CHRONIC INJURY: ANTIBODY, COMPLEMENT, AND FIBROSIS

Abstract# 1090 Poster Board #-Session: P20-III

ROLE OF COMPLEMENT IN ALLOGRAFT REJECTION AND INDUCTIVE ANTIBODY THERAPY. Keri Csencsits,[1] Sherri Chan Wood,[1] Guanyi Lu,[1] Gregory L. Stahl,[2] D. Keith Bishop.[1] *[1]General Surgery, University of Michigan School of Medicine, Ann Arbor, MI; [2]Department of Anesthesia, Brigham and Women's Hospital, Boston, MA.*

Background: The role of complement (C') in allograft rejection is not well understood. C' deposition (C4d) within the transplant has been associated with human allograft rejection, but causative effects and the pathways of C' activation employed have not been established. In addition, recent evidence suggests that C' mediated cytolysis may be necessary for the tolerance inducing activities of monoclonal antibody (mAb) therapies. Hence, the purpose of this study was to determine the roles of the individual C' activation pathways in unmodified rejection as well as during inductive mAb therapy.

Methods: WT mice and mice deficient in the classical C' pathway (C1q-/-), the alternative pathway (Factor D-/-) or all C' pathways (C2/Factor B-/-) received heterotopic cardiac allografts. Allograft recipients were left untreated or were given inductive anti-CD4 (GK1.5) or anti-CD40L (MR1) mAb, both of which markedly prolong graft survival in WT mice. Kinetics and pathology of rejection, Th1/Th2 priming (ELISPOT), and alloantibody responses were assessed.

Results: Untreated C1q-/-, Factor D-/-, and C2/Factor B-/- mice rejected cardiac allografts at the same rate as control WT mice, indicating that C' was not required for acute rejection. Th1, Th2, and alloantibody responses were comparable between all groups at the time of rejection. However, rejection in C1q-/- and C2/Factor B-/- mice appeared more severe as manifested by total myocyte necrosis. The enhanced severity of rejection in C1q-/- and C2/Factor B -/- recipients was evident as early as 5 days post-transplant, which was prior to loss of graft function. In addition, IgG alloantibody responses were accelerated in all C' deficient strains. Hence, C' appeared to be protective, rather than deleterious in the unmodified rejection response. When C1q-/- or C2/Factor B-/- allograft recipients were given inductive anti-CD4 or anti-CD40L mAb, allograft survival was prolonged as seen in WT recipients.

Conclusions: These observations indicate that activated C' components may mediate protective activities that outweigh potentially deleterious C' activities in the context of transplantation. Further, these observations indicate that C' mediated cytolysis of T cells is not requisite for the protective activities of anti-CD4 or anti-CD40L mAb therapy. Hence, other mechanisms of action contribute to the immunosuppressive effects of these inductive mAb therapies.

Abstract# 1091 Poster Board #-Session: P21-III

MEMORY ALLOANTIBODY RESPONSE AND COMPLEMENT-DEPENDENT CARDIAC GRAFT INJURY IN PREGNANCY-SENSITIZED RECIPIENTS. Zhiping Qian,[1] Jinhuan Liu, Karen Fox-Talbot, Barbara A. Wasowska, William M. Baldwin III. *[1]Transplantation Laboratory, Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD.*

Previous pregnancy is a known risk factor for alloantibody production. Some recent studies have reported that deposits of the complement split products C4d and C3d are more frequent in multiparous than nulliparous female patients. The purpose of this study is to investigate the effects of pregnancy-induced alloantibodies on complement deposition and graft rejection in a highly immunogenic rat strain combination. C6-sufficient and -deficient female PVG.1U (RT1.A^u) rats were mated with allogeneic PVG.R8 (RT1.A^a) males or control isogeneic PVG.1U (RT1.A^u) males. Three weeks after parturition, experimental and control females received cardiac allografts from female PVG.R8 donors. After transplantation, low dose Cyclosporin A (CsA, 5mg/kg on alternate days) was used for immunosupression. Alloantibodies were measured by a sensitive flow cytometry assay. Allogeneic, but not control isogeneic, pregnancy elicited a weak, transient IgG alloantibody response that declined before transplantation. Experimental female recipients produced a rapid, vigorous IgM and IgG alloantibody response to the transplant in spite of CsA immunosuppression. C6-sufficient recipients rejected their transplants in an accelerated rate (average 5 days, n=6), compared with control animals (7 days, n=4). In contrast, all allografts in C6-deficient recipients functioned until sacrifice at 90 days (n=5). Most (4 of 5) of experimental C6-deficient recipients continued to produce IgM and IgG alloantibodies for 90 days. At the time of sacrifice C3d was diffusely deposited on the vascular endothelium of the graft. This continuous humoral response was correlated with significant neointimal formation. In summary, previous pregnancy leads to memory alloantibody responses that are resistant to CsA treatment. Membrane attack complex (MAC) formation is required for irreversible acute rejection. When MAC formation is blocked, previous pregnancy increases chronic graft vasculopathy.

Abstract# 1092 Poster Board #-Session: P22-III

COMPLEMENT C1Q DEFICIENCY PROLONGS VASCULARIZED SOLID ORGAN GRAFT SURVIVAL. Kenneth Christopher,[1] Yurong Liang,[1] Rachel DeFina,[1] Thomas F. Mueller,[1] David L. Perkins.[1] *[1]Laboratory of Molecular Immunology, Brigham and Women's Hospital, Boston, MA.*

Our laboratory has demonstrated large increases in graft C1q following murine cardiac transplantation. To evaluate the effects of the classical (C1q,C2,C4), lectin (C2,C4) and alternative (Bf) pathways during transplantation, we used donor and recipient mice deficient in C1q, C4 and double deficient mice lacking complement factor B (Bf) and complement 2(C2). In this study, early complement components in graft rejection are evaluated and the source of complement components is studied by analyzing infiltrating cells.

Methods: Heterotopic cardiac transplantation is performed on 8-12 week old male mice. Grafts are harvested on day 1 though 7. Cell trafficking studies are performed following Balb donor >GFP transgenic B6 recipients and grafts harvested at 12, 24 and 36 hrs. Cell suspensions prepared from the graft are incubated with PE conjugated antibodies to cell markers and sorted via flow cytometry. RNA is individually isolated from GFP$^+$ and GFP$^-$ cell fractions, and grafts. RNA is treated with DNase and reverse transcribed to cDNA. Expression of immune genes and complement is determined by Real Time PCR.

Results: Graft Mean Survival Time (in days)
C1qa$^{-/-}$B6>Balb(18.5); Balb>C1qa$^{-/-}$B6(14.5)
C4$^{-/-}$B6>Balb(8.5); Balb>C4$^{-/-}$B6(8.5)
C2$^{-/-}$Bf$^{-/-}$B6>Balb(12.3); Balb>C2$^{-/-}$Bf$^{-/-}$B6(7.5)
B6>Balb(8.5); Balb>B6(7.5)

Thus, deficiency of C1qa, but not C4 or Factor B, significantly prolongs graft survival. Interestingly, deficiency of C1q in either the donor or recipient was sufficient to prolong survival.

Complement (Bf, C3, C4) expression in the graft following C1q$^{-/-}$B6> Balb or B6>Balb is similar at day 1 and 7. Cell trafficking shows infiltrating cells produce C5aR and C1qb at early time points (3 hrs post transplant). At 24 hrs, infiltrating cells produce C5aR, C1qb, C3aR, Bf, and calreticulin. Whereas, isolated graft cells at 24 hrs produce C4, C1qc, C1qa, CRRY, C3, CD91, Factor H, C1-inhibitor, and also calreticulin. By analyzing subsets of infiltrating cells, we show that C5aR, and Properdin are produced by infiltrating Gr1$^+$ and F480$^+$ cells, whereas, calreticulin, C1qb, and C3aR, is produced by infiltrating Gr1$^-$ and F4/80$^+$ cells.

Conclusion: Infiltrating and graft cells differ in complement gene expression and both sources are important to produce the components of the complement cascade. C1q production in C1q-/- grafts following transplant is produced by infiltrating Gr1$^-$F480$^+$ macrophages. Importantly, C1q deficiency prolongs graft survival suggesting that pathogen immunity and graft rejection may involve overlapping mechanisms.

Abstract# 1093 Poster Board #-Session: P23-III

ROLE OF ALLOANTIBODY IN CD4$^+$ T CELL DEPENDENT IMMUNE DAMAGE OF HEPATOCYTES. Keri E. Lunsford,[1] Phillip H. Horne,[1] Yue Wang,[1] Donghong Gao,[1] Anna M. Eiring,[1] Ginny L. Bumgardner.[1] *[1]Department of Surgery, Division of Transplantation, The Ohio State University Medical Center, Columbus, OH.*

Allogeneic hepatocytes initiate both CD4- and CD8-dependent rejection responses; however, the mechanisms by which these pathways mediate immune damage of allogeneic hepatocytes have yet to be determined. This study addresses the hypothesis that *acute* immune damage of allogeneic hepatocytes by the CD4-dependent pathways is mediated by alloantibody.

Methods. Purified (98%) transgenic hA1AT-FVB/N (H-2^q) hepatocytes were transplanted into C57BL/6, B cell knockout (BCKO), CD8 KO or C57BL/6.SCID recipient mice (all H-2^b). Hepatocyte survival was determined by detection of the secreted reporter product, hA1AT, in host serum by ELISA. In order to confine the studies to the CD4-dependent rejection pathway, C57BL/6 and BCKO recipients were depleted of CD8$^+$ T cells prior to transplantation by treatment with anti-CD8 mAb (2.43, 0.25 mg, d-4, d-2). Host serum was assayed for the presence of donor-reactive IgG alloantibodies by incubation of serum with FVB/N splenocytes and flow cytometry. Kaplan Meier statistics were used to compare survival between experimental groups.

Results: Readily detectable donor-reactive alloantibody production was observed in CD8 KO recipients. Hepatocyte median survival time (MST) was significantly prolonged in CD8-depleted BCKO mice (MST=35 days) but not in CD8-depleted C57BL/6 (MST=14 days) or CD8 KO (MST=14 days) recipient mice ($p<.001$, $p<.001$ respectively). In order to determine whether alloantibody in the absence of T cells mediates damage of allogeneic hepatocytes, SCID mice were transfused with high titer pooled alloantibody or control serum from naïve mice. High titer pooled alloantibody was generated by FVB/N splenocyte sensitization of C57BL/6 mice. Pooled serum was administered by ip injection to C57BL/6.SCID mice with functioning hepatocellular allografts. Survival of allogeneic hepatocytes in control SCID mice was indefinite (>100 days). Reconstitution of SCID mice by infusion of alloantibody resulted in prompt hepatocellular allograft loss with MST=7 days.

Conclusions: These data demonstrate that allogeneic liver parenchymal cells elicit the production of donor-reactive alloantibody which is sufficient (in the absence of T cells) to cause acute immune damage. Further, B cells critically impact CD4-dependent immune damage of hepatocytes. The collective results support the conclusion that alloantibody mediates CD4-dependent immune damage of liver parenchymal cells.

Abstract# 1094 Poster Board #-Session: P24-III

INJURY OF KIDNEY XENOGRAFTS FROM α1,3-GALACTOSYL-TRANSFERASE GENE-KNOCKOUT (GalT-KO) PIGS INTO BABOONS IS CHARACTERIZED BY GLOMERULAR THROMBOTIC MICROANGIOPATHY. A. Shimizu,[1,2,3] K. Yamada,[1] K. Yazawa,[1] J. Cheng,[3] S. C. Robson,[4] H. J. Schuurman,[3] D. H. Sachs,[1] R. B. Colvin.[2] *[1]Transplantation Biology Research Center, Massachusetts General Hospital/Harvard Medical School, Boston, MA; [2]Pathology, Massachusetts General Hospital/ Harvard Medical School, Boston, MA; [3]Immerge BioTherapeutics, Cambridge, MA; [4]Center for Immunobiology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: GalT-KO miniature swine have been generated recently, and kidneys from these animals were transplanted into baboons. In this study, we assessed kidney xenograft pathology during the initial injury after transplantation. **Methods:** All kidney grafts were from GalT-KO miniature swine. Eleven baboons were thymectomized and splenectomized prior to transplantation, and received a kidney graft together with a vascularized thymic lobe as part of a tolerance-inducing regimen, or a composite thymokidney graft prepared 2 months before transplantation. Three baboons received only a kidney graft. All recipients were treated with an immunosuppressive conditioning regimen including T cell depletion, human anti-human CD154mAb (ABI793, Novartis Pharma AG), steroids and MMF with/without cobra venom factor. Morphologic and immunohistochemical studies were performed on biopsy or graftectomy samples. **Results:** There were no episodes of hyperacute rejection observed: in a number of animals the first indications of mild and slowly progressing dysfunction of the life-supporting kidney xenograft were observed between 2nd week and 2nd month after transplantation. An open biopsy (n=5) or graftectomy (n=3: cases with infection or cerebral infarction) sample was obtained at that periods. In all 8 xenografts, histologic sections showed changes of focal and segmental mild thrombotic microangiopathy in the glomeruli. The damaged glomeruli were characterized by mild activation of endothelium (increase of tissue factor expression), $TUNEL^+$ apoptosis, focal destruction of $CD31^+$ capillary network, and formation of fibrin-$CD62p^+$ platelet thrombi. Thrombotic areas in glomeruli showed mild deposits of IgM and C4d, but IgG and other complement components were almost negative. There was no or only minimal damage to peritubular capillaries, arterial endothelial cells and tubular epithelial cells. In general, cellular infiltration if present was minimal. **Conclusions:** The initial injury in GalT-KO kidney xenografts includes the microvascular endothelium in the glomeruli, and not the peritubular capillaries and/or arterial endothelium and tubular cells. The histologic picture resembles glomerular thrombotic microangiopathy. The mechanism of endothelial cell injury appears multifactorial, involving both immune and non-immune factors. Additional studies should focus on the pathogenesis of endothelial cell injury.

Abstract# 1095 Poster Board #-Session: P25-III

DOES DONOR ANTIGEN-SPECIFIC HYPOREACTIVITY PREDICT CHRONIC REJECTION-FREE SURVIVAL IN SOLID ORGAN RECIPIENTS? - A DECADE OF FOLLOW UP. Nancy Reinsmoen,[1] Angela Burnette,[1] Karen Mohler,[1] Kay Savik,[2] Lois McHugh,[2] Arthur Matas.[2] *[1]Pathology, Duke University Medical Center, Durham, NC; [2]Surgery, University of Minnesota, Minneapolis, MN.*

Recipients (recips) who develop donor antigen-specific hyporeactivity posttx have been shown to have a lower incidence of chronic rejection (CR) than those recips who remain responsive to donor antigen; the predictive value of hyporesponsiveness to donor antigen identifying a CR free-state is high for kidney, heart, and lung recips (91-100%) with a 2-4 year follow up. Since the incidence of CR increases with time posttx in all organ groups, the aim of our study was to determine if long term graft survivors who developed donor antigen-specific hyporeactivity by 12 - 18 mos posttx, continued to remain CR-free up to 12 years posttx. A total of 422 kidney, 41 lung, and 45 heart recips (tx'ed 1990-2001) were tested. CR was defined as biopsy-proven in kidney, bronchiolar obliterans syndrome in lung, and coronary artery disease in heart recips. Donor antigen-specific hyporeactivity was defined as a significantly (>60%) decreased post- (12-18mos) vs. pretx proliferative response to donor cells (or cells expressing donor class II antigens) while response to third party cells remained unchanged. Hyporeactivity to donor antigens was identified for 26% (108/422) of kidney, 39% (16/41) of lung and 53% (24/45) of heart recips at the 12-18 mos posttx time point. Since an acute rejection (AR) episode is a risk factor for CR, kidney recips were grouped based on any previous AR episodes. Of those recips with previous AR (N=153), CR developed in 26% of hyporesponsive recips and 41% of recips who remained responsive to donor antigen. Responder status did not appear to influence the incidence of CR in AR-free recips. Thus in kidney recips with a previous AR, the odds of developing CR were 1.92 times higher for recipients who remained responsive to donor antigens than for those who developed hyporeactivity. In lung recips, the median time to CR was 38.4 mos for those who developed hyporeactivity vs. 15.4 mos for those who remained responsive. In heart recips, 13% of hyporesponsive recips developed CR vs. 43% of those who remained responsive (p=0.02). In conclusion, the incidence of CR increases with time posttx for recips of all three organ groups. Determining the donor antigen response status relatively early posttx (12-18 mos) is a useful indicator to predict which recips are more likely to remain CR-free or develop CR at a significantly later time point. This allows the opportunity for intervention aimed at slowing or averting the CR process.

Abstract# 1096 Poster Board #-Session: P26-III

REDUCTION OF CD4 ATTACHMENT AND INFILTRATION INTO THE PERIVASCULAR SPACE OF ARTERIES AND THE MYOCARDIUM LEADS TO A SIGNIFICANT REDUCTION OF TRANSPLANT VASCULOPATHY AFTER CARDIAC TRANSPLANTATION IN RATS. Markus Richter,[1] Markus Barten,[1] Hartmut Bittner,[1] Thomas Walther,[1] Jan Gummert,[1] Friedrich Mohr.[1] *[1]Heart Center of Leipzig, University of Leipzig, Leipzig, Germany.*

Transplant vasculopathy (TVP) is characterized by vascular smooth muscle cell (VSMC) migration and proliferation in the subintimal space of graft arteries. This activation of VSMC could be mediated by infiltration of CD4 and CD8 positive (+) leucocytes into the perivascular space (PVS) and/or the myocardium. We investigated the reduction of CD4+ and CD8+ leukocytes infiltration and the correlation to the extent of neointima under CSA, FK506 or MMF.

Methods: After heterotopic cardiac transplantation (Lewis to Fisher) animals were divided into 4 groups: CSA 3mg/kg/d (n=74), MMF 40mg/kg/d (n=96), FK506 0.3mg/kg/d (n=96) and controls (no therapy, n=74). 3-4 animals of each group were sacrificed in intervals of 1-4 days up to day 60. Using immunohistochemistry, CD4+ and CD8+ were analysed at endothelium and in PVS of intra- (s) and epicardial (L) arteries and in myocardium. TVP in coronary arteries was expressed as mean vessel occlusion (mvo). **Results:** CSA, FK506 and MMF reduced CD4+ and CD8+ leukocytes at the endothelium, in PVS of s and L arteries, in myocardium and they reduced mvo of the arteries, compared with controls. MMF further significantly reduced mvo and CD4+ cells in the PVS of s and L arteries compared with FK506 and CSA ($p<0.05$). Between FK506 and CSA we found no significant difference, neither in the reduction of the CD4+ cells nor in the extent of TVP. There was a significant correlation between the CD4+ cells in the PVS, at the endothelium and in the myocardium and the resulting neointima (R=0.8) but none with the CD8+ cells in the PVS.

Conclusion: Infiltration of CD4+ leukocytes after transplantation seems to be a trigger for VSMC migration, proliferation and neointima formation. MMF attenuates transendothelial migration of leucocytes and reduces mvo. Immunosuppression with MMF after cardiac transplantation seems to be an option to prevent chronic rejection.

Abstract# 1097 Poster Board #-Session: P27-III

INVOLVEMENT OF EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT) IN CHRONIC RENAL ALLOGRAFT DYSFUNCTION. Michael Zeisberg,[1] Anil Chandraker,[2] Mohamed H. Sayegh,[2] Raghu Kalluri.[1] *[1]Center for Matrix Biology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [2]Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital and Harvard Medical School, Boston, MA.*

Chronic graft dysfunction is now the leading clinical problem following renal transplantation. The principal histopathological lesion seen in this disease is tubular loss with concurrent interstitial fibrosis, similar to lesions typical of fibrosis in primary nephropathies. Recent studies suggested that transition of tubular epithelial cells into activated fibroblasts (EMT) was an important contributor to the progression of renal fibrosis and that reversal of EMT could prevent the decline of renal function. Here we attempted to elucidate the contribution of EMT in a rat model of chronic allograft dysfunction. We transplanted kidneys from Fisher 344 rats (allografts) or LEW rats (isografts) orthotopically into LEW rats. Transplanted rats received a short course CsA treatment and fibrosis was recorded by H&E staining, Masson Trichrome Staining as well as by immunohistochemistry after 30 and 120 days. Expression of fibroblast specific protein1 (FSP1) in tubular epithelial cells was considered evidence for EMT. After 30 days allograft and isograft kidneys both displayed slightly increased numbers of interstitial fibroblasts, whereas EMT did not occur at this stage. After 120 days, tubular epithelial cells expressing FSP1 (indicating EMT) were sporadically present (0.4 cells per high power field). In allografts, severe fibrosis and tubular atrophy were associated with substantial numbers of tubular epithelial cells undergoing EMT (5.2 cells per high power field) and abundant presence of interstitial fibroblasts.

These findings suggest that EMT contributes to the progression of chronic renal allograft dysfunction. Our results also indicate that EMT is mediated by both alloantigen-dependent and alloantigen-independent factors. Future studies will determine, if EMT will be a useful therapeutic target to prevent progression of chronic renal allograft dysfunction.

Abstract# 1098 Poster Board #-Session: P28-III

POSSIBLE CONTRIBUTION OF ENDOTHELIAL-MESENCHYMAL-TRANSITION (EMT) TO CARDIAC FIBROSIS IN HEART FAILURE AND CHRONIC ALLOGRAFT REJECTION.

Elisabeth Höcht,[1] Oleg Tarnavski,[1] Michael Zeisberg,[1] Seigo Izumo,[1] Anil Chandraker,[2] Xueli Yuan,[2] Mohamed H. Sayegh,[2] Raghu Kalluri.[1] *[1]Center for Matrix Biology, Gastroenterology and Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [2]Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital and Harvard Medical School, Boston, MA.*

Chronic rejection manifested pathologically as chronic allograft vasculopathy and cardiac fibrosis is the most common cause of failure of transplanted hearts. Fibrosis of the heart is also the underlying cause of diastolic dysfunction, which is present in up to 40% of heart failure patients. We hypothesized that in addition to resident fibroblasts, endocardial or endothelial cells of the microvasculature could contribute to cardiac fibrosis by converting into activated fibroblasts via an endothelial-mesenchymal transformation (EMT)- like mechanism. Here we first established two independent mouse models of cardiac fibrosis and screened the ensuing heart tissues by Masson Trichrome Staining as well as by immunohistochemistry in order to detect evidence for EMT. Heterotopic transplantation of heart allografts in the abdominal cavity in mice with class II major histocompatibility difference (BM12 into B6) between donor and recipient leads to development of chronic vasculopathy and cardiac fibrosis due to chronic rejection 6-8 weeks after transplantation. As a second model we used cardiac fibrosis due to pressure overload which was achieved by banding of the ascending aorta. Hearts of these mice display substantial fibrosis after one week and an increased left ventricular enddiastolic pressure four weeks after aortic banding. In both models cardiac fibrosis was associated with a robust increase of vimentin-positive fibroblastoid cells. Interestingly, a substantial number of the vimentin positive cells also stained positive for the endothelial cell markers CD31 and endoglin. To further characterize these fibroblast populations, we stained these tissues with antibodies for α-smooth muscle actin (αSMA, a marker for myofibroblasts) and fibroblast specific protein 1 (FSP1, a marker of all fibroblasts). Interestingly, the numbers of αSMA positive and FSP1 positive cells were substantially less than the population of vimentin positive cells. While FSP1 positive cells were only sparsely present in normal hearts, they were robustly increased in fibrotic hearts. Some of these cells, which were localized within the endocardial layer or directly adjacent to the endocardial surface, stained also positive for the endothelial cell marker CD31, suggesting intermediate stages of cells in transition to becoming fibroblasts. These data are highly suggestive that EMT is a mechanism that contributes to fibrosis in the heart.

Abstract# 1099 Poster Board #-Session: P29-III

PREVENTION OF EPITHELIAL TO MESENCHYMAL TRANSDIFFERENTIATION BY VITAMIN D.

Debra A. Hullett,[1] Tina M. Stern,[1] Arjang Djamali,[2] Hans W. Sollinger,[1] Bryan N. Becker.[2] *[1]Surgery, University of Wisconsin-Madison, Madison, WI; [2]Medicine, University of Wisconsin-Madison, Madison, WI.*

Emerging evidence suggests that the pathologic fibrotic changes associated with chronic allograft nephropathy (CAN) are mediated by epithelial cells that undergo an epithelial to mesenchymal transdifferentiation (EMT). Renal tubular epithelial cells lose E-cadherin and acquire a-smooth muscle actin (a-SMA) expression and deposit extracellular matrix. We have previously shown that $1,25(OH)_2D_3$, the active metabolite of vitamin D, prevents the development of CAN in a rat (F344 to Lewis) renal allograft model. To study the underlying mechanism(s) by which $1,25(OH)_2D_3$ prevents fibrotic changes associated with CAN, we sought to develop an in vitro model of EMT.

A normal rat kidney tubular epithelial cell line (NRK52E) was cultured for 5 days on plastic in the presence or absence of $1,25(OH)_2D_3$ (50 nM) or TGFb-1 (10 ng/ml). Cells were monitored for morphology and assayed for E-cadherin and a-SMA mRNA and protein expression by RT-PCR and immunohistochemistry.

Addition of TGFb-1 to the cell culture media resulted in a profound change in cell morphology by 48h. Cells lost their cobblestone appearance and acquired an elongated fibroblast morphology. RT-PCR showed decreased E-cadherin expression. Immunohistochemistry revealed decreased E-cadherin protein expression. This was accompanied by increased a-SMA expression. Addition of vehicle or $1,25(OH)_2D_3$ to the cultures resulted in no changes in morphology or E-cadherin and a-SMA expression. In striking contrast, simultaneous addition of TGFb-1 and $1,25(OH)_2D_3$ to the cultures prevented EMT at 48h and significantly decreased TGFb-1-induced morphologic changes at day 5 of culture. RT-PCR and immunohistochemistry showed maintenance of E-cadherin expression at 48h. These in vitro data suggest one mechanism underlying the beneficial effect of $1,25(OH)_2D_3$ in the F344 to Lewis model of CAN may be this compound's ability to prevent EMT in renal epithelial cells. The results also suggest that $1,25(OH)_2D_3$ may be an effective therapy for the prevention of CAN.

Abstract# 1100 Poster Board #-Session: P30-III

GENOMIC ANALYSES OF PROTEINURIC CONSOMIC RATS: POTENTIAL SURROGATE MARKERS OF CHRONIC ALLOGRAFT NEPHROPATHY.

Asher D. Schachter,[1,2] Isaac S. Kohane.[2] *[1]Nephrology, Children's Hospital, Boston, MA; [2]Children's Hospital Informatics Program, Children's Hospital, Boston, MA.*

Proteinuria is a sensitive and specific marker of chronic allograft nephropathy. The pathophysiology of proteinuria is arguably multigenic in that it has been associated with over a dozen cytokines/growth factors, several transcription factors, and altered T cell subsets. To this end, the Program for Genomic Applications (PGA) has bred a partial panel of consomic rats to provide chromosome-specific animal models that have been phenotyped, genotyped, and in some cases undergone expression array analysis. We have analyzed the PGA renal protocol data to determine chromosomes of interest in the pathogenesis of proteinuria. **METHODS:** Consomic strains for which the degree of proteinuria was significantly lower in consomic (SS-xBN, where x= inserted chromosome) strains vs. parental background (SS) strains were considered to represent chromosomes of interest. For the chromosomes of interest for which gene expression arrays studies were performed on renal tissue, gene expression data were subjected to regression normalization, filtered (3-fold difference) for genes differentially expressed between SS and SS-xBN strains, and clustered by known function (EASE software). In addition, each rat chromosome of interest was mapped to human chromosomes (MAPVIEW), and a PubMed search was performed to detect publications demonstrating an association between mapped genes and proteinuria. All data were analyzed in the R programming environment. Significance was defined as non-parametric p value < 0.05. **RESULTS:** Significantly elevated proteinuria excretion levels were detected in male rats, and in SS-18BN rats stratified by gender. The rat Y chromosome was not identified as a chromosome of interest by our filter criteria. Therefore rat chromosomes 18 and X were further analyzed. SS-18BN vs. SS gene expression array analyses revealed that the main functional clusters included **immune-related genes (RT-1 antigen binding gene, phospholipase A2, and fibroblast growth factor (FGF) receptor 2)** and **G-protein coupled receptor genes**. PubMed searches of human genes mapped from rat chromosomes X and 18 included **CD154, FGF 1, tissue inhibitor of metalloproteinase 1 (TIMP1 collagenase inhibitor), and a glucocorticoid receptor subunit gene** that have been previously associated with proteinuria. **CONCLUSIONS:** Consomic studies can identify novel biomarkers and provide global profiles of molecular processes involved in proteinuria that is in turn a marker of chronic allograft nephropathy.

Abstract# 1101 Poster Board #-Session: P31-III

MMF SIGNIFICANTLY REDUCES THE TRANSENDOTHELIAL MIGRATION OF GRAFT INFILTRATING LEUCOCYTES. A COMPARATIVE STUDY WITH CSA AND FK506 AFTER EXPERIMENTAL CARDIAC TRANSPLANTATION.

Markus Richter,[1] Hartmut Bittner,[1] Thomas Walther,[1] Volkmar Falk,[1] Gummert Jan,[1] Mohr Friedrich.[1] *[1]Heart Center of Leipzig, University of Leipzig, Leipzig, Germany.*

Transendothelial migration of graft infiltrating leucocytes seems to play a pivotal role not only in the destruction of the graft but also in the activation of endothelial and smooth muscle cells, leading to the migration and proliferation of VSMC from media to intima. These events cause chronic rejection. Purpose of the study was to evaluate graft infiltrating leucocytes (CD4, CD8, CD11a, CD18) after cardiac transplantation in rats under immunosuppressive therapy **Methods:** 340 animals were transplanted and divided by random into four groups of therapy: CSA 3mg/kg/d (n=74), MMF 40 mg/kg/d (n=96), FK506 0.03 mg/kg/d (n=96) and a control group receiving no immunosuppressive therapy (n=74). 3-4 animals of each group were sacrified in intervals of 1-4 days up to day 60. FACS analysis of leucocytes (CD4, CD8, CD11a, CD18) from peripheral blood was performed in the same intervals like sacrification. Immunohistochemistry on frozen specimens was performed using a mAb against CD4,CD8,CD11a, CD18. Positive stained cells were analysed at the endothelium and in the perivascular space of intra- and epicardial arteries. Statistical analysis was performed using the areas under the curves over the time of 60 days by an extended t-test. **Results:** While **i**n peripheral blood the amounts of stained leucocytes where not statistically different among the study groups, the amounts of graft infiltrating leucocytes (CD4,CD8,CD11a and CD18) were reduced under MMF therapy by 80% compared to the control groups already from day 1 after grafting. CSA and FK506 needed 12 to 20 days to achieved this reductive effect. The reduction of leucocytes under the therapy of MMF was statistically significant compared with control , CSA and FK 506 treated animals. **Conclusion:** This significant reduction of graft infiltrating leucocytes reflects the superior of MMF over CSA and FK506 of CSA, FK506 and MMF. Chronic rejection was statistically reduced under MMF therapy. The reduction of transendothelial migrating leucocytes probably is responsible for the reduction of chronic rejection under MMF.

Abstract# 1102 Poster Board #-Session: P32-III

MYCOPHENPLATE MOFETIL INHIBITS INTIMAL HYPERPLASIA AND ATTENUATES THE EXPRESSION OF GENES FAVOURING SMOOTH MUSCLE CELL PROLIFERATION AND MIGRATION. Julian R. Waller,[1] Nicholas R. Brook,[1] Michael L. Nicholson.[1] *[1]Transplant Surgery, University of Leicester, Leicester, United Kingdom.*

Aims: Allograft vasculopathy, remains the leading cause of late graft failure following heart transplantation. MMF inhibits the development of intimal hyperplasia. This study assessed the effect of combinations of mycophenolate mofetil, calcineurin inhibition and sirolimus on the development of intimal hyperplasia, reflective of modern clinical immunosuppression.

Methods: Male Sprague-Dawley rats received MMF (30mg/kg/day) and either tacrolimus (0.1mg/kg/day) or cyclosporin (5mg/kg/day) or sirolimus (0.05mg/kg/day). All animals underwent common carotid artery balloon angioplasty and were explanted at 2 weeks. Intima/medial ratios were calculated using computer-aided planimetry. Gene expression was assessed with competitive RT-PCR at 2 weeks for MMP-2, MMP-9, TIMP-1, collagen III and TGF-b. Sections were stained with picro-sirius red and extracellular matrix deposition was quantified using an image intensifier.

Results: MMF in combination rapamycin was associated with the greatest reduction in intimal thickening median intima medial ratio 0.79 (range 0.45-0.86), compared to its combination with either cyclosporin, 1.41 (range 1.06-1.68, P<0.02) or tacrolimus 0.93 (range 0.81-1.37, P<0.05) and untreated controls, 1.47 (1.02-2.04, P<0.005). Treatment with mycophenolate mofetil and rapamycin significantly inhibited all pro-fibrotic genes studied compared to controls (P<0.01) but there were no differences between tacrolimus and cyclosporin groups. MMF and sirolimus significantly attenuated extracellular matrix deposition compared to tacrolimus and cyclosporin (P<0.023).

Conclusion: The synergistic benefits of mycophenolate mofetil in combination with sirolimus are preferential over those observed with either cyclosporin or tacrolimus. The inhibition of pro-fibrotic gene expression may explain this beneficial effect.

Abstract# 1103 Poster Board #-Session: P33-III

BILIRUBIN SUPPRESSES NEOINTIMA FORMATION BY INHIBITION OF VASCULAR SMOOTH MUSCLE CELL PROLIFERATION. Robert Öllinger,[1,2] Martin Bilban,[1] Alberto Froio,[1] James McDaid,[1] Anna Erat,[1] Eva Csizmadia,[1] Angela Liloia,[1] Miguel P. Soares,[1] Anny Usheva,[3] Kenichiro Yamashita,[1] Fritz H. Bach.[1] *[1]Surgery, Harvard Medical School, BIDMC, Boston, MA; [2]Transplant Surgery, University of Innsbruck, Innsbruck, Tyrol, Austria; [3]Endocrinology, Harvard Medical School, BIDMC, Boston, MA.*

Introduction. Induction of heme oxygenase 1 (HO-1) ameliorates the development of chronic allograft arteriopathy, which normally involves oxidative stress and smooth muscle cell proliferation. Based on the observation that healthy individuals with higher levels of bilirubin develop less atherosclerosis, we hypothesized that the antioxidant bilirubin, a product of HO-1 mediated heme catabolism, would have a salutary effect on balloon injury (BI) mediated neointima formation in vivo and inhibit vascular smooth muscle cell proliferation in vitro.

Methods. BI was performed in the left common carotid artery of congenitally hyperbilirubinemic Gunn rats and wild-type Wistar rats (congenic with the Gunn rats). The artery was harvested two weeks after BI and neointima formation was assessed by quantifying luminal cross-sectional area narrowing (LCAN). In vitro, proliferation of primary vascular smooth muscle cells (VSMC) was measured by 3H-thymidine incorporation in the presence or absence of bilirubin. Cell cycle progression was monitored by DNA content analysis; apoptosis by the Annexin V binding assay. Protein levels were detected by western blot.

Results. BI mediated neointima formation was significantly reduced in Gunn rats (serum bilirubin levels: 12.0 ± 2.5 mg/dl) as compared to control Wistar rats (LCAN 0.18 vs. 0.44; $p < 0.01$). Systemic administration of biliverdin, the precursor of bilirubin, to normal rats also suppressed neointima proliferation (LCAN 0.29 vs. 0.43; $p < 0.05$). In vitro, bilirubin inhibited VSMC proliferation in a dose-dependent manner. Bilirubin (200 μM) arrested VSMC cell cycle progression at the G0/G1 phase, while it did not increase apoptosis (p=0.2816). Inhibition of cell cycle progression was mediated by reduced phosphorylation of p38 MAPK, down-regulation of cdk2, cyclin D1, E and A as well as decreased phosphorylation of Rb. The effects of bilirubin were independent of HO-1 expression, as demonstrated in HO-1-/- VSMCs.

Conclusions. Bilirubin suppresses both BI induced neointima formation in vivo and VSMC proliferation in vitro. Increasing levels of bilirubin by systemic adminstration may have a beneficial effect on VSMC proliferation in chronic allograft arteriopathy and may become a potent strategy for the treatment of chronic rejection.

Abstract# 1104 Poster Board #-Session: P34-III

COMBINED TREATMENT WITH εPKC ACTIVATOR AND δPKC INHIBITOR REDUCES ISCHEMIA REPERFUSION INJURY AND GRAFT CORONARY ARTERY DISEASE CAUSED BY PROLONGED ISCHEMIA IN RAT CARDIAC ALLOGRAFTS. Masashi Tanaka,[1] Feny Gunawan,[1] Anthony D. Caffarelli,[1] Koichi Inagaki,[2] Theo Kofidis,[1] Daria Mochly-Rosen,[2] Robert C. Robbins.[1] *[1]Deapartment of Cardiothoracic Surgery; [2]Department of Molecular Pharmacology, Stanford University, Stanford, CA.*

Ischemia-reperfusion (I/R) injury is a risk factor for the development of graft coronary artery disease (GCAD). In a model of acute MI, treatment with epsilon PKC (εPKC) activator and delta protein kinase C (δPKC) inhibitor reduces I/R injury. Whether these regulators can reduce I/R injury in the transplanted heart and subsequent development of GCAD has not been determined. We investigated the ability of PKC regulators to reduce I/R injury and GCAD using two different ischemic times (30 min and 120 min) in rat cardiac allografts. **Methods:** Hearts of PVG rat ($RT1^c$) were heterotopically transplanted into ACI rats ($RT1^a$). After cardioplegic arrest of the donor heart, 2 mL of εPKC activator (1.5 nmol) solution was injected antegrade into the coronary artery system. Hearts were procured and bathed in εPKC activator (0.5 μM) for 30 and 120 min at 4°C. Before reperfusion, 1 ml of δPKC inhibitor (30 nmol) solution was injected into the recipient IVC. To analyze I/R injury, grafts were procured after 4 h of reperfusion (n=6/group) and analyzed for superoxide production by the spin-trapping method; for myeloperoxidase (MPO) activity, TNF-α, IL-1β, and MCP-1 production by ELISA; and for cardiomyocyte apoptosis by TUNEL and caspase-3, -8, and -9 activities. To analyze GCAD, another set of animals underwent equal ischemic times and treatment strategies, survived, treated with cyclosporine A, and then after 90 days analyzed for GCAD indices (n=8/group). Controls were treated with normal saline. **Results:** In the control groups, all measures of I/R injury and GCAD for the 120 min ischemia group were significantly increased over the 30 min group. For the 30 min ischemia groups, there was no statistical difference between treated vs. control. For the 120 min ischemia groups, MPO activity, TNF-α, IL-1β, MCP-1 production, cardiomyocyte apoptosis, caspase-3 and -9 activities, and all three GCAD indices decreased significantly in the treated group vs. contols; additionally, these values were equivalent to the 30 min controls. However, superoxide production and caspase-8 activity did not differ significantly between the treated and control groups with 120-min ischemia. **Conclusions:** Combined treatment with εPKC activator and δPKC inhibitor reduces I/R injury and GCAD caused by prolonged ischemia; therefore, this pharmacologic treatment may be useful for clinical cardiac transplantation.

Abstract# 1105 Poster Board #-Session: P35-III

LOW DOSE INHALATION OF CARBON MONOXIDE PREVENTS THE DEVELOPMENT OF CHRONIC ALLOGRAFT NEPHROPATHY IN RAT RENAL ALLOGRAFTS. Joao Seda Neto,[1] Hideyoshi Toyokawa,[1] Anna Romanosky,[1] Kei Kimizuka,[1] Donna Stolz,[1] Atsunori Nakao,[1] Noriko Murase.[1] *[1]Surgery, University of Pittsburgh, Pittsburgh, PA.*

Background: Chronic allograft nephropathy (CAN) is the primary cause for late kidney allograft loss. Carbon monoxide (CO), a product of heme metabolism by heme oxygenases, is known to impart protection against oxidative stress. We hypothesized that CO could minimize the chronic fibro-inflammatory process and protect kidney allografts from CAN. **Methods:** Lewis kidney grafts were orthotopically transplanted into binephrectomized syngeneic (Group 1) or allogeneic (BN) rats. Allograft recipients received short course FK506 (0.5 mg/kg, d0-6, IM) and were maintained in room air (Group 2) or exposed to CO (Group 3) at 20 ppm for 30 days after transplant. Animals were sacrificed 30 and 80 days to assess efficacy of inhaled CO. **Results:** Syngeneic grafts maintained normal kidney function throughout the experiment. Group 2 control animals had continuous decline in creatinine clearance (Ccr) and concomitant increase of proteinuria (Table). CO-treated animals had significantly better Ccr with minimum proteinuria. Histological examination confirmed the development of progressive CAN in air-exposed controls. CO exposure abrogated the development of interstitial fibrosis (ci) and tubular atrophy (ct) with negligible collagen IV and TGF-β1 stain. In addition, diffuse ED-1^+ macrophage and localized B and T cell infiltration seen in control allografts were virtually eliminated with CO inhalation. In vitro analyses revealed that CO-treated recipients had significantly less proliferation against donor peptides via the indirect allorecognition pathway and less anti-donor IgG antibodies, compared to controls. Intragraft mRNA levels for chemokines (RANTES, MIP-1α), chemokine receptors (CCR1, CXCR3, CXCR5), IL-2 and ICAM-1 were significantly decreased in CO-treated allografts compared to controls. **Conclusions:** The study demonstrates that exposure of kidney graft recipients to CO at a low concentration can abrogate chronic fibro-inflammatory changes associated with CAN and improve long-term renal graft function

	Ccr ml/min	Protein mg/24h	Banff grade ci	Banff grade ct	RANTES ◊	IL-2◊	Anti-donor IgG§
ISO	1.7±0.1	1.7±1.1	0.1±0.3	0±0	1.0±0.9	0.4±0.6	2.0±0.6
AIR	0.2±0.1	410±157	0.8±0.4	1.6±0.5	28.4±13.7	18.5±12.0	14.0±5.5
CO	1.3±0.2*	2.0±0.7*	0±0*	0.6±0.5*	10.2±9.2*	5.3±4.4*	3.8±2.0*

*p<0.05 AIR, ◊ mRNA fold increase vs normal, §MFI mean fluorescence intensity

Abstract# 1106 Poster Board #-Session: P36-III

CARBON MONOXIDE INHALATION PROTECTS HEART ALLOGRAFT FROM CHRONIC REJECTION. Atsunori Nakao,[1] Hideyoshi Toyokawa,[1] Kei Kimizuka,[1] Tetsuma Kiyomoto,[1] Robert J. Bailey,[1] Michael A. Nalesnik,[1] Noriko Murase.[1] *[1]Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA.*

Chronic rejection (CR) remains as the major obstacle in successful organ transplantation. Heme oxygenase system has been shown to provide protection against several inflammatory reactions including CR. Carbon monoxide (CO), a by-product of heme catalysis, also exerts similar cytoprotective effects against a variety of stimuli. This study evaluated the efficacy of CO in preventing CR in rat heart transplantation (HHT) model. **Methods**: Fully allogeneic HHT was performed in LEW to BN rat combination under short course FK506 (0.5 mg/kg, d0-6). Recipients were either kept in air or exposed to CO (20 ppm) for 28 days continuously after HHT. Cytokine mRNA levels, flowcytometry, mixed lymphocyte reaction (MLR), graft histopathology, and graft survival were analyzed. **Results** (Table): In recipients treated with CO, blood carboxyhemoglobin (COHb) levels were maintained at 10 % during CO exposure, compared to 1.4 % in air. CO treatment was remarkably effective in prolonging heart graft survival to a median of >100 days from 45 days in air control group. Severe histopathological changes associated with CR seen in control allografts were significantly reduced in CO-treated allografts. Th1 type cytokine (IL-2, IFNγ) upregulation in the spleen of air group at d28 was significantly downregulated with CO, associated with a considerable reduction of splenic CD25 (IL-2R) expression. CO-treated recipients developed donor-specific hypo-responsiveness in MLR by 28d, while maintaining vigorous third-party alloreactivity. Antidonor IgG antibodies in recipient serum at d50 were significantly increased in air- compared to CO-treated recipients. In addition, early short CO exposure (d0-13) was more effective in prolonging graft survival than later exposure (d13-28), suggesting that CO exposure during early acute phase is important for graft survival. **Conclusion**: Low dose CO inhalation to allograft recipients was effective in prolonging heart allograft survival and preventing the development of CR via the downregulation of Th1 type cytokine responses.

	mean graft survival	severity of CR	IL-2mRNA (d28, spleen)	IFNγmRNA (d28, spleen)	%CD25+ cells	MLR (cpm, d28)	antidonor Ab (X-mean, d50)
normal	N/A	N/A	1.0± 0.6	1.0±0.6	3.4±0.4	6450	0.6±0.1
air	45 days	++++	4.6±1.5	2.5±0.9	4.5±0.3	6371	5.6±3.3
CO	>100 days	++	2.6±0.2*	1.2±0.3*	2.9±0.5*	1956*	1.1±1.1*

*p<0.05 vs air, mRNA levels; fold increase vs. normal spleen

Abstract# 1107 Poster Board #-Session: P37-III

VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN RENAL ALLOGRAFT REJECTION AND CYCLOSPORINE TOXICITY. B. Handan Ozdemir,[1] A. Nihan Haberal,[1] Beyhan Demirhan,[1] Remzi Emiroglu,[2] Aydin Dalgic,[2] Mehmet Haberal.[2] *[1]Pathology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]General Surgery and Transplantation, Baskent University Faculty of Medicine, Ankara, Turkey.*

Vascular endothelial growth factor (VEGF) is an endothelial mitogen with increased expression in inflammation and vasculopathy. The aim of this study was to clarify the relationship between VEGF expression and macrophage infiltration in renal allografts and to assess the role of VEGF on interstitial fibrosis (IF).

The expression of VEGF and the proportion of macrophage in the interstitium were examined immunohistochemically in 92 cases including 4 cases with non-rejected kidneys, 40 cases with acute rejection (AR), 29 cases with chronic allograft nephropathy (CAN), 10 cases with borderline changes (BD) and 9 cases with CsA toxicity. The follow-up biopsies of all cases with AR were evaluated for the progression of IF.

In the non-rejected group, intraglomerular VEGF expression was observed in endothelial, parietal and visceral cells. Tubular VEGF expression was positive only in few tubules of 2 cases. There was a significant positive correlation between the interstitial macrophage infiltration and tubular VEGF expression ($p<0.01$). In serial sections interstitial VEGF colocalized with the expression of CD-68 positive macrophages. There were significant differences between the cases with AR, CAN and CsA toxicity for the expression of tubular and interstitial VEGF ($p<0.001$). Tubular VEGF expression was marked in 17/29 cases (58.6%) of CAN, 9/50 cases (18%) of AR and 3/9 cases (33.3%) of CsA toxicity. A significant difference was found between AR grades with regard to tubular and interstitial VEGF expression ($p<0.01$). Patients with AR whose initial biopsies showed marked tubular and interstitial VEGF expression developed IF earlier than those whose initial biopsies showed low grade tubular and interstitial VEGF expression ($p<0.05$). In addition a significant VEGF expression was observed in the mesangial cells of cases with CAN.

The results suggest that macrophage infiltration and hypoxia due to chronic allograft vasculopathy may play an important role in the induction of VEGF that leads to early interstitial fibrosis

Abstract# 1108 Poster Board #-Session: P38-III

ROLE OF MATRIX METALLOPROTEINASES 2 AND 9 IN SKIN AND CARDIAC ALLOGRAFT REJECTION. Hiroyuki Amano,[1] Shoji Koga,[1] Satoshi Hirohato,[2] Masayoshi Miura,[1] Suneel Apte,[2] Robert Senior,[4] Hiroshi Toma,[3] Andrew C. Novick,[1] Robert L. Fairchild.[1] *[1]Urology, The Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, OH; [2]Biomedical Engineering, Cleveland Clinic Foundation, Cleveland, OH; [3]Urology, Tokyo Women's Medical Univ., Tokyo, Tokyo, Japan; [4]Immunology, Washington Univ., St. Louis, MO.*

INTRODUCTION: The factors directing T cell infiltration into allografts to mediate rejection remain poorly defined. Matrix metalloproteinases (MMPs) are enzymes that digest extracellular matrix proteins. Activated T cells express MMP2 and MMP9. The goal of this study was to investigate the role of these MMPs in the rejection of allografts.

METHODS: C57BL/6 (H-2b) mice and MMP-9 deficient mice on a 129 background received heterotopic heart or skin allografts from completely MHC mismatched donors. Immunohistochemistry was performed to identify cells infiltrating the grafts. Recipients were depleted of CD4+ or CD8+ T cells by treatment with specific mAb before transplantation. RNA expression of MMP2 and MMP9 was tested by ribonuclease protection analyses. Gel zymography was performed to detect the presence of proenzyme and active forms of MMP2 and MMP9 in graft tissue homogenates. In-situ zymography was also performed to detect and to investigate the localization of active form of MMP2 and MMP9 in graft tissue

RESULTS: Heart allografts were rejected in wild type recipients on day 7-8 and in MMP9-deficient recipients from day 11-22 post-transplant. When compared to allografts retrieved from wild-type recipients, rejecting allografts from MMP-9 deficient mice had decreased infiltration with T cells and macrophages. In wild-type recipients, rejecting allografts had high levels of MMP9 and low levels of MMP2. MMP9 activity was absent in recipients depleted of CD4+ T cells. Using antibody-mediated depletion of allograft recipients, expression of MMP9 was only observed during CD4+ T cell mediated rejection. In-situ zymography in the wild type graft indicated co-localization of active MMPs and CD4+ T cells. However, there were no co-localization between MMPs and CD8+ T cells. In the graft of MMP9 deficient recipient, there was no co-localization between CD4+ T cells and MMPs. These results suggest CD4+ T cell produce active MMP9 during acute rejection.

CONCLUSIONS: MMP-9 activity is required for optimal rejection of allografts and is dependent on CD4+ T cells. The results suggest that T cell graft infiltration may be attenuated by interfering with these enzymes.

Abstract# 1109 Poster Board #-Session: P39-III

THE NOVEL AGENT PIRFENIDONE ATTENUATES THE PRO-FIBROTIC MOLECULAR ENVIRONMENT GENERATED BY CALCINEURIN-INHIBITORS IN THE RAT SALT-DEPLETION MODEL. Nicholas R. Brook,[1] Julian R. Waller,[1] Gareth R. Bicknell,[1] Michael L. Nicholson.[1] *[1]Transplant Surgery, University of Leicester, Leicester, United Kingdom.*

Background

Chronic renal allograft dysfunction (CRAD) is the major cause of allograft loss following the first post-transplant year and is in part due to the nephrotoxic effects of calcineurin inhibitor (CNI) immunosuppression. We employed the salt-depleted model to examine the potential antifibrotic effects of the novel agent pirfenidone on CNI-induced CRAD.

Methods

Male Sprague-Dawley rats (350-500g) were salt-depleted for seven days prior to the introduction cyclosporine (CyA) (15mg/kg/day) or tacrolimus (FK) (6mg/kg/day), with or without addition of varying doses of pirfenidone. Serum creatinine and urinary protein/creatinine ratios were measured for 28 days. After sacrifice, collagen deposition was calculated, and mRNA expression TGF-beta, collagen III, MMP-2, MMP-9 and TIMP-1 was assessed by reverse transcriptase PCR.

Results

CyA (94 vs 61 µmol/l, P=0.002) and FK (76 vs 61 µmol/l, P=0.01) increased serum creatinine compared to the positive controls, but the effect was more marked for CyA (P=0.009). Pirfenidone significantly attenuated serum creatinine for both drug treatments at 28 days (non-dose-dependent). CyA reduced MMP-2 mRNA expression, an effect abrogated by pirfenidone (P=0.009). The reverse was found for the profibrotic TIMP-1 (P= 0.015). Collagen III (P=0.026) and TIMP1 (P=0.04) mRNA expression was reduced below FK-alone levels and control levels when pirfenidone was added to FK. Collagen III deposition was not significantly different between any groups.

Conclusions

The underlying mechanism of the action of the novel antifibrotic agent pirfenidone is attenuation of profibrotic gene expression. It also reduces serum creatinine in salt-depleted rats treated with cyclosporine and tacrolimus in this model of CNI-induced nephrotoxicity.

Abstract# 1110 Poster Board #-Session: P40-III

POST-TRANSPLANT LYMHPOPROLIFERATIVE DISEASE AFTER ALLOGENEIC SPLEEN TRANSPLANTATION IN MINIATURE SWINE. Frank J. M. F. Dor,[1] Karen E. Doucette,[2] Nicolas J. Mueller,[2] Robert A. Wilkinson,[2] Junaid A. Bajwa,[1] Isabel M. McMorrow,[1] Yau-Lin Tseng,[1] Kenji Kuwaki,[1] Stuart L. Houser,[3] David H. Sachs,[1] Jay A. Fishman,[2] Christene A. Huang,[1] David K. C. Cooper.[1] *[1]Transplantation Biology Research Center, MGH/Harvard Medical School, Boston, MA; [2]Infectious Diseases Division, MGH/Harvard Medical School, Boston, MA; [3]Department of Pathology, MGH/Harvard Medical School, Boston, MA.*

Background: Post-transplant lymphoproliferative disease (PTLD) is an important complication of immunosuppression after transplantation (Tx). In our miniature swine model, we have studied the potential of the spleen to induce tolerance. We observed PTLD after spleen Tx across full major histocompatibility complex barriers. We describe the time course of events before clinical features became apparent and discuss useful markers of the disease.

Methods: Recipient pigs were immunosuppressed with whole body irradiation (100cGy), thymic irradiation (700cGy), and cyclosporine given intravenously daily to maintain a trough level of 400-800ng/mL for 45 days. Native splenectomy and spleen Tx were performed on day 0. Two control pigs received the identical immunosuppressive regimen and underwent native splenectomy, but did not undergo spleen Tx. Recipients were monitored intensively with complete blood count, lymphocyte profiles (flow cytometry), viral load of porcine lymphotropic herpes virus-1 (PLHV-1) in blood and tissues, serum IgM levels, and CyA trough levels. Histological examination was performed on tissues obtained at routine biopsy (day 28) and at necropsy.

Results: After spleen Tx, 2 of 7 pigs developed PTLD (29%) on days 30 and 32, respectively. One pig developed a malignant proliferation of host-type B lymphocytes, whereas the other had proliferation of donor-type B lymphocytes. The 2 pigs had significantly better T cell depletion by the day of spleen Tx, and had higher trough levels of cyclosporine during the first week than pigs that did not develop PTLD and the control animals. Early changes associated with PTLD included upregulation of PLHV-1 in blood and tissues, and increases in the number of leukocytes and B (CD3-CD16-) cells, and in the total serum IgM. These changes occurred before clinical features of the disease could be detected.

Conclusions: This is the first report to describe the occurrence of PTLD after spleen Tx. This study provides data regarding time course and risk factors for PTLD in a clinically-relevant large animal model. This model may provide a useful system for studies of the pathogenesis of PTLD.

Abstract# 1111 Poster Board #-Session: P41-III

TELOMERE SHORTENING IN RENAL TUBULAR EPITHELIAL CELLS IN RESPONSE TO CYCLOSPORINE A IN VITRO – A PARAMETER OF REDUCED KIDNEY TRANSPLANT SURVIVAL IN VIVO? Gabriele Schratzberger,[1] Christian Koppelstaetter,[1] Paul Jennings,[2] Anna Maria Wolf,[3] Walter Pfaller,[2] Gert Mayer.[1] *[1]Internal Medicine/Div. of Nephrology, University Hospital Innsbruck, Innsbruck, Austria; [2]Physiology, University Innsbruck, Insbruck, Austria; [3]Internal Medicine/Div. of Gastroenterology, University Hospital Innsbruck, Innsbruck, Austria.*

Telomeres, the non-coding region at the end of chromosomes, are required for the stability of chromosome ends and non genome erosive cell-replication. Telomeres are shortened by each cell division and environmental stress factors and hence have been implicated in processes such as aging and cancer development. The immunosuppressive drug Cyclosporine A (CsA) has revolutionized transplant medicine in the last decades. However, CsA induced nephrotoxicity still represents a major therapeutic challenge. Chronic CsA nephropathy is characterized by a decrease in glomerular filtration rate (GFR), tubular atrophy, interstitial fibrosis and progressive renal dysfunction. In vitro cell culture techniques allow the direct determination of toxicity at a cellular and molecular level, thus allowing each of the effects of CsA to be investigated independently. In this study we have investigated the effect of the widely used immunosuppressive reagent Cyclosporine A on telomere length in a renal proximal tubular cell line (HK-2). HK-2 cells were cultured in growth medium containing 0, 1 and 10 µg/ml CsA. These concentrations have previously been shown to be non-toxic to confluent HK-2 monolayers. HK-2 cells were therefore seeded in low concentration and CsA was applied one day after seeding to the proliferating cells up to day 6. Genomic DNA was isolated on the sixth day with magnetic beads extraction and analyzed by quantitative PCR for relative telomere length. CsA caused a dose dependent decrease in total DNA content over the three concentrations and 10µg/ml CsA resulted in a significant telomere shortening as compared to control cells (unpaired T-Test).

From these results it can be postulated that CsA causes a shortening of telomere length in proliferating proximal tubular epithelial cells and thus could be a factor in the limited life span of transplanted kidneys, where patients are treated for prolonged periods with CsA. Telomere length may act as a parameter in the process of organ function deterioration and therefore a reduced kidney transplant survival. At present it is unclear if the shortening of telomere length is a CsA specific event or whether other chronic nephrotoxins can also induce this phenomenon.

Abstract# 1112 Poster Board #-Session: P42-III

PREVALENCE OF HCV VIREMIA IN THE ABSENCE OF ANTI-HCV ANTIBODIES AMONG SOLID ORGAN DONORS IN A LARGE METROPOLITAN TRANSPLANT CENTER. Marek Nowicki,[1] Sali Aswad,[1] Claudia Chinchilla,[1] Livier Corado,[1] Nasreen Khan,[1] Hamid Shidban,[1] Rafael G. Mendez,[1] Robert Mendez.[1] *[1]National Institute of Transplantation, Los Angeles, CA.*

Background Nucleic Acid Testing (NAT) is currently not required for solid organ donors screening. It was reported that a persistent HCV viremia in the absence of anti-HCV antibodies detectable by the contemporary screening assays do exist (MMWR 2003, Apr 4;52(13), Transfusion, 2003, Jul;43(7)). The prevalence of such a pattern is unknown among organ donors. Determining the frequency of transplantations from such donors and the risk for transmitting HCV to recipients is important in evaluating whether additional prevention measures are warranted.

Aim To estimate the frequency of HCV viremia among anti-HCV antibody negative organ donors in a large transplantation center.

Methods We tested 575 donors available for organ donations between 10/2002 and 11/2003. All specimens were tested for anti-HCV by 2 HCV EIAs (Abbott Laboratories, Chicago and Ortho Diagnostics, Raritan NJ) and for HCV RNA using Transcription-Mediated Amplification TMA assay (*VERSANT*, Bayer Diagnostics, Tarrytown, N.Y.). Anti-HCV negative HCV RNA positive specimens were re-tested for HCV RNA and anti-HCV using another aliquot from the same donor.

Results Among 575 donors with specimen available for testing, 34 were repeatable anti-HCV reactive, and 33 were HCV RNA positive (Table). Six donors were repeatedly HCV RNA positive (TMA signal strength 451,751 - 1,244,796) in the absence of anti-HCV antibodies. For 4 donors we quantified their HCV viremia using bDNA and quantitative TMA assays (in house qualitative TMA, Bayer Diagnostics, Berkeley, CA). Their HCV viral load ranged from 50 to 22,250 copies/ml and their genotypes were 2b (n=1), 3a (n=1), and 1 (n=3). One donor had HCV RNA too low for genotyping (LIPA, Bayer Diagnostics, Tarrytown, N.Y.).

Total tested	Anti-HCV(-) HCV RNA(-)	AntiHCV(+) HCV RNA(+)	Anti-HCV(+) HCV RNA(-)	Anti-HCV(-) HCV RNA(+)
n=575	n=535 (93.0%)	n=27 (4.7%)	n=7 (1.2%)	n=6 (1.0%)

Conclusions We found that 1% of prospective organ donors were HCV RNA positive in the absence of detectable anti-HCV antibodies. Such a frequency is higher than those reported for blood donors and most likely reflects differences in pre-testing screening and epidemiology of blood versus organ donor populations. Our data suggests that NAT may contribute to the reduction of HCV transmission in the transplantation setting.

Abstract# 1113 Poster Board #-Session: P43-III

FK778, A SYNTHETIC MALONONITRILAMIDE, IS A POTENT ANTI-CYTOMEGALOVIRAL AGENT WITH A UNIQUE MECHAMISM OF ACTION THAT IS REVERSIBLE WITH ADDITION OF URIDINE. Kenneth A. Andreoni,[1] David L. Evers,[2] Xin Wang,[2] Shu-Mei Huong,[2] Eng-Shang Huang.[2] *[1]Abdominal Transplantation, Dept of Surgery, University of North Carolina, Chapel Hill, NC; [2]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.*

FK778, a malononitrilamide derived from A77 1726, is an immunosuppressant related to its parent compound, Leflunomide. It may act through inhibition of de novo pyrimidine synthesis or tyrosine protein kinases. We sought to determine if FK778 had similar anti-Cytomegalovirus (CMV) properties to its parent compound.

Methods: Plaque-forming assays using fibroblasts infected with Towne and two clinical strains of CMV were performed. IC50s were calculated for FK778, Ganciclovir (Ganc), or no drug (ctrl). Western Blot analyses were performed for examination of the immediate early viral proteins IE1& IE2, early UL84, and late proteins UL94 and gB, as well as phosphorylation of the EGF-R, PI3-K, Akt and p70s6K. The activities of NF-κB and Sp1 were measured by EMSA. Uridine was added to test for reversal of inhibition.

Results: FK778 had an IC50 comparable to Ganc (1 to2 µM) and well below its cellular toxicity (200 µM). Western Blot Analysis showed near complete inhibition of the Early Viral protein, UL84 and the Late Proteins UL94 and gB. At later time points, FK778 did significantly decrease IE2 and had a modest reduction of IE1. Uridine (100 µM) was able to completely reverse the antiviral effects of FK778. At 24 hrs post infection (hpi), phosphorylation of PI3-K (p85), Akt, and p70s6K began to decrease, reaching very low levels by 48 hpi. NF-κB and Sp1 activities were partially inhibited at their early phase (<4 hpi), and reduced to background by 12 hpi by FK778.

Discussion: We demonstrate that FK778 is as effective an anti-CMV drug as Ganc in our in-vitro model. FK778's IC50 against clinical strains of CMV is 50 to 100 times less than its reported safe and immunosuppressive serum concentrations in humans. Its reversal by uridine is consistent with specific inhibition of dihydroorotate dehydrogenase (DHODH), a required enzyme in the de novo biosynthesis of pyrimidines thus blocking viral DNA replication. The major mechanism of FK778's antiviral effect is the inhibition of viral protein and DNA synthesis. It also shows

partial inhibition of both phases of CMV-mediated PI3-K signaling and NF-κB and Sp1 activities. These results are in contrast to that reported for A77 1726: inhibition of assembly of complete virions despite the presence of abundant viral proteins. FK778 has a unique anti-CMV mechanism of action compared to other currently used drugs giving it promise as an ideal adjuvant agent in transplant patients.

Abstract# 1114 Poster Board #-Session: P44-III

TELOMERE LENGTH AS A NOVEL PREDICTOR OF DONOR ORGAN SURVIVAL IN RENAL TRANSPLANTATION. Gabriele Schratzberger,[1] Christian Koppelstaetter,[1] Rainer Oberbauer,[2] Bernd Mayer,[3] Heinz Regele,[4] Gert Mayer.[1] *[1]Internal Medicine/Division of Nephrology, University Hospital Innsbruck, Innsbruck, Austria; [2]Internal Medicine/Division of Nephrology, University Hospital Vienna, Austria; [3]Emergentec Business Solutions, Vienna, Austria; [4]Pathology, University Hospital Vienna, Vienna, Austria.*

Major problems in renal transplantation are limited organ availability on the one hand and late graft loss due to chronic allograft nephropathy on the other hand. To overcome limited organ availability, the use of kidneys from elderly donors has become more and more frequent. Even though it is known that on average these organs are more susceptible to late allograft failure, some of them maintain an excellent function for long periods of time. Thus, alternative strategies have been sought to characterize true "organ age" rather than relying on absolute years of age to predict organ survival and outcome. Telomeres represent non-coding regions at the ends of the chromosomes, and aging as well as environmental stress factors have been shown to shorten telomeres. Therefore, we measured telomere length in donor kidneys as a novel approach to assess donor "organ age".

In this study, we investigated mean telomere length in zero-hour biopsies from 78 individual renal transplant donors (22 living, 56 cadaveric) and compared it to donor age and to serum creatinine levels at 12 and 30 months posttransplant. Therefore, genomic DNA was extracted from paraffin-embedded biopsy slides, and relative telomere length was determined by quantitative PCR using specific primer pairs for telomeric DNA and a single copy house-keeping gene, of which a ratio was then calculated.

Results show that comparing telomere length and donor age revealed only a weak correlation (r = - 0.160). Moreover, age as well as telomere length were significantly associated with serum creatinine levels one year posttransplant, showing higher creatinine in patients receiving kidneys from donors with higher age or lower telomere length. Finally, after 2 1/2 years telomere length showed a stronger correlation with serum creatinine than donor age had with serum creatinine (r = -0.29 vs. r = -0.258).

From these results we conclude that the assessment of telomere length in zero-hour donor biopsies may represent a novel, more reliable predictor for renal transplant survival rather than donor age in the long run.

Abstract# 1115 Poster Board #-Session: P45-III

PORCINE VON WILLEBRAND FACTOR ANTIGEN DEFICIENCY WITH PULMONARY INTRAVASCULAR MACROPHAGE DEPLETION ABROGATES HYPERACUTE PULMONARY XENOGRAFT DYSFUNCTION. Edward Cantu,[1] Sarah Gibson,[1] Aaron Wyse,[1] Christine Lau,[1] Jeffery Gaca,[1] Gonzalo V. Gonzalez-Stawinski1,[1] Kamran Baig,[1] Timothy Nichols,[2] William Parker,[1] R. Duane Davis.[1] *[1]Department of Surgery, Duke University Medical Center, Durham, NC; [2]Department of Cardiology, University of North Carolina, Chapel Hill, NC.*

Objectives: Endothelial activation results in significant release of porcine von Willebrand factor (pvWF) in pulmonary xenografts. pvWF activates human and primate platelets within minutes even in the absence of sheer stress. Although elimination of porcine pulmonary intravascular macrophages (PIMs) prolongs pulmonary xenograft survival, pulmonary xenografts demonstrate diffuse microvascular thrombosis at 24 hours. We sought to define the time course and pattern of xenograft injury evident after coagulant and inflammatory modulation.

Methods: An orthotopic single lung pig-to-baboon transplant model was utilized. Donors were vWF antigen deficient (n=2), human membrane cofactor protein transgenic (hMCP) and PIM depleted (n=5), or vWF antigen deficient and PIM depleted (n=3). Graft survival was defined as continuous pulsatile flow of ≥ 50 ml/min.

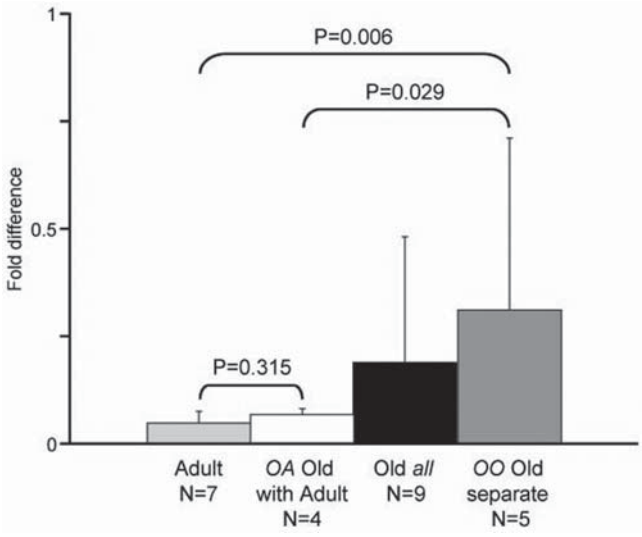
Results: Von Willebrand Factor deficiency alone was not protective with severe and widespread interstitial hemorrhage evident at 3 hours. Lifetable analysis demonstrated significant (p=0.0062) differences in survival between groups with mean survival of 5.5± 0.5 h for vWF deficiency alone, 22.6 ± 0.7 h for hMCP PIM depleted grafts and 67.4 ± 25.8 h for vWF deficient, PIM depleted grafts. One of the pvWF deficient xenografts lacking PIM's survived for 3.0 days and another survived for 4.6 days, with both times being longer than any other survival of a pulmonary xenograft reported to date. Depletion of PIM's from vWF deficient lungs, like depletion of PIM's from hMCP lungs, resulted in abrogation of the coagulopathy associated with pulmonary xenotransplantation. No differences in complement activation were identified between the PIM depleted groups.

Conclusions: Coagulant and inflammatory modulation through PIM depletion and pvWF deficiency results in prolonged survival. These data confirm the importance of porcine PIM's in pulmonary xenograft dysfunction and provide initial evidence that pvWF probably plays a critical role in the "delayed" (24 hours) dysfunction observed in pulmonary xenotransplantation using PIM depleted, hMCP-expressing organs. In particular, aberrant interactions between pvWF and the recipient's platelets are apparently a major factor contributing to decreased graft flow during pulmonary xenotransplantation.

Abstract# 1116 Poster Board #-Session: P46-III

DIFFERENTIAL GENE EXPRESSION PATTERNS IN HUMAN KIDNEYS WITH AGE: A POTENTIAL TOOL FOR EVALUATING MARGINAL DONORS? Anette Melk,[1,2] Elaine Mansfield,[3] Philip F. Halloran,[1] Minnie Sarwal.[3] *[1]Div. of Nephrology, University of Alberta, Edmonton, AB, Canada; [2]Div. of Pediatric Nephrology, University of Heidelberg, Heidelberg, Germany; [3]Div. of Pediatric Nephrology, Stanford University, Palo Alto, CA.*

Purpose: Given the powerful effect of donor age on kidney graft outcomes, we evaluated the molecular basis of renal aging. *Methods:* We used cDNA microarrays (~42,000 spots) to identify age-associated gene expression patterns in human kidneys. Samples included young (8 weeks-8 years, N=4), adult (31-46 years, N=7), and old kidneys (71-88 years, N=9). *Results:* Expression of ~500 genes differed significantly among the three groups. Old compared to adult kidneys showed increases in genes controlling extracellular matrix synthesis/turnover and inflammation, and decreases in genes associated with energy metabolism, cell integrity and mitochondrial function. Compared to adults, young kidneys had high expression of fetal collagen COL3A1 and other genes affecting extracellular matrix and growth/proliferation, and reduced expression of cell senescence and inflammation genes. Old kidneys had more glomerulosclerosis, tubular atrophy, interstitial fibrosis, and fibrous intimal thickening in small arteries. Quantitative supervised analysis using pathology scores and gene profiles of adult and old kidneys identified 255 significantly correlating genes. Both the genes correlating with age and with pathology scores clustered old kidneys into two groups: old kidneys that segregated away from adults (OO) and ones that clustered with adult kidneys and hence more closely resemble them (OA). Expression differences for 6 selected genes (FN1, MMP7, TNC, SERP3A, BPHL, CSPG2) were confirmed by quantitative RT-PCR, and independently confirmed in 17 additional kidneys. p53 inducible gene CSPG2 performed best in separating OO kidneys from adults and OA samples (Fig1). *Conclusions:* We propose that the genes identified here will be useful in quantitiating biological aging processes that contribute to kidney pathology post transplant. Aging markers with functional significance may be able to predict performance of marginal donor tissues.



Abstract# 1117 Poster Board #-Session: P47-III

EXPRESSION OF FORKHEAD ACTIVIN SIGNAL TRANSDUCER-1 (FAST-1) IN ACTIVATED INFILTRATING MACROPHAGES IN ARTERIES FROM PATIENTS WITH CARDIAC ALLOGRAFT VASCULOPATHY. Sheri E. Kelemen,[1] Howard J. Eisen,[1] Michael V. Autieri.[2] *[1]Cardiology, Temple University School of Medicine, Philadelphia, PA; [2]Physiology, Temple University School of Medicine, Philadelphia, PA.*

Introduction: Transcriptional analysis of gene expression in coronary arteries from patients with cardiac allograft vasculopathy (CAV) is a promising approach to identify targets to treat this disease.

Methods: Complimentary DNA microarray analysis of human coronary arteries from a patient with CAV was compared to coronary arteries form a non-transplant patient without heart failure or other cardiovascular diseases. Differences in gene expression were identified by fold change analysis, and verified by immunohistochemistry and western blot.

Results: Messenger RNA expression of the Forkhead Activin Signaling Transducer-1 (FAST-1) was not present in normal, but increased 7.7 fold in the coronary artery with CAV. FAST-1 is an important transcription factor for the Transforming Growth Factor-β (TGF-β) signaling pathway. Immunohistochemistry revealed expression in endothelial cells, smooth muscle cells, and predominantly infiltrating macrophages in the media and neointintima of the vessel. Cytokine stimulation of a human macrophage cell line, THP-1 was utilized to further characterize FAST-1 macrophage expression. Western blot analysis determined constitutive expression of FAST-1 in unstimulated THP-1 macrophages, with expression increased 67% in LPS stimulated, and 50% in TNF-α stimulated macrophages. No change in expression was noted in G-CSF, IFN γ, T-leukocyte conditioned media, or PDGF-stimulated cells.

Conclusion: FAST-1 mRNA and protein is elevated in coronary arteries from patients with CAV. Considering the role of TGF-β in cellular activation and the development of CAV, FAST-1 expression or activity may represent an important participant in the pathophysiology of this disease and an attractive target for modalities to treat CAV.

Abstract# 1118 Poster Board #-Session: P48-III

RIBAVIRIN COUPLED TO HEMOGLOBIN PROTECTS AGAINST EXPERIMENTAL VIRAL HEPATITIS: THERAPY FOR HEPATITIS C VIRUS POST-TRANSPLANT. Gary A. Levy,[1] Anand Ghanekar,[1] Nancy F. Ng,[2] Adam Levy,[1] Laisum Fung,[1] M. James Phillips,[1] Pieter Biessels,[2] Caroline Woods,[2] Gord Adamson,[2] David N. Bell.[2] *[1]Multi Organ Transplant Program, Toronto General Hospital, Toronto, ON, Canada; [2]Hemosol Inc., Mississauga, ON, Canada.*

Recurrent hepatitis C virus (HCV) is a serious problem post-transplant. Side effects of interferon-ribavirin combination therapy limit the sustained viral response achievable in a given HCV patient population. Coupled ribavirin/hemoglobin offers the potential of a therapy with improved safety and efficacy by targeting the delivery of ribavirin to key tissues (hepatocytes and macrophages) which possess receptors for binding and internalization of hemoglobin-haptoglobin complexes infected by viral infections such as HCV. To evaluate the effect of a ribavirin-hemoglobin conjugate (RBV-Hb, HRC 203) in a mouse model of viral hepatitis induced by the coronavirus, MHV-3. Ribavirin was covalently coupled to highly purified hemoglobin HbAo at a mean molar drug ratio of 8:1 (ribavirin:hemoglobin). The RBV-Hb was evaluated for retention of haptoglobin binding and cellular uptake *in vitro*, as well as the ability to recover bioactive ribavirin following enzymatic cleavage from RBV-Hb. The RBV-Hb was complexed to haptoglobin prior to administration to MHV-3 infected Balb/c mice, and carried 1/3 of the drug dose that was used for the Ribavirin group. The RBV-Hb-treated group was compared to control infected untreated and Ribavirin-treated groups for survival, clinical behavior, viral titer, biochemistry and liver histopathology. RBV-Hb was specifically internalized by cultured human and mouse hepatic cells but not by control non-hepatic cells. Ribavirin enzymatically cleaved from RBV-Hb retained *in vitro* bioactivity similar to unmodified ribavirin. Untreated and MHV-3 infected mice all developed clinical and biochemical signs of acute viral hepatitis and died by day 4 post infection (ALT_{max} 7000 IU/L). Livers recovered from untreated and infected mice showed greater than 90% necrosis. In contrast, survival was enhanced in both Ribavirin and RBV-Hb treated and MHV-3 infected mice with a marked reduction in biochemical (ALT_{max} 1000 IU/L) and histologic evidence of hepatic necrosis (<10%). Clinically, RBV-Hb treated mice behaved normally at all time points, in contrast to Ribavirin treated mice which developed lethargy and abnormal fur texture compatible with drug toxicity or ongoing viral infection. A beneficial effect of RBV-Hb was shown on the course of MHV-3 infection as demonstrated by prolonged survival, improved clinical behavior, and a reduction in biochemical and histologic disease.

Abstract# 1119 Poster Board #-Session: P49-III

THE SIGNIFICANCE OF HEPATIC ISCHEMIA-REPERFUSION INJURY ON LIVER TUMOR DEVELOPMENT - ACTIVATION OF CELL INVASION AND MIGRATION PATHWAYS. Kwan Man,[1] Kevin T. P. Ng,[1] Chung-Mau Lo,[1] Joanna W. Y. Ho,[1] Yi Zhao,[1] Chris K. W. Sun,[1] Terence K. W. Lee,[1] Xian-Liang Li,[1] Sheung-Tat Fan.[1] *[1]Surgery, University of Hong Kong, Hong Kong, China.*

Objective:

The aim of this study is to investigate the role of hepatic ischemia-reperfusion injury on liver tumor development in a rat liver tumor model by the comparison of tumor development, cell signaling pathways related to adhesion, migration, invasion and agiogeness, and stellate cell activation among the rats undergoing major hepatectomy with or without hepatic ischemia-reperfusion injury.

Materials and Methods:

Inbred male Buffalo rats (230-280g) were used. Rats were injected with hepatoma cell line (CRL1601, 1.5 $X10^6$) via the right portal vein (1) without major hepatectomy and partial hepatic ischemia-reperfusion; (2) after major hepatectomy (left and caudate lobes); (3) after 60 minutes' ischemia (right and median lobes) and 60 minutes' reperfusion; and (4) after 60/60 ischemia-reperfusion and subsequently major hepatectomy. The rats were sacrificed at days 20, 25 and 30 after cell injection. The liver and tumor tissues were sampled for morphological examination, detection of proliferation (PCNA), angiogenesis (VEGF) and stellate cell activation (a-SMA and desmin), and cell signaling pathway related to adhesion, migration and invasion (FAK, Rho and ROCK) by real time quantitative RT-PCR, Western blot and immunostaining. The tumor development and tumor size were compared among the groups.

Results:

Liver tumors usually developed with multiple small nodules (3-4mm) in right lobes at day 25 in Group (1). Early development of tumor was found in the other 3 groups. Significantly larger tumor size was mainly found in Group (4). In Group (4), the tumor nodules usually occupied the whole right lobes. Significant liver regeneration and tumor cell proliferation were found in Groups (2) and (4). Significant up-regulation of mRNA levels of Rho and ROCK were found in Groups (4), (3) and (2) compared to Group (1) both in tumor and non-tumor tissues by real time quantitative RT-PCR. Consistently, phospho-FAK and ROCK were also activated in Groups (4), (3) and (2) together with higher levels of VEGF by Western blot. Significant activation of stellate cells was mainly found in Group (4).

Conclusions:

Hepatic ischemia-reperfusion injury on small liver remnant exacerbated liver tumor development by significant activation of cell adhesion, invasion and migration pathways. Therefore, it is important to develop prophylactic strategy to prevent tumor recurrence after LDLT for hepatocellular carcinoma patients.

Abstract# 1120 Poster Board #-Session: P50-III

EPITHELIAL-MESENCHYMAL TRANSDIFFERENTIATION DURING LATE DETERIORATION OF HUMAN KIDNEY TRANSPLANTS: POTENTIAL ROLE OF TUBULAR CELLS IN FIBROGENESIS. Attapong Vongwiwatana,[1] Adis Tasanarong,[1] David C. Rayner,[2] Anette Melk,[1] Philip F. Halloran.[1] *[1]Department of Medicine; [2]Pathology, University of Alberta, Edmonton, AB, Canada.*

We hypothesized that tubular epithelial cells (TECs) contribute to fibrogenesis via epithelial-mesenchymal transdifferentiation (TEMT) in kidney transplants developing tubular atrophy/interstitial fibrosis (TA/IF). Biopsies from 10 kidneys with TA/IF, as defined by Banff CT CI CV scores and impaired function, were studied by immunohistochemistry and compared to their implantation biopsies, and to 6 protocol biopsies from transplants with stable function. Epithelial markers (E-cadherin, cytokeratin) were reduced and E-cadherin expression was redistributed from the basolateral membrane to the cytoplasm and apical membrane. New expression of mesenchymal markers [vimentin, S100A4 (human homologue of fibroblast specific protein-1)] and collagen synthesis marker (heat shock protein-47, HSP-47) was significantly demonstrated in cytoplasm of TECs, both in deteriorating and atrophic tubules. Double immunostaining showed coexpression of cytokeratin and vimentin, S100A4, and HSP-47 in some TECs, suggesting an intermediate stage of TEMT. Moreover, TEMT features correlated with serum creatinine, the degree of TA/IF, history of T cell-mediated rejection (TCMR), calcineurin inhibitor effect (hyalinosis), and proteinuria.

Conclusion: TECs apparently contribute to fibrogenesis in kidney transplants, via TEMT. TEMT changes correlates with graft function and extent of fibrosis. The association of TEMT with TCMR as well as post-transplant stresses (i.e. CNI effect, proteinuria) suggests that damage to TECs either by immunologic or non-immunologic processes leads to loss of epithelial features and induction of fibroblast features.

	IHC results		**Correlation with clinical parameters** (P values)				
	Allograft nephropathy	Implantation biopsies	CI&CT score	Creatinine	History of rejection	CNI effect	Proteinuria
E-cadherin loss	37.7 ± 5.0 %*	0.8 ± 1.2 %	0.001	0.001	0.03	0.04	0.006
Cytokeratin loss	40.6 ± 7.8 %*	0.6 ± 1.3 %	0.001	0.001	0.004	0.001	0.001
Vimentin	30.6 ± 10.6 %*	0 %	0.001	0.001	0.02	0.03	0.008
HSP-47	14.9 ± 4.4 %*	0%	0.001	0.001	0.02	0.04	0.003
S100A4	33.6 ± 9.4 %*	0%	0.001	0.001	0.007	0.006	0.01

*P < 0.05

Abstract# 1121 Poster Board #-Session: P51-III

ANALYSIS OF REGULATORY SIGNALS OF LIVER REGENERATION -SIGNALS REGULATING HEPATOCELLULAR PROLIFERATION AND GROWTH. Sanae Haga,[1] Shin Enosawa,[1] Keita Terui,[1,2] Wataru Ogawa,[4] Hiroshi Inoue,[4] Michitaka Ozaki.[1,3] *[1]Dept. of Innovative Surgery, Nat'l Res Inst for Child Health & Develop, Segaya, Tokyo, Japan; [2]Department of Pediatric Surgery, Chiba University Graduate School of Medicine, Inohana, Chuo-ku, Japan; [3]Department of Food and Health Science, Okayama University Graduate School of Medicine and Dentistry, Shikata, Okayama, Japan; [4]Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Chuo-ku, Kobe, Japan.*

Liver regeneration is one of the most important issues in liver transplantation. Rapid regeneration with good function will definetly be advantageous to the recipients. On the contrary, impaired regeneration of liver graft will affect patients' survival as well as graft function. Jak/Stat pathway and PI3K/PDK/Akt pathway are important in cell proliferation and cell growth, respectively. These signals, activated immediately after hepatectomy, definitely play crucial roles during liver regeneration. However, the functional roles of these signals during liver regeneration have not been understood so far.

In the present study, we have examined roles of Jak/Stat and PI3K/PDK/Akt signals in liver regeneration using liver-specific Stat3-KO (LS3-KO) mice and liver-specific PDK1-KO (LSPDK-KO) mice.

In 70% hepatectomized mice, wild type mice and LS3-KO mice did not show significant defference in liver regeneration 72 hr after 70%PH, though mRNA of cyclinD1 is reduced and mitogenic response (evaluated by PCNA and mitosis) is very weak in the liver of LS3-KO mice.

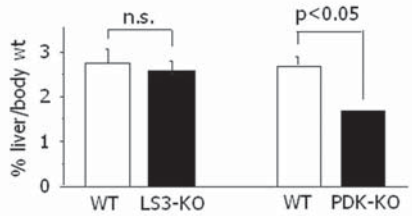
On the contrary, 67% of LSPDK-KO mice died within 72 hr after 70% PH because of liver failure, and 33% of the survived mice showed insufficient liver regeneration (Figure). Interestingly, though LSPDK-KO mice showed good mitogenic response after PH, they failed to regenerate liver as an organ.

Compensatory liver cell grwoth (increase of liver cell size) was observed in LS3-KO mice, but not in LSPDK-KO mice after PH. In addition, serum levels of AST/ALT were significantly elevated in hepatectomized LSPDK-KO mice.

Jak/Stat pathway is important in liver cell proliferation, but does not seem to be essential for liver regeneration. However, PI3K/PDK/Akt signals must be essential in maintaining good liver regeneration by compensating impared liver cell proliferation and in preventing liver failure after PH.

Figure

PI3K/PDK/Akt plays crucial roles in liver regeneration.



Abstract# 1122 Poster Board #-Session: P52-III

HEMATOPOIETIC STEM CELL TRANSPLANTATION PREVENTS LYMPHOMA IN Atm DEFICIENT MICE. Jessamyn Bagley,[1] John Iacomini.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA.*

PURPOSE: Ataxia-telangiectasia (A-T) is an autosomal recessive disease caused by mutations in the gene encoding ataxia-telangiectasia mutated (*ATM*), characterized by progressive cerebellar degeneration, variable immunodeficiency, and susceptibility to hematologic malignancies. Leukemia and lymphoma are major causes of morbidity and mortality in A-T patients. In mice, an introduced mutation in *Atm* leads to a phenotype that recapitulates many of the symptoms of A-T, and $Atm^{-/-}$ mutant mice develop fatal thymic malignancies as early as 9 weeks of age. By 20 weeks of age, the majority of $Atm^{-/-}$ mutant mice develop thymic lymphomas which prove fatal by 30 weeks of age. In this study we investigated whether replacement of the hematopoietic stem cell compartment would be sufficient to prevent the development of lymphoma.

METHODS: Because $Atm^{-/-}$ mice are extremely sensitive to irradiation, we first set out to develop a host preparative regimen that does not require irradiation to achieve engraftment of wild-type donor bone marrow. $Atm^{-/-}$ and wild-type littermate mice were treated with a depleting dose of anti-CD4 and anti-CD8 antibodies and 200 mg/kg cyclophosphamide before reconstitution with C3H bone marrow cells. Chimeric mice were followed long-term to determine the incidence of thymic lymphoma.

RESULTS: $Atm^{-/-}$ mice that underwent conditioning alone developed thymic lymphoma and were sacrificed (MST = 11.5 weeks, range 6-22 weeks after transplant, n=12). In contrast, $Atm^{-/-}$ mice reconstituted with C3H bone marrow displayed prolonged survival (MST >60 weeks, n=21, P<0.001). In this group, only one mouse was confirmed to have died of thymic lymphoma 13 weeks after transplant based on post-mortem examination. We were unable to detect thymic lymphoma in two mice in this group that died at 30 and 43 weeks after bone marrow transplantation. The remaining mice survived greater than 52 weeks after bone marrow transplantation, or were sacrificed at earlier time points without evidence of thymic lymphoma.

CONCLUSIONS: These data suggest that replacement of the $Atm^{-/-}$ hematopoietic compartment by bone marrow transplantation prevents the development of thymic lymphoma. Therefore, hematopoietic stem cell transplantation may prove to be of therapeutic benefit in A-T patients.

Abstract# 1123 Poster Board #-Session: P53-III

BENEFICIAL EFFECTS OF PANCREAS TRANSPLANTATION ON PROGRESSIVE DESTRUCTION OF PANCREATIC BETA-CELLS IN SPONTANEOUSLY DIABETIC SDT RAT. Gang Miao,[1] Toshinori Ito,[1] Fumihiro Uchikoshi,[1] Yusuke Akamaru,[1] Tetsuma Kiyomoto,[1] Hiroshi Komoda,[1] Jinghai Song,[1] Masumi Nozawa,[1] Hikaru Matsuda.[1] *[1]Surgery, Department of Surgery (E1), Osaka University Graduate School of Medicine, Osaka, Japan.*

Background: Type 2 diabetes is characterized by a combination of insulin resistance and alterations in beta-cell function. The latter may be ascribed to the deleterious effect of chronic hyperglycemia, a process referred to as glucotoxicity. Both clinical and experimental data have demonstrated that pancreas transplantation (PTx) can improve insulin sensitivity. However, it remains unclear that whether PTx could protect islets from glucose toxicity in type 2 diabetes. We hypothesized that pancreas transplantation might protect islets from glucose toxicity in a spontaneously diabetic type 2 model, SDT (Spontaneously Diabetic Torii; $RT1^a$) rat.

Methods: nondiabetic 10-week-old and diabetic (defined as blood glucose level over 250mg/dl) 25-week-old (onset age of diabetes) male SDT rats served as donors and recipients.

Results: In the control SDT rats, islets gradually decreased and blood glucose levels were progressively increased (>600mg/dl after 40 weeks of age). In the PTx rats, the onset age of diabetes was significantly prolonged [>47.5±18.2(PTx, graft age) vs. 25.2±3.9(control) weeks.]. Importantly, 2 of 6 recipients have enjoyed normoglycemia after PTx for more than 70 weeks. Both glucose tolerance and insulin sensitivity were improved in PTx recipients. By immunohistochemical staining, insulin-secreting islets were clearly observed in the naive pancreas with 48 weeks of age from PTx recipients, whereas no islets could be found in the pancreas with over 40 weeks of age from the control SDT rats. These results indicate that there may be a possibility of beta-cell regeneration in the damaged naive islets under the normoglycemic condition achieved by pancreas transplantation.

Conclusion: Pancreas transplantation has the potential to rescue impaired islets in spontaneously diabetic SDT rats.

Abstract# 1124 Poster Board #-Session: P54-III

GENE EXPRESSION PROFILING OF LIVER REGENERATION IN A MOUSE HEPATECTOMY MODEL. Christopher T. Barry, Ryan McTaggart, John P. Roberts, Sandy Feng. *Transplantation, UCSF, San Francisco, CA.*

Understanding the genetic programs responsible for liver regeneration post hepatectomy has important clinical implications for living related and cadaveric split liver transplantation. We use DNA microarrays to define gene expression changes over time in a mouse hepatectomy model. Mice, in triplicate, were subjected to 2/3 hepatectomy and liver specimens were snap frozen at times 0, 2, 4, 6, 12, 48, 72, 168 and 240 hours post operatively. Liver mRNA was purified and a reference mixture was made from all pooled time points. Samples from each individual time point were compared to the reference mixture by hybridizing to mouse DNA microarrays containing 23,000 elements. Data were analyzed by calculating fluorescence intensity ratios for individual genes and also by subjecting the entire timecourse of arrays to a hierarchical clustering algorithm. Gene expression profiles were compared to liver weight growth curves to correlate gene classes associated with the initiation and termination of the regeneration process. By 2 hours, stress response genes such as ubiquitin and GADD-45 and hepatocyte specific genes such as lipin and carbonic anhydrase are activated; these genes are not upregulated in a parallel sham hepatectomy time course. At 4 hours, previously described regeneration genes such as HIP-2 and p21 are upregulated. Later time points are characterized by increased expression of hepatocyte specific genes. The terminal time points implicate candidate genes, such as GHRH and RAB-25, and pathways responsible for the termination of regeneration. Cluster analysis reveals the dynamic interplay of genetic programs over the course of the entire regeneration process.

Abstract# 1125 Poster Board #-Session: P55-III

TRANSLATIONAL ENHANCEMENT IMPROVES GLUCOSE-DEPENDENT HEPATIC INSULIN EXPRESSION AND REDUCES HYPERGLYCEMIA IN STZ-INDUCED DIABETIC RATS. Philipp C. Nett,[1] Hans W. Sollinger,[1] Tausif Alam.[1] *[1]Department of Organ Transplantation, UW Hospital and Clinics, Madison, WI.*

Introduction: Gene-therapy based hepatic insulin production is a promising strategy in the treatment of insulin dependent diabetes mellitus (IDDM). We have previously shown that hepatocytes engineered with Ad.3SAM2 including the liver-specific albumin promoter coupled with 3 glucose inducible regulatory elements (GIRE)s from the S-14 gene and the modified proinsulin, improved glucose tolerance tests and corrected fasting hyperglycemia in streptozotocin (STZ)-induced diabetic rats. However, due to insulin insufficiency, postprandial hyperglycemia was not fully corrected.

Material and Methods: To increase the insulin output we generated a new insulin gene construct (3SATEM) containing an additional translational enhancer sequence derived from vascular endothelial growth factor (VEGF). The capacity of insulin expression of both constructs 3SAM2 and 3SATEM were tested *in vitro* and *in vivo*.

Results: Primary rat hepatocytes ($1x10^6$cells) exposed to 27.5mM glucose and transduced with Ad.3SATEM showed a 3.1-fold increase of glucose-dependent insulin secretion (435±45ng/ml) over a period of 24 hours compared to Ad.3SAM2 (142±21ng/ml), while glucose responsiveness was still maintained. *In vivo* studies in STZ-induced diabetic rats demonstrated that treatment with Ad.3SATEM in contrast to Ad.3SAM2 significantly increased insulin serum concentration under both postprandial (14.9±1.9µU/ml) and fasting (5.9±1.2µU/ml) conditions ($P<0.001$), thus accelerating kinetics to restore postprandial euglycemia and improving re-feeding tests. Both Ad.3SAM2- and Ad.3SATEM-treated groups showed a significant reduction of postprandial hyperglycemia and reduced weight-loss compared to diabetic control rats.

Conclusion: Our data demonstrate that the enhanced translational efficacy of the preproinsulin construct (3SATEM) substantially improved the output of hepatic insulin secretion and accelerated kinetics to restore postprandial euglycemia *in vivo*. Furthermore, this study substantiate the feasibility of gene therapy-based treatment for IDDM and provides novel information on the potential use of translational enhancement in an insulin gene construct that yields a therapeutically relevant amount of insulin to correct diabetic hyperglycemia in IDDM.

Abstract# 1126 Poster Board #-Session: P56-III

LIVER-SPECIFIC INSULIN PRODUCTION IN STZ-INDUCED DIABETIC MICE AFTER SYSTEMIC PLASMID DNA GENE DELIVERY. Philipp C. Nett,[1] Hans W. Sollinger,[1] Tausif Alam.[1] *[1]Department of Organ Transplantation, UW Hospital and Clinics, Madison, WI.*

Introduction: Gene-based therapy for the treatment of insulin dependent diabetes mellitus (IDDM) requires the development of a surrogate beta-cell that can synthesize and release insulin in response to glucose challenge in the physiological range. We have previously shown that hepatocytes genetically altered with a viral vector to produce transgenic insulin improved glucose tolerance tests and corrected fasting hyperglycemia in streptozotocin (STZ)-induced diabetic animals. Some viral vectors transduce hepatocytes efficiently and express transgenes at high levels; however, non-viral vectors have the advantage of being less toxic, less immunogenic and further lack the risks inherent to viral vectors. Recent studies provided evidence that intravascular delivery of plasmid DNA results in an effective gene transfer to hepatocytes.

Material and Methods: To test whether the systemic delivery of plasmid DNA is sufficient to correct diabetic hyperglycemia, STZ-treated diabetic mice were injected via the tail vein by proinsulin plasmid DNA including the liver-specific albumin promoter coupled with 3 glucose inducible regulatory elements (GIRE)s from the S-14 gene and the modified proinsulin with (3SATEM) or without (3SAM2) an additional translational enhancer sequence derived from vascular endothelial growth factor (VEGF).

Results: Compared to diabetic control mice, both 3SATEM- and 3SAM2-treated diabetic groups showed a normalization of fasting blood glucose levels, a significant reduction of postprandial hyperglycemia and reduced weight-loss. Treatment with 3SATEM in contrast to 3SAM2 significantly increased insulin serum concentration under both postprandial (3SATEM: 8.0±4.8µU/ml, and 3SAM2: 2.8±1.0µU/ml) and fasting (3SATEM: 2.4±0.3µU/ml, and 3SAM2: 0.3±0.4µU/ml) conditions (both $P<0.05$), thus accelerating kinetics to restore postprandial euglycemia.

Schlussfolgerung: Our data demonstrate that high levels of insulin expression can be achieved in hepatocytes by plasmid DNA injection via the tail vein avoiding the inherent risks of gene delivery by viral vectors. Additionally, the enhanced translational efficacy of our preproinsulin construct (3SATEM) substantially improves the output of hepatic insulin secretion and accelerated kinetics to restore postprandial euglycemia *in vivo*.

Abstract# 1127 Poster Board #-Session: P57-III

EMERGENCE OF A $CD4^+CD28^-$ GRANZYME B^+, CMV-SPECIFIC T CELL SUBSET AFTER RECOVERY OF PRIMARY CMV-INFECTION. Ester M. M. van Leeuwen,[1,2] Ester B. M. Remmerswaal,[2] Rene A. W. van Lier,[2] Ineke J. M. ten Berge.[1] *[1]Department of Internal Medicine, Divisions of Nephrology and Clinical Immunology & Rheumatology, Academic Medical Center, Amsterdam, Netherlands; [2]Laboratory for Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands.*

Cytotoxic $CD4^+CD28^-$ T cells form a rare subset in human peripheral blood. The presence of $CD4^+CD28^-$ cells has been associated with chronic viral infections but how these particular cells are generated is unknown. We here show that in primary CMV-infections $CD4^+CD28^-$ T cells emerge just after cessation of the viral load, indicating that infection with CMV is the major cause of the formation of $CD4^+CD28^-$ T cells. In line with this, we found these cells only in CMV-infected persons. $CD4^+CD28^-$ cells had an antigen-primed phenotype and expressed the cytolytic molecules granzyme B and perforin. Importantly, $CD4^+CD28^-$ cells were to a large extent CMV-specific since proliferation was only induced by CMV-antigen but not by recall antigens such as purified protein derivative (PPD) or tetanus toxoid (TT). $CD4^+CD28^-$ cells only produced IFNg after stimulation with CMV-antigen whereas $CD4^+CD28^+$ cells also produced IFNg in response to varicella zoster virus (VZV) and PPD. Thus $CD4^+CD28^-$ T cells emerge as a consequence of CMV-infection.

Abstract# 1128 Poster Board #-Session: P58-III

INNATE IMMUNE RESPONSE IS ACTIVATED VIA TLR4 SIGNALING IN BAL FLUIDS OF HUMAN LUNG TRANSPLANT RECIPIENTS. Sabarinathan Ramachandran,[1] Kalpaj R. Parekh,[1] G. A. Patterson,[1] T. Mohanakumar.[1,2] *[1]Department of Surgery, Washington University School of Medicine, St Louis, MO; [2]Pathology & Immunology, Washington University School of Medicine, St Louis, MO.*

Introduction: Innate immune responses mediated by TLRs have been shown to be linked to the adaptive immune responses. Single immunoglobulin IL-1R-related molecule (SIGIRR) has been shown to negatively regulate the TLR activation. The objective of the study was to identify the TLR receptor through which innate immune response is activated following human lung transplantation and study the expression profile of SIGIRR in epithelial cells following cold storage.

Methods: BAL samples were collected from 5 patients' post-op day 1 following lung transplantation and 5 patients with stable lung function. Cells were harvested from these BAL samples by centrifugation, RNA extracted using TRIZOL reagent and reverse transcribed. cDNA derived from 20ng of RNA was analyzed by quantitative real-time PCR using primers specific for TLRs 2,3,4,5,6,7,8, 9, 10 TIRAP and TOLLIP. The samples were normalized based on the expression levels of b-actin. Human airway epithelial cells were stored at 4 C for 4 hours and then cultured at 37 C for 2 hrs to study the effect of cold storage on SIGIRR expression by quantitative real-time PCR.

Results: BAL samples obtained immediately post transplantation showed 2 – 5 fold increase in expression of TLR4, which correlated with an increased expression of the adapter proteins, TIRAP and TOLLIP. However no increase in the expression of other TLRs was observed indicating that TLR4 is the major pathway through which innate immunity is activated immediately following human lung transplantation. Cold storage of the epithelial cells isolated from donor trachea showed a three fold reduction the expression of SIGIRR.

Conclusion: An increased activation of the innate immune response through the TLR4 signaling was observed in the BAL samples from lung transplant recipients. Preliminary results also showed that cold storage of the epithelial cells may result in decreased expression of SIGIRR, a negative regulator of TLR.

Abstract# 1129 Poster Board #-Session: P59-III

LONG-TERM CYCLOSPORINE TREATMENT IMPAIRS URINE CONCENTRATING MECHANISM BY INHIBITING AQUAPORINS AND UREA TRANSPORTERS. Chul Woo Yang,[1] Can Li,[1] Bum Soon Choi,[1] Sun Woo Lim,[1] Bo Kyoung Sun,[1] Yong Soo Kim,[1] Jin Kim,[2] Byung Kee Bang.[1] *[1]Division of Nephrology, Kangnam St.Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea; [2]Anatomy, The Catholic University of Korea, Seoul, Republic of Korea.*

Chronic cyclosporine (CsA) nephropathy is associated with a urine concentrating defect of tubular dysfunction, but the mechanism is unknown. We investigated the effect of CsA treatment on the expression of aquaporins (AQPs) and urea transporters (UTs) in a rat model of chronic CsA nephropathy. Rats were maintained on a low-salt diet (0.05% sodium) and treated daily for four weeks with olive oil (vehicle, 1 mL/kg s.c.) or CsA (15 mg/kg s.c.). The expression of AQPs (AQP-1–4) and different isoforms of UTs (UT-A1, UT-A2, UT-A3, and UT-B) was examined with Western blot analysis and immunohistochemistry. The expression of aldose reductase (AR) and Na-K-ATPase-alpha1, apoptotic cell death, histology, and functional parameters (urine volume, urine osmolality, sodium excretion, and free-water reabsorption) were also examined. CsA-treated rats showed an increase in urine volume (two-fold) and fractional excretion of sodium, and a decrease in urine osmolality (56.8%) and free-water reabsorption compared with vehicle-treated rats. Compared with vehicle-treated rats, rats given CsA showed a significant decrease in the expression of AQP-2 in the inner medulla, and AQP-1, AQP-3 and AQP-4 in throughout the kidney, but not AQP-2 expression in cortex and outer medulla. Similarly, the expression of UT-A2, UT-A3, and UT-B also decreased in CsA-treated rat kidney. These changes were accompanied by a significant down regulation of AR and Na-K-ATPase-alpha1, and an increase in the number of TUNEL-positive cells. The number of TUNEL-positive cells was well correlated with the expression of AQP-2 and UT-A3 of inner medulla. These findings suggest that the long-term treatment of CsA impairs urine concentrating capacity by inhibiting AQPs and UTs expression.

Abstract# 1130 Poster Board #-Session: P60-III

DOWN REGULATION OF GENE EXPRESSION IN THE GRAFT LIMB BY RNA INTERFERENCE. Yuki Sato,[1] Takashi Ajiki,[1] Yoji Hakamata,[1] Takashi Murakami,[1] Eiji Kobayashi.[1] *[1]Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Kawachi, Tochigi, 329-0498, Japan.*

Background. Genetic modification of the graft organ has great potential as a novel therapeutic approach. We have recently developed a non-viral gene transfer method to donor grafts using the organ-selective technique. Because RNA interference has been an attractive strategy for silencing the specific gene expression, we applied it to down-regulate the endogenous gene expression in the graft limb by small interference RNA (siRNA).

Methods. Green fluorescent protein (GFP) Tg LEW rats and wild LEW rats were used as donors and recipients, respectively. Just before the transplantation, 200 microgram of siRNA-GFP was rapidly injected into the limb grafts through femoral vein using 22-gauge catheters. The orthotropic limb transplantation was then conducted, and the GFP expression was evaluated in the graft by the fluorescent microscopy under 489-nm excitation light.

Results. While the degree of down-regulation of GFP was various in the graft, 30% of GFP positive muscle fibers was completely down-regulated in the injected area by siRNA at 2 days post transplantation. These RNAi-mediated gene suppression was transient and GFP expression was recovered at day 7.

Conclusions. This is a first report showing that siRNA transfer into the limb graft was achieved by catheter-based intra-venous rapid injection method. This procedure may allow the endogenous or exogenous target gene to be modified and could be applied for genetic controlling of the grafted organ.

Abstract# 1131 Poster Board #-Session: P61-III

MARKED PROLONGATION OF ISLET ALLOGRAFT SURVIVAL IN NOD MICE WITH HIGH DOSE RAPAMYCIN AND CD154 PLUS ICOSL BLOCKADE. Mohammed Javeed I. Ansari,[1,3] Arezou Khosroshahi,[3] Indira Guleria,[3] Hideo Yagita,[2] Hugh Auchincloss, Jr.,[1] Mohamed H. Sayegh.[3] *[1]Department of Surgery, Massachusetts General Hospital, Boston; [2]Juntendo University, Tokyo, Japan; [3]Transplantation Research Center, Brigham & Women's Hospital and Children's Hospital, Boston.*

Introduction: NOD mouse has proven to be an excellent model to study the pathogenesis and therapy of autoimmune diabetes including islet transplantation, however, these mice tend to be markedly resistant to tolerance induction. Rapamycin forms an important component of the Edmonton protocol for clinical islet transplantation and has been shown to have unique properties in facilitating tolerance induction in other organ/tissue transplant models. The CD154-CD40 and ICOS-B7h costimulatory pathways play an important role in alloimmunity, and combined CD154 and ICOS blockade is synergistic in promoting long-term allograft survival in cardiac transplant recipients. We tested the efficacy of high dose Rapamycin in combination with T cell costimulatory blockade of CD154 and ICOSL in fully allogeneic islet grafts in NOD recipients.

Methods: Approximately 800 islets from BALB/c donor mice were transplanted in spontaneously diabetic (blood glucose >500mg/dL on at least 2 consecutive days) NOD mice. Rapamycin was administered in a dose of 3mg/kg on each day from day 0-7 followed by alternate day injections until day 28. Blocking anti-CD154 (MR1) and anti-ICOSL (HK5.3) mAbs were injected in doses of 0.5mg on day 0 or day 4 respectively, followed by 0.25mg on alternate days for 3 additional doses. Blood glucose was monitored post-transplantation, initially daily for 2 weeks and on alternate days thereafter. A blood glucose reading of >200mg dL on two consecutive readings was considered as rejection of islet allograft.

Results & Discussion: High dose Rapamycin significantly prolonged islet allograft survival in NOD mice (MST 32, n=5, P=0.0382). CD154 or ICOSL blockade alone did not prolong islet allograft survival significantly, however marked prolongation of islet allograft survival was seen on combining high dose Rapamycin with anti-CD154 mAb (MST 93.5, n=4, P=0.0069) or high dose Rapamycin with both anti-CD154 and anti-ICOSL mAbs (MST 104, n=4, P=0.0069).

Conclusion: Combination therapy with high dose Rapamycin and CD154 plus ICOSL costimulatory blockade has synergistic effect in facilitating prolonged islet allograft survival in NOD mice. These results provide the rationale to test this protocol in non-human primates and ultimately in human islet transplant recipients.

Abstract# 1132 Poster Board #-Session: P62-III

***IN VIVO* QUANTITATIVE NONINVASIVE BIOLUMINESCENCE IMAGING OF INTRAPORTAL TRANSPLANTED ISLETS.** Juan L. Contreras,[1] Tandra R. Chaudhuri,[2] Amanda S. Starged,[2] Hongju Wu,[2] Cheryl A. Smyth,[1] Guadalupe Bilbao,[1] David T. Curiel,[3] Devin Eckhoff,[1] Kurt R. Zinn.[2] *[1]Surgery, UAB, Birmingham, AL; [2]Radiology, UAB, Birmingham, AL; [3]Human Gene Therapy, UAB, Birmingham, AL.*

Pancreatic islet transplantation (PIT) has been demonstrated to reverse type I diabetes. However, significant loss of islet mass occurs in the peritransplant period that require the infusion of high numbers of islets to achieve insulin-independence. To asses potential therapeutic approaches to enhance the efficiency of PIT, an accurate *in vivo* measure of islet mass is needed. Based on recent technological advances, it is possible to obtain quantitative, noninvasive, *in vivo* imaging of cells expressing Lucifearse. We hypothesized that this method can also be used to asses intraportal islet mass after transplantation. **Methods**: Islets were isolated from male Lewis rats. Hand-picked islets were infected with a targeted adenoviral vector encoding firefly Luciferase or LacZ (RGD and polylysine motifs incorporated in the fiber knob, AdRGDpk7Luc), which allowed high transfection efficiency (96% islet cells) using a minimal viral dose (0.1 VP/cell). 24 hours after infection, different islet doses were infused into the portal vein in streptozotocin-induced diabetic syngeneic recipients. Bioluminiscence imaging was correlated with islet dose, glucose levels, and functional islet mass (glucose disposal rates obtained from serial metabolic testing). **Results**: No significant changes in islet viability, islet mass or *in vitro* glucose stimulated insulin release were demonstrated after islet infection with AdRGDpK7Luc. After transplantation of 2000 IEQ/rat, 100% of the recipients presented euglycemia (nonfasting glucose <200 mg/dL) within 7 days compared with animals given 1500 IEQ (glucose 220-279 mg/dL) or 1000 IEQ (glucose=>300 mg/dL). A direct correlation between islet dose, luciferase readings, and glucose disposal rates was demonstrated (day 7: 2000 IEQ= mean 1.97 E+06 photons/sec; Kg-2.96±0.6 *versus* 1000 IEQ=mean 5.2+05 photons/sec; Kg 1.4±0.5). No luciferase readings were demonstrated outside the liver or in animals given islets infected with AdRGDpk7LacZ. No significant interference in iselt engraftment or functionality was demonstrated in animals given islets infected with AdRGDpK7Luc (Kg=2.96±0.6 compared with mock infected islets=3.1±0.8). **Conclusions**: It is possible to quantitatively assess intraportal transplanted islet mass *in vivo* using noninvasive bioluminescence imaging.

Abstract# 1133 Poster Board #-Session: P63-III

DONOR T-CELL CHIMERISM IS ASSOCIATED WITH ABROGATION OF RECURRENT AUTOIMMUNITY IN ISLET GRAFTED NOD MICE. Donghua Wang,[1] Qixin Shi,[1] Gregg A. Hadley,[1] Donna L. Farber,[1] Stephen T. Bartlett.[1] [1]*Dept. of Surgery, University of Maryland School of Medicine, Baltimore, MD.*

Introduction: Type I diabetes is a T cell-mediated autoimmune disease that causes destruction of insulin-producing β cells in the pancreas. Islet cell transplantation is the most promising cure for Type I diabetes; however, recurrent autoimmunity has been showed to play a role in clinical disease recurrence. We have developed a novel and clinically relevant strategy for inducing long-term islet graft survival in diabetic NOD mice by initial host T cell depletion with anti-lymphocyte serum (ALS) followed by administration of donor pancreatic lymph node cells (PLNC) along with islets. In the present study, we sought to elucidate the role of ALS treatment in promoting the chimerism of donor T cells and to establish whether the mice were rendered tolerant to the islet grafts. **Methods:** Pre-diabetic or diabetic NOD (Thy1.2$^+$) mice were treated with or without ALS on day -6, -4, and -2. On day 0, they were adoptively transferred with PLNC from NOR x NOD.NON F1 (Thy1.1$^+$) mice. For islet grafting, freshly isolated NOR islets were injected via the portal vein of diabetic NOD hosts. Blood glucose was regularly assessed and FACS analyses were performed to monitor the chimeric status and donor and host T cell phenotype. **Results:** ALS treatment resulted in dramatic depletion of the host peripheral blood T cells followed by gradual repletion. Following adoptive transfer into untreated NOD hosts, F1 donor T cells rapidly disappeared from the host peripheral blood within two weeks. However, if host mice were treated with ALS prior to adoptive transfer, a high level of donor T cells in the host peripheral blood (5-20%) persisted for more than 100 days. Despite gradual reduction in donor T cell chimerism, blood glucose levels remained normal. Following adoptive transfer, the donor CD4 T cells in host peripheral blood retained a naïve phenotype while the host CD4 T cells underwent transient upregulation of activation/memory markers. Transfer of splenocytes from diabetic NOD mice into NOD.SCID hosts resulted in diabetes within 20-24 days. However, transfer of splenocytes from ALS/PLNC-treated mice with long-surviving islet allografts resulted in no diabetes in NOD.SCID hosts up to 70 days post-transfer. **Conclusions:** These data demonstrate that ALS treatment is necessary for long-term engraftment of donor T cells and that such chimerism is associated with protection from recurrent autoimmunity.

Abstract# 1134 Poster Board #-Session: P64-III

ISLET-KIDNEY TRANSPLANTATION: DETERMINING THE MINIMUM NUMBER OF ISLET EQUIVALENTS NEEDED TO CURE DIABETES IN INBRED MINIATURE SWINE. Matthew Nuhn,[1] Emma Samelson-Jones,[1] Chisako Kamano,[1] Vaja Tchipashvili,[1] Koji Yazawa,[1] Shuji Nobori,[1] Parsia A. Vagefi,[1] David H. Sachs,[1] Kazuhiko Yamada.[1] [1]*Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

Background: We have previously demonstrated the cure of surgically induced diabetes mellitus (DM) by the transplantation of prevascularized, composite islet-kidneys (IK) across allogeneic barriers in inbred miniature swine, using 5,000 islet equivalents (IE)/kg recipient weight. In contrast, the same number of IE did not cure DM if administered directly into the portal vein or beneath the renal capsule, across the same immunologic barrier. Clinical studies have also required injection of much higher numbers of IE (25,000 IE/kg) to cure DM. **Methods:** Donors were subjected to 70% partial pancreatectomy; islets were isolated and cultured for 3 days. Autograft islets were then injected under the right kidney capsule and allowed to develop vasculature over the next 6-8 weeks before allogeneic IK transplantation (Tx). Recipients of IKs were totally pancreatectomized 3-5 days prior to IK Tx and received a 12-day course of FK-506, starting on the day IK Tx. Of 7 swine recipients, 3 received 2,000 IE/kg, 2 received 3,500 IE/kg and 2 received 5,000 IE/kg across a two-haplotype, fully MHC-mismatched barrier. Graft function was assessed by daily blood glucose and creatinine (Cr) level, biopsies and immunologic assays. **Results:** All 3 recipients of 2,000 IE/kg maintained normal renal function, however, never achieved normoglycemia. All of these recipients completed the 12-day course of FK-506. The first recipient of 3,500 IE/kg required exogenous insulin for the first few days following IK Tx and then maintained a blood glucose of 120-180 mg/dl without exogenous insulin. CML and MLR at day 30 and thereafter showed donor specific unresponsiveness. The second recipient in this group immediately became normoglycemic, however, was sacrificed on POD 2 due to an unexplained neurologic event. Both recipients of 5,000 IE/kg IKs had normal renal function and maintained normoglycemia after cessation of FK-506. No insulin was required. **Conclusion:** These data indicate that 3,500 IE/kg is the minimal effective dose of vascularized islets capable of curing surgically induced DM as part of a tolerance protocol of renal transplantation. This number is much lower than the number of IEs required to cure DM using intraportal injection of free islets, suggesting the clinical potential of this technology for treatment of type I DM with renal failure.

Abstract# 1135 Poster Board #-Session: P65-III

A LOW DONOR ISLET MASS IS SUFFICIENT TO RESTORE NORMOGLYCEMIA IN DIABETIC NOD MICE AFTER PRETRANSPLANT INDUCTION OF A SUBSTANTIAL LEVEL OF DONOR CHIMERISM. Zhiguang Guo,[1] Hannes Kalscheuer,[1] Yisheng Pan,[1] Bin Luo,[1] Haken Sozen,[1] David E. R. Sutherland,[1] Bernhard J. Hering.[1] [1]*Department of Surgery, University of Minnesota, Minneapolis, MN.*

Two or more donors are required for a successful human islet allotransplantation under the current Edmonton protocol. However, human islet autotransplant recipients after pancreatectomy need fewer islets per recipient than the islet allotransplant recipients. In this study, we determined whether the islet mass required to reverse diabetes could be significantly reduced in diabetic NOD mice after establishing a substantial level of donor lymphocyte chimerism (>40%), as compared with the islet mass required for successful allogeneic islet transplantation in immunosuppressed diabetic NOD mice. Fludarabine phosphate (400 mg/kg) and cyclophosphamide (200mg/kg) were given at day -1. Donor BALB/c bone marrow cells were transplanted into diabetic NOD mice at day 0. Anti-CD154 mAb were given from day 0 to day 14. Different numbers of islets were transplanted in diabetic NOD mice with >40% donor chimerism 3 weeks post-BMT. We also transplanted different numbers of islets into diabetic NOD mice under immunosuppression comparable to the Edmonton protocol. Diabetic NOD mice were treated with ALS (0.3 ml) on day –1 and 0, and rapamycin (1 mg/kg/day) and low-dose tacrolimus (0.5 mg/kg/day) beginning on day –2.

At 3 weeks posttransplant, diabetes was not reversed in any NOD mice that received 100 islets and immunosuppression (N=3). When 200 islets were given, only 16 % of the mice achieved normoglycemia (N=12). When the number of transplanted islets was further increased to 300 and 400, the percentage of mice that achieved normoglycemia was increased to 50% (N=12) and 75% (N=8). However, in diabetic NOD mice with > 40% donor lymphocyte chimerism, 83% of mice reversed diabetes 3 weeks after receiving 100 donor islets (N=6). When the number of transplanted islets was increased to 200 (N=9), 300 (N=8), and 400 (N=8), all recipient mice achieved normoglycemia and significantly increased body weight in these three groups.

Our results demonstrate that a high number of islets is required to reverse diabetes in spontaneously diabetic NOD mice with immunosuppression. However, a low islet number is sufficient to restore normoglycemia in diabetic NOD mice with a substantial level of donor chimerism. These data suggest that a small number of islets are sufficient to reverse diabetes in those diabetic NOD mice whose alloimmune response and autoimmune response were abrogated by pretransplant induction of a substantial level of donor chimerism.

Abstract# 1136 Poster Board #-Session: P66-III

ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE EFFECTS OF THE GLUCOCORTICOID-INDUCED LEUCINE-ZIPPER PROTEIN GILZ ON ISOLATED PANCREATIC ISLETS (PI). Juan L. Contreras,[1] Hongju Wu,[2] Guadalupe Bilbao,[1] Christopher Eckstein,[1] Carlton Young,[1] Carlo Riccardi,[3] David Curiel,[2] Judith Thomas,[1] Devin Eckhoff.[1] [1]*Surgery, UAB, Birmingham, AL;* [2]*Human Gene Therapy, UAB, Birmingham, AL;* [3]*Clinical and Experimental Medicine, University of Perugia, Perugia, Italy.*

The production of proinflammatory cytokines (PIC) and chemokines contributes to PI dysfunction and death after PI transplantation (Tx). GILZ is a key component of the IL-10 and glucocorticoid signal pathways. In this regard, GILZ has anti-inflammatory effects and down-regulates both NF-κB and AP-1. Here, we investigated the capacity of GILZ to prevent loss of functional islet mass (FIM) after Tx. **Methods**: Hand-picked human PI were transfected with human GILZ-pcDNA3 or GFP-pcDNA using lipofectamine, and incubated 24 hours later with PIC (human IFN-γ+IL-1β+TNF-α). for up to 4 days. PI viability was analyzed by membrane-integrity staining in dissociated single cells, PI mass by DNA/Insulin content, apotosis by ELISA. GILZ expression was confirmed by RT-PCR. Monocyte chemoattractant protein 1 (MCP-1) was assessed by ELISA. NF-κB and AP-1 were tested with a luciferase reporter gene. To examine GILZ effects *in vivo*, PI were isolated from C57BL/6 mice, transfected with murine GILZ, and transplanted into the portal vein (400 IEQ/mouse) in STZ-induced diabetic BALB/c (n=8). No immunosuppression was used. **Results:** PI viability after PIC exposure was significantly higher in PI expressing GILZ (day 4 = 62.3±8%) compared with GFP-expressing PI (35.8±5, P<0.05) or non-treated PI controls (31.3±6, P<0.05). Similarly, islet mass was higher (% DNA/insulin reduction in GILZ-PI=37.4±8; GFP-PI=66.2±6, P<0.05). In contrast, apoptosis was greater in controls (Enrichment Factor=8.3±2.2) compared with PI expressing GILZ (4.2±1.8, P<0.05). MCP-1 levels analyzed from culture supernatants were higher in control PI (no treatment=78.4, GFP=73.2± 8 pg/mL) compared with PI treated with GILZ (27.8±6, P<0.05). GILZ treatment reduced the activation of NF-κB (12224±4553 RLU/mg protein/second) *versus* controls (38451±6334, P<0.05). Significant down-regulation of AP-1 was also demonstrated. PI allograft survival (non-fasting glucose < 200 mg/dL) was significantly prolonged in GILZ-PI treated recipients (mean survival =18.4±2.6 days) compared with GFP (5.23±3.3) or Mock (6.1±2.5, P<0.05). **Conclusion:** GILZ promoted PI survival by interference with NF-κB and AP-1 activation. GILZ expression protected islets exposed to PIC and significantly delaged allograft rejection. PI treatment with GILZ represents a novel approach to improve the rusults in PI Tx.

Abstract# 1137 Poster Board #-Session: P67-III

A NOVEL APPROACH TO XENOTRANSPLANTATION COMBINING SURFACE ENGINEERING AND GENETIC MODIFICATION OF ISOLATED ADULT PORCINE ISLETS (PI).

Devin E. Eckhoff,[1] Dong Xie,[2] Cheryl A. Smyth,[1] Cristopher Eckstein,[1] Guadalupe Bilbao,[1] Carlton J. Young,[1] Anthony Thompson,[1] Firoz Rahemtulla,[3] David L. Curiel,[4] Juan L. Contreras.[1] *[1]Surgery, UAB, Birmingham, AL; [2]Engineering, UAB, Birmingham, AL; [3]Prosthodontics and Biomaterials, UAB, Birmingham, AL; [4]Human Gene Therapy, UAB, Birmingham, AL.*

Effective cytoprotection to PI would circumvent the major tissue limitation for islet transplantation (ITx). Cell-surface engineering with polyethylene glycol (PEG) and albumin prevent aggregation after exposure to incompatible serum. On the other hand, PI cytoprotection against xenoreactive reactions has been demonstrated after gene transfer of the anti-apoptotic and anti-necrotic Bcl-2 gene. In this study, we assessed survival and function of surface-engineered PI that overexpressed Bcl-2. **Methods**: Incorporation of albumin-capped PEG-di-succimidyl-succinate (PEGDS) into the PI surface was performed 12 hours after isolation by directly adding PEGDS into the PI suspension. Genetic modification was accomplished by adenoviral-mediated gene transfer of Bcl-2. Surface-modification was performed 12 hours after the infection. PI cytoprotection was evaluated by intracellular LDH release after 24 hours exposure to human serum, islet viability by MTT assay, and islet mass by DNA/insulin content. PI functionality was assessed by static incubation and after intraportal ITx (5000 IEQ) into diabetic NOD-SCID mice reconstituted with human lymphocytes (5 x 10^8/i.p./15 days before the ITx). **Results**: No significant change in PI viability was found at 1, 3, and 5 days after PEGDS treatment, as analyzed by MTT assay. PI treated with PEGDS 6K at 40% led to significant reduction in LDH release, compared with controls (35.3±5.1% vs. 76.8±8%, P<0.001). Further cytoprotection was accomplished by Bcl-2 (25.3±2.2%), and Bcl-2+PEGDS (5.23±1.1%, P<0.05). The combination of surface and genetic modification reduced loss of islet mass (day 3=61.2±5.6 % of pre-treatment islet mass) compared with controls (17.3±5.5%, P<0.001). Improved PI functionality was demonstrated after treatment with PEGDS (stimulation index=2.1±0.4 *vs*. controls 0.4±0.2, P<0.05), Bcl-2 (2.3±0.3), or both (3.5±0.4). Hyperglycemia (non-fasting glucose >200 mg/dL) was found in 100% of the animals given control PI (n=10). In contrast, stable euglycemia was found in 40% of mice treated with PEGDS, 50% with Bcl-2, and 70% with Bcl-2+PAGDS. **Conclusions:** Surface-engineering with functionalized PEG-derivatives in combination with genetic modification with Bcl-2 significantly reduced PI loss after ITx. Application of this novel technology may improve results in xenoislet transplantation.

Abstract# 1138 Poster Board #-Session: P68-III

FUNCTIONAL BENEFITS AFTER TRANSPLANTATION OF HEMATOPOIETIC STEM CELL ENRICHED BONE MARROW INTO ISCHEMIC MYOCARDIUM ARE NOT DUE TO TRANSDIFFERENTIATION.

Leora B. Balsam,[1] Amy J. Wagers,[2] Theo Kofidis,[1] Irving L. Weissman,[2] Robert C. Robbins.[1] *[1]Cardiothoracic Surgery, Stanford University, Stanford, CA; [2]Pathology and Developmental Biology, Stanford University, Stanford, CA.*

Recent reports suggest that c-kit$^+$ stem cell enriched bone marrow can regenerate myocardium and vasculature in mouse models of infarction. This has led to clinical trials of whole bone marrow and fractionated bone marrow transplantation into infarcted and end-stage failing hearts. We tested the hypothesis that this cell population would regenerate cardiac tissue and provide long-term functional benefit after infarction.

Methods: The c-kit$^+$ population of bone marrow cells was enriched by magnetic cell sorting from green fluorescent protein (GFP) transgenic C57BL mice. After creation of an infarct by ligation of the left anterior descending coronary artery, 1x10^6 cells were injected into the border-zone myocardium of C57BL mice. Hearts were analyzed histologically at 10 days (n=6) and 30 days for GFP$^+$ cells (n=5). Confocal microscopy along with staining for cardiomyocyte-specific (connexin-43 and α-sarcomeric actin), endothelial (vWF), and hematopoietic markers (c-kit, CD45, Gr-1, B220, CD3) was performed. At 2 and 6 weeks, left ventricular dimensions and fractional shortening (FS) were measured by echocardiography in another group of infarcted control (n=7) and cell-treated mice (n=11). At 6 weeks, invasive hemodynamics were also measured.

Results: At 10 days, abundant GFP$^+$ cells were present in the infarct and border-zone. The majority were c-kit$^-$CD45$^+$Gr-1$^+$ and none expressed cardiomyocyte or endothelial markers. At 30 days, rare GFP$^+$ cells were noted, and these were either c-kit$^-$CD45$^+$Gr-1$^+$ or c-kit$^-$CD45$^+$B220$^+$, again with no expression of cardiac tissue-specific markers. At 2 weeks, there was a trend toward smaller ventricular end-diastolic and end-systolic dimensions in cell-treated animals, and at 6 weeks this was statistically significant. FS did not differ at 2 weeks (21.7±11.9 vs. 21.2±8.2), but at 6 weeks, was higher in cell-treated animals (21.2±9.4 vs. 13±7.3). Invasive hemodynamic measures, including HR, MAP, LVEDP, +dP/dt, and –dP/dt did not differ between groups.

Conclusion: Treatment of post-infarcted hearts with c-kit enriched bone marrow cells does not result in myocardial or vascular regeneration; rather, these cells adopt traditional hematopoietic fates. Cell treatment may be of benefit, limiting left ventricular dilation and preserving function, but this is not due to transdifferentiation. Further mechanistic studies are warranted before this treatment technique is carried over to the clinical arena.

Abstract# 1139 Poster Board #-Session: P69-III

AN ESSENTIAL ROLE OF IFN-γ IN PRIMARY NON-FUNCTION OF ISLET GRAFTS IN THE LIVER OF MICE.

Masayuki Sato,[1] Yohichi Yasunami,[1] Yoshiichiroh Nakamura,[1] Masahiko Nakano,[1] Kentaroh