients between 4/1/1994 to 3/31/2001 with a known history of amiodarone treatment prior to transplant (n=13,204). The primary outcome was re-transplantation within 60 months following cardiac transplantation. Follow-up time was truncated at the time of death or at 60 months after transplantation. The probability of re-transplantation was calculated using the Kaplan-Meier method. Hazard ratios were estimated using a covariate-adjusted Cox proportional hazard regression model. For risk factors exhibiting non-proportional hazard over time, hazards were modelled either as a function of time or by stratification.

Results

13,204 patients met the study criteria, with 2,720 (20.6 %) patients having history of amiodarone use prior to transplantation. There were 120 re-transplantations in the 60 months follow-up period. The cumulative probability of re-transplantation at 60 months was 1.32% among amiodarone users compared to 1.53% among nonusers (p=0.61, figure). Under the proportional hazard assumption, multivariate analysis revealed no significant difference in the risk of re-transplantation for amiodarone use patients with a hazard ratio of 1.00 (95% CI: 0.62-1.62,p = 0.99).

Conclusion

Pre-transplant use of amiodarone does not appear to be associated with an increased risk of cardiac re-transplantation.



Abstract# 1078 **Poster Board #-Session: P8-III**

ASYMPTOMATIC CYTOMEGALOVIRUS ACTIVATION LEADS TO ACUTE REJECTION IN HEART TRANSPLANT RECIPIENTS DESPITE ANTI-VIRAL PROPHYLAXIS. Luciano Potena,[1] Cecile Holweg,[1] Helen I. Luikart,[1] Edward S. Mocarski,[2] John P. Cooke,[1] David B. Lewis,[3] Hannah A. Valantine.[1] *[1]Cardiovascular Medicine; [2]Microbiology and Immunology; [3]Paediatrics Immunology, Stanford University, Stanford, CA.*

Background Anti-cytomegalovirus (CMV) prophylaxis significantly decreased morbidity and mortality rates associated with CMV disease after heart transplantation (HT) but the impact of asymptomatic CMV reactivation is unknown. In this prospective study we hypothesize that asymptomatic CMV activation predisposes to acute rejection (AR)

Methods 36 consecutive HT recipients (43±18 yr, 72%males) were enrolled. Immunosuppression consisted of prophylaxis with daclizumab, and a regimen of cyclosporine, prednisone and mycofenolate or sirolimus. All seropositive recipients received CMV prophylaxis, consisting of 4 weeks of intravenous ganciclovir. In addition, seronegative recipients receiving the graft from seropositive donor received CMVIgG. Endomyocardial biopsies (EMB) were taken weekly during the first month, and monthly thereafter. At each EMB time point peripheral blood leucocytes (PBL) were isolated for CMV-DNA PCR analysis.

Results Patients were followed for 251±153 days; 490 EMBs and 454 blood samples were available for analysis. Although none of the patients presented clinical CMV disease, we found 24 (66%) patients with multiple positive CMV PCRs in PBL, thus indicating CMV reactivation. This group of patients had higher 6-months rejection score index than CMV negative ones (0.79±0.44 vs. 0.51±0.35, P=0.07). Similarly, estimated incidence of AR≥1B was 90±7% in CMV positive patients and 53±17% in CMV negative ones (P=0.02). Limiting the analysis to AR ≥ 3A, estimated AR incidence was 50±11% in CMV positive vs 20±13% in CMV negative (P=0.09) patients. Time to first positive CMV PCR was significantly shorter than time to first AR positive biopsy (53±51 vs. 104±92 days P=0.05), consistent with CMV reactivation preceding AR. Among multiple clinical and demographic characteristics evaluated, cold ischemia time (RR for each 30 min = 1.81; P<0.01) and CMV reactivation (RR= 5.31; P=0.04) were the only independent predictors of early AR.

Conclusions Early asymptomatic CMV reactivation is associated with the subsequent development AR. These data suggest a role for CMV in rejection, which may result from direct consequences of viral replication or from an active interplay between host adaptive immune response to CMV and the alloimmune response to the graft. Anti-viral prophylaxis with ganciclovir appears to prevent acute CMV disease but leaves the recipient open to other consequences of viral persistence.

Abstract# 1079 Poster Board #-Session: P9-III

IS THIRD-TIME RETRANSPLANTATION JUSTIFIABLE? Jonah Odim, Hillel Laks, Simin Bahrami, Ana Banerji, Kaushik Mukherjee, and the UCLA Heart Transplant Team. *Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; UNOS Registry.*

Introduction: Repeat heart transplantation may carry higher risk than primary engraftment.

Purpose: We tested the hypothesis that third time cardiac allograft transplantation is associated with prohibitive mortality and morbidity.

Patients: The cohort of all third time cardiac retransplants performed in the United States and reported to UNOS from 1987 to 2002 (N = 10) ws reviewed in addition to our single center experience. The primary endpoints were early and late mortality.

Results:

Comparison of mortality outcome after 1st, 2nd and 3rd cardiac allografts

Descriptor	1st Tx	2nd Tx	3rd Tx*
	UNOS 2000	UNOS 1987-1998	UNOS 1987-2002
Female	587/2198 (27%)	19%	2/10 (20%)
CHD	146/2198 (7%)		3/10 (30%)
Mean age (yr)	~58**	49	26.8
Interval b/w Tx		1st and 2nd Tx	2nd and 3rd Tx
≤6 m		171/597 (29%)	1/10 (10%)
>6 m		426/597 (71%)	9/10 (90%)
Mortality			
<30 d	2970/11346 (26%) ***	~33%	0
31 d - 1 y	2827/11346 (25%)***	~33%	2/10 (20%)
>1 y	5549/11346 (49%)***	~33%	1/10 (10%)

Tx, transplant; CHD, congenital heart disease; b/w. between, * based on OPTN data as of July 11, 2002; ** median age; *** UNOS 1987-2001 data

Of the ten patients undergoing third-time retransplants, pre-operatively, one was VAD dependent, four were on IV inotropes, and two had creatinine levels greater than 2.5. Additionally, four were male recipients of female donor hearts and the mean donor ischemic time was 2.6 hours. In our center experience, 3 patients underwent a third heart transplant. There was no early or hospital mortality. One patient died late from TCAD and another following a fourth allograft.

Conclusion: The mortality rate for third-time heart allograft recipients is acceptable. These favorable results may be influenced by small sample size, younger age, case selection, and operations at select, high-volume institutions with significant experience. Further study is warranted

Abstract# 1080 Poster Board #-Session: P10-III

INFLUENCE OF GROWTH FACTORS GENE POLYMORPHISMS ON ACUTE AND CHRONIC REJECTION AFTER PEDIATRIC HEART TRANSPLANTATION. Sylvie Di Filippo,[1] Steven A. Webber,[2] Anat Tambur,[3] Robert Ferrel,[4] Kevin McDade,[1] Gerard J. Boyle,[2] Susan A. Miller,[2] Adriana Zeevi.[1] *[1]Transplant Pathology, Biomedical Science Tower, Pittsburgh, PA; [2]Cardiology, Childrens Hospital of Pittsburgh, Pittsburgh, PA; [3]Pathology, Rush Medical Center, Chicago, IL; [4]School of Public Health, University of Pittsburgh, Pittsburgh, PA.*

The aim of this study was to assess the effect of growth factors gene polymorphisms on acute rejection (AR) and graft coronary disease (CAD) in pediatric heart transplant (HTx) recipients.

Genotyping using PCR specific primers were performed for TGFβ1 (codons 10 and 25) and VEGF-2578 gene polymorphisms in 111 patients who underwent a HTx at the mean age of 7.5 ± 6.7 years. Acute rejection (AR) was defined as ISHLT grade > 2. Patients were defined as rejectors if they had recurrent AR (more than 2 episodes), steroid-resistant AR or graft dysfunction within the first year post-Tx; the other cases were defined as non-rejectors.Thirty-nine patients were considered Rejectors (35%) and 31 developed graft coronary disease (CAD) (28%), 22 to 149 months posttransplant (mean follow-up 79months).

The proportion of rejectors was higher in the TGFβ1codons10-25 high-producer group than in the intermediate/low-producer group (respectively 40% versus 14%, p= 0.026). Allele AA for VEGF was associated with a higher incidence of acute rejection (55.5% rejectors versus 30.6% rejectors in patients with AC or CC allele, p= 0.05).

CAD was observed in 2/21 (9.5%) of HTx recipients TGFbeta1codon10-25 low-producers while 29/90 (32.2%) of TGFbeta1codon10-25 high-producers developed CAD (p= 0.037). This difference was also observed when TGFβ1codon25 gene polymorphism was analyzed (11 low-producers with no CAD and 90 high producers with 31 CAD, p= 0.03). VEGF-2578 genotype was not different among patients with or without coronary disease.

In summary, TGFbeta codon10-25 high producers have an increased risk of both AR and CAD. Allele AA in the VEGF -2578 was linked with AR but did not influence CAD frequency. Further larger studies are needed to confirm these results and analyze the impact of VEGF on pediatric graft coronary disease.

Abstract# 1081 Poster Board #-Session: P11-III

ROUTINE INVASIVE MONITORING MIGHT NOT BE NEEDED AFTER HEART TRANSPLANTATION IN CHILDREN. Sylvie Di Filippo,[1] Pascale Boissonnat,[2] Francois Sassolas,[1] Jean Ninet,[2] Gerard Champsaur,[2] Andre Bozio.[1] *[1]Pediatric Cardiology, Hopital Cardiovasculaire Louis Pradel, Lyon, France; [2]Cardiothoracic Surgery, Hopital Cardiologique Louis Pradel, Lyon, France.*

Background: Many centers still recommend invasive endomyocardial biopsy (EMB) monitoring to detect acute rejection in pediatric heart-transplant recipients.

Aim: The aim of the study was to determine the usefullness of routine EMB early and late after heart transplantation (HTx) in children.

Material and methods: Thirty-eight patients (mean age at HTx= 11.8 years, mean follow-up= 6.8 years) underwent 278 EMB, 227 as routine surveillance EMB (R-EMB) and 51 as non-routine EMB (NR-EMB) for symptoms or changes in non-invasive tests. All were given ciclosporine-based triple immunosuppression. Histological ISHLT grade > II was defined as high-grade acute rejection (AR)and ISHLT = or < II as low-grade.

Results: During the first 3 months postHTx, 44 of 93 EMBs were positive for AR, ranging 32 of 80 R-EMB (41%) and 12 of 13 NR-EMBs (92%). Fourteen high-grade were diagnosed, 7 occurring on R-EMB (9% of early R-EMB, 50% of early high-grade rejections).

Between the 3rd and the 12th month postHTx , 3 of 8 AR occurred on R-EMB (2 high-grade) and 5 on NR-EMB (1 high-grade).

Beyond 1-year postHT, 31 of 146 EMBs were positive for AR, 7 of 117 R-EMB (5%) and 24 of 29 NR-EMB (83%). Fifteen were high-grade rejections, 14 occurring on NR-EMB; only one of the 117 late R-EMB was positive for high-grade AR (0.08%).

Changes in Echocardiographic and Doppler measurements were the most frequent indication for NR-EMB; Echo-Doppler abnormalities were recorded in 81% of high-grade AR and 63% of low-grade AR. Clinical symptoms were present in 54% of high-grade AR but only 5% of low-grade. ECG changes were less frequent:18% of high-grade and 26% of low-grade AR. Ten NR-EMB were performed for suspected AR and were negative: echographic changes were present in 50%, clinical symptoms in 40% and ECG modifications in 20% of these EMB indications.

Conclusion: In our experience, routine EMB could detect unexpected significant acute rejection in the first 3 months after HTx in children, but did not yield to significant information beyond the first year postHTx. Non-invasive monitoring should be preferred in the long-term follow-up after HTx in the young.

Abstract# 1082 Poster Board #-Session: P12-III

USING GENE CHIPS TO DISCRIMINATE AMONG REJECTION, NON-REJECTION AND CHAGAS' DISEASE REACTIVATION IN HEART TRANSPLANTS. Andrey Morgun,[1] Natalia Shulzhenko,[1] Ainhoa Perez-Diez,[2] Polly Matzinger,[2] Maria Gerbase-DeLima.[1] *[1]Pediatrics Department, UNIFESP, Sao Paulo, SP, Brazil; [2]LCMI, NIAID, NIH, Bethesda, MD.*

Currently, the diagnosis of acute cardiac rejection (AR) is based on histology of endomyocardial biopsies (EMB). However, its main drawback is a low sensitivity. Moreover, AR is morphologically identical to post-transplant cardiac Chagas' disease reactivation (CHR) unless the parasite is seen. The aim of this study was to determine possible differences in gene expression patterns among rejection, no rejection, or CHR. We divided 54 EMB into the three groups on the basis of their histology and positivity for T. cruzi. We isolated RNA, amplified it, labeled with Cy5 or Cy3, and hybridized to microarray chips containing 14,000 genes. We then divided the samples into two sets, using the first as a training set to build gene predictors and the second as a test set to validate the results. The gene analysis of the training set allowed us to correctly classify rejection vs. no rejection in 87% of cases, based on 70 discriminating genes ($p<0.001$). Using the genes revealed from the training set, 83% of the test set EMB were predicted correctly, and all EMB with rejection were correctly classified by the array (100% sensitivity). Interestingly, 75% of CHR samples were also classified as rejections ($p<0.01$), revealing the similarity of immune/inflammatory expression profiles between AR and CHR. However, a comparison of genes expressed by CHR and AR biopsies disclosed 25 genes that discriminated between them ($p<0.001$), leading to 93% of correctly classified samples in the training set and 80% in the test set. Based on these microarray results, we used real-time RT-PCR to evaluate the expression of 10 target genes encoding immunity/inflammation and cellular energy-related molecules and 2 control genes, in the non-amplified RNA samples analyzed by the microarray and in 30 additional EMB. Among these 10 target genes, the expression of 7 genes confirmed differences observed in microarrays, 2 genes showed a trend, and 1 did not show any difference. Analysis of differences in gene expression between clinical situations revealed a high correlation between the microarray and RT-PCR results ($r=0.94$, $p<0.001$), and a detailed gene-by-gene analysis showed that the difference between molecular profiles of AR and CHR lies within an area of non-immune but tissue related gene expression. These results are valuable not only for the diagnosis of AR, and differential diagnosis of AR and CHR, but also for the better understanding of these pathological processes.

Abstract# 1083 Poster Board #-Session: P13-III

LEUKOCYTE GENE EXPRESSION SIGNATURE OF CMV VIREMIA IN CARDIAC ALLOGRAFT RECIPIENTS IS DISTINCT FROM THAT SEEN DURING ALLOGRAFT REJECTION. Mario C. Deng,[1] Mandeep Mehra,[2] Sharon Hunt,[3] Hannah Valantine,[3] Richard Miner,[4] Julie Phillips,[5] Jay G. Wohlgemuth,[5] David Chernoff,[5] Eleanor Woodward,[5] Howard J. Eisen.[6] *[1]Columbia University, New York, NY; [2]Ochsner Clinic, New Orleans, LA; [3]Stanford University, Stanford, CA; [4]UCSF, San Francisco, CA; [5]Expression Diagnostics Inc, South San Francisco, CA; [6]Temple University, Philadelphia, PA.*

Purpose: CMV infection in cardiac transplant recipients has been associated with accelerated graft failure and morbidity related to visceral CMV infection. CMV can trigger specific immune responses which may overlap with immune activation profiles associated with acute allograft rejection. This study compared gene expression patterns in peripheral blood mononuclear cells (PBMCs) associated with CMV viremia and acute rejection.

Methods: Adult cardiac transplant patients were prospectively enrolled in the Cardiac Allograft Rejection Gene Expression Observational (CARGO) multi-center study. Subjects were followed at post-transplant visits with cardiac biopsy (read by 3 pathologists blinded to clinical data) and simultaneous sampling of PBMC RNA and plasma. Plasma was tested for CMV using Roche's AMPLICOR quantitative DNA PCR assay. PBMC expression profiles were determined using real-time PCR on 200 leukocyte genes. Profiles of patients with CMV viremia and patients with acute rejection were compared.

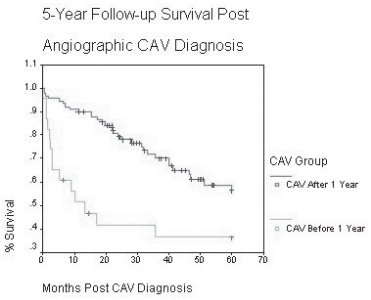
Results: Of 171 samples from 104 patients, 13(8%) had CMV detected by quantitative PCR. Eighteen genes correlated with CMV viremia ($p < 0.05$), including T-cell activation (Granzyme B, LAG3) and anti-viral response and host defense (Viperin, IFNg) genes. A subset of these genes had previously been correlated with CMV infection, though a subset of genes not previously known to be associated with CMV infection was also identified. No correlation was detected between plasma CMV PCR positivity and acute rejection (ISHLT Grade 2 or greater) at time of sample acquisition or at future visits. Gene expression profiles seen in PBMCs from subjects with CMV detectable by PCR did not overlap with those seen in acute rejection using a quantitative clinically validated 14 gene acute rejection diagnostic assay.

Conclusions: CMV infection in cardiac allograft recipients has a distinct molecular signature in PBMCs that is independent from that of acute rejection. The gene expression profile of CMV viremia has confirmed previously described genes but also identified a new subset of genes associated with antiviral immune response patterns and with CMV-specific gene expression, indicating that the unique molecular signatures for CMV and acute rejection may be clinically useful in determining optimal immunosuppression.

Abstract# 1084 Poster Board #-Session: P14-III

POOR OUTCOME OF EARLY DEVELOPMENT OF CARDIAC ALLOGRAFT VASCULOPATHY AFTER CARDIAC TRANSPLANTATION. Jignesh K. Patel,[1] Jonathan Nakashima,[1] Jaime D. Moriguchi,[1] Brandy T. Oeser,[1] Angela Marquez,[1] Hillel Laks,[1] Jon A. Kobashigawa.[1] *[1]University of California at Los Angeles, Los Angeles, CA.*

Cardiac allograft vasculopathy (CAV) after heart transplantation may have different presentations. It can progress fulminantly over weeks or be indolent with gradual progression over years. Patients who have angiograms before 1 year usually do so because of unstable clinical symptoms, abnormal ECGs, or positive cardiac enzymes. To determine if there is a difference in early (less than 1 year) versus late (greater than 1 year) angiographic development of CAV on outcome, we reviewed 114 heart transplant patients (on cyclosporine-based immunosuppression) between January 21, 1990 and July 15, 1998, who were found to have initial CAV (>30% stenosis) on angiography. The mean age of the patients was 52.2±12.1 with 22.8% female. These patients were followed for 5-year survival from the time of initial diagnosis of CAV. Patients with CAV in the first year had significantly less 5-year follow-up survival compared to those patients with diagnosed CAV greater than 1 year, $p<0.002$ (see figure).



Conclusion: Heart transplant patients with early angiographic development (less than 1 year) of CAV appear to have decreased 5-year follow-up survival compared to those patients who develop CAV later. More aggressive immunosuppression and risk factor reduction should be considered in these high risk patients.

Abstract# 1085 Poster Board #-Session: P15-III

HEMODYNAMIC MEASURES ARE AN ESSENTIAL COMPONENT OF THE ENDOMYOCARDIAL BIOPSY PROCEDURE. Frank W. Smart,[1] Rajko Radovancevic,[1] Igor D. Gregoric,[1] Branislav Radovancevic,[1] O. H. Frazier.[1] *[1]Department of Cardiopulmonary Transplantation, Texas Heart Institute, Houston, TX.*

With managed care pressure to reduce cost following cardiac transplant many centers are considering omitting routine hemodynamic measurements at the time of endomyocardial biopsy. (EmBx). To evaluate the safety of such a practice we retrospectively reviewed 704 consecutive heart transplant recipients who underwent scheduled EmBx. Among these patients 57 (8%) were identified as having rejection as defined by a reduced ejection fraction, reduced cardiac output or significantly elevated pulmonary capillary wedge pressure; but in whom the ISHLT EmBx score was ≤2. These patients received augmented immune suppression for cellular negative rejection (CNR), and subsequently improved. There was no difference in long term survival following this therapy. There were no other pre or post operative predictors that would have predestined these patients to allograft dysfunction.

	CNR n=57	Control n=647	P value
Pre Op PRA >10%	2.9%	7.7%	0.463
Allograft Vasculopathy	41%	40%	0.906
CMV Infection	33%	22%	0.099
Survival in Years	7.67±4.89	6.73±5.59	0.261

Allograft vasculopathy was not affected by the presence of CNR and there was a trend toward a higher incidence of CMV but this was at any time and not necessarily associated with the episode of CNR. In conclusion we believe the routine use of hemodynamic measures at the time of EmBx is an essential part of the screening procedure. While reducing these measurements will reduce cost approximately 8% of patients would be placed at an increased risk of allograft loss, and no pre or post-operative measure routinely employed will identify the at risk group.

Abstract# 1086 Poster Board #-Session: P16-III

IS BNP A RELIABLE PREDICTOR FOR ACUTE REJECTION AFTER HEART TRANSPLANTATION? Peter Landwehr,[1] Ingo Kaczmarek,[1] Ioannis Adamidis,[1] Markus Mueller,[1] Jan Groetzner,[1] Peter Ueberfuhr,[1] Bruno Meiser,[1] Bruno Reichart.[1] *[1]Cardiac Surgery, Ludwig-Maximilians-University, Klinikum Grosshadern, Munich, Germany.*

BACKGROUND: BNP levels are elevated in heart insufficiency and are a predictor for cardiac mortality. Recent findings suggest that enhanced BNP plasma levels could form a basis for a noninvasive test for cardiac allograft rejection. Aim of this study was to reveal a correlation between BNP-levels and acute rejection.

METHODS: From 2001 to 2003 a total of 472 BNP measurements were taken immediately before routine endomyocardial biopsies. For statistical analysis patients were devided into four quartiles (n=118 per group) according to their BNP-levels. Further covariables were age, fractional shortening (FS), left ventricular end diastolic diameter (LVEDD) and serum creatinine.

RESULTS: Mean BNP was 105.7±127 pg/ml with 28.8±9; 55.3±6.9; 89±15.9 and 248.6±186.5 pg/ml for the quartiles. Creatinine levels were significantly different among the quartiles (1.50±0.53; 1.59±0.64; 1.61±0.56 and 2.22±1.13 mg/dl; $p<0.001$). The highest correlation was found between BNP and creatinine levels ($p<0.001$) and age, whereas differences in ISHLT grading between the quartiles were evident ($p=0.044$) but not highly significant. There was no correlation between BNP and FS or LVEDD.

CONCLUSION: Circulating BNP is elevated in heart transplant recipients. BNP increases with renal insufficiency. As a marker for acute rejection BNP is not reliable because its level depends on many influencing factors and great interindividual differences occur. This study is limited due to a low number of rejection episodes (no 3A or higher occured in the investigational period) but still BNP seems to be a marker for wall stress and volume overload and not primarily for allograft rejection.

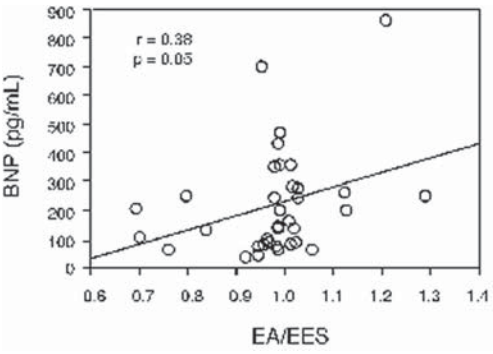
Abstract# 1087 Poster Board #-Session: P17-III

VENTRICULAR-VASCULAR UNCOUPLING AND EXPRESSION OF B-TYPE NATRIURETIC PEPTIDE IN HEART TRANSPLANTATION. Mandeep R. Mehra, Richard V. Milani, Moriah Richie, Patricia A. Uber, Myung H. Park, Robert L. Scott, Hector O. Ventura. *Cardiomyopathy and Heart Transplantation Center, Ochsner Clinic Foundation, New Orleans, Louisiana.*

Background. Allograft adaptation to a foreign circulation is imperfect as noted from decreased cardiac reserve and persistence limitations to stress. Effective arterial elastance (Ea), a measure of afterload, provides a reliable estimate of aortic impedance. End systolic elastance (Ees) is a load independent measure of ventricular performance and its interaction with the periphery. Their ratio (Ea to Ees) characterizes ventricular-vascular coupling and a value close to unity signifies maximal ventricular work (and thereby poor mechanical efficiency). The purpose of this investigation was to correlate mechanical efficiency of work with expression of B-type natriuretic peptide (BNP), a specific marker of ventricular stress and strain.

Methods. We studied 40 consecutive primary heart transplant recipients who were stable and free from rejection. Echocardiography was performed in all patients and Ea, Ees and their ratio (Ea/Ees) was obtained by the single-beat method. BNP levels were measured by a point of care assay. We examined correlates of BNP expression by assessing Ea/Ees while correcting for mean arterial pressure (MAP), body mass index (BMI), left ventricular mass index (LVMI), ejection fraction (EF) and serum creatinine.

Results. BNP levels were significantly and positively correlated with an increasing Ea/Ees ratio (see figure).



On multivariable analysis, this relationship persisted independently (t = 2.1, p=0.04) while BMI, MAP, LVMI, EF and serum creatinine were insignificant predictors.

Conclusion. This investigation indicates that the transplanted heart demonstrates poor contractile efficiency and operates at maximal left ventricular work. This is paralleled by a tandem increase in expression of BNP and suggests that elevation in this stress peptide is at least partly explained by ventriculo-vascular uncoupling in heart transplantation, independent of alterations in blood pressure.

Abstract# 1088 Poster Board #-Session: P18-III

IMPACT OF LATE ACUTE REJECTIONS ON LONG-TERM CARDIAC ALLOGRAFT FUNCTION. Michael Dandel,[1] Manfred Hummel,[1] Hans B. Lehmkuhl,[1] Rudolf Meyer,[1] Christoph Knosalla,[1] Onnen Grauhan,[1] Roland Hetzer.[1] *[1]Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, Berlin, Germany.*

Background: The impact of acute rejections (ARs) occurring during late post-transplant periods on graft function is barely known. To provide more information on this very controversial issue we investigated the influence of late ARs and both left ventricular (LV) function and progression of transplant coronary arteriopathy (TxCA).

Methods: In 552 patients (post-transplant times 2-16 years) we analyzed all cases of biopsy-proven late AR that arose after the second post-transplant year. The late ARs were detected by both routine endomyocardial biopsies (EMBs) performed during annual cardiac catheterisations and diagnostic EMBs performed whenever AR was suspected clinically and/or echocardiographically. Late AR analysis included prevalence, severity (both histological and functional) and potential relation to new appearance or aggravation of TxCA.

Results: During a mean observation time of 7.5 ±4.9 years a total number of 160 EMBs were graded as ISHLT ≥1A. In 33 patients the positive EMBs were associated with severe LV dysfunction accompanied by hemodynamic alterations. These patients represented 60.6% of all patients with post-transplant times of > 2 years in whom severe LV dysfunction was detected during the study period. The severity of graft dysfunction was not significantly related to the histological severity grade (ISHLT classification). All 33 patients with severe LV dysfunction had intense vascular reaction in addition to cellular rejection and in 16 patients (48.5%) humoral (vascular) rejection was dominant. LV dysfunction was completely reversible in only 2 of these 33 patients. Seven (21.2%) died due to AR-related graft failure (GF). In the other 24 patients LV function remained moderately altered after AR. Shortly (19.8 ±11.2 months) after AR, 8 of them developed accelerated TxCA; other 11 showed aggravation of preexistent TxCA. Sudden cardiac death within 2 and 18 months after late AR occurred in 6 of these 24 patients. The total of 13 rejection-related deaths represented 59.1% of all deaths due to GF (n=22) that occurred beyond the 2nd post-transplant year during the study period. The mean number of late ARs/year/patient was significantly higher in those which developed angiographic TxCA after the 2nd post-transplant year than in those without TxCA at that time ($p < 0.01$).

Conclusions: AR beyond the second post-transplant year is an important cause of late acute and chronic allograft dysfunction. The new appearance or aggravation of TxCA is at least partially related to late ARs.

Abstract# 1089 Poster Board #-Session: P19-III

MOLECULAR ADAPTATIONS OF ENDOTHELIAL CELLS TO ISCHEMIA/ REPERFUSION INJURY AFTER HEART TRANSPLANTATION. Gaurang Shah,[1] Frank Middleton,[2] Maria Azizian,[1] Daivd Bruch,[1] Dilip Kittur.[1] *[1]Department of Surgery, SUNY Upstate medical University, Syracuse, NY; [2]Department of Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, NY.*

Introduction: The brunt of ischemia/ reperfusion injury (I/R) after heart transplantaion is taken by endothelial cells and can have long-term consequences leading to atherosclerosis. However, endothelial cells contribute a fraction of the total cells in transplanted hearts, we resorted to laser microdissection of endothelial cells from the hearts to determine the molecular adaptations of endothelial cells to I/R after transplantation.

Materials and Methods: Vascularized heart allografts were exchanged between C57BL/6 mice donors and Balb/C mice recipients. Animals were sacrificed after 24 hours and coronary vessels were isolated from heart allografts and normal C57BL/6 mice. Endothelial cells were dissected out from coronary vessels using the Leica® laser dissector software.

Total RNA was extracted and amplified for hybridization to the Mouse U74A GeneChip. We used Robust Multichip Analysis to normalize and quantify the expression levels of each gene.

Results: In day 1 transplanted heart endothelial cells (THEC), there were 59 and 32 genes with 1.5 fold increased and decreased expression, respectively. These genes were involved in critical cellular metabolic pathways (Table 1).

Conclusion: In response to oxidative stress, THEC cells may increase expression of ETC genes to boost production of ATP and increase vesicular trafficking to eliminate oxidatively damaged proteins and improve cell viability. Because recent evidence indicates that supplementation with serine protease inhibitors (such as aprotinin) greatly reduces ischemia reperfusion damage, the reduced expression of this class of transcripts in the THEC cells may be an important event in the initiation of the ischemia reperfusion cascade, and the basis for the efficacy of this treatment.

Table 1

Metabolic Pathway	Specific Genes	Fold Change
Electron Transport Chain	Nicotinamide nucleotide transhydrogenase, ATP V0 subunit, Succinate dehydrogenase subunit	1.7 ↑
Oxidative stress response	Glucose regulated protein, peroxiredoxin 1 and 6, Glutathione peroxidase 3	1.5 ↑
Intercellular communication / vesicular transport	Monocarboxylic acid transporter, vesicle associated membrane protein 3, synapsin I	1.5 ↑
Cytochrome 450 families	CY 450 2, 3, 4	1.5 – 1.8 ↓
Serine protease inhibitors	Members 1-1, 1-2, 1-3, 1-4, 1-5	1.5 – 1.7 ↓
Fibrinogen, plasminogen, kininogen families	Fibrinogen, plasminogen and kininogen families genes	1.8 ↓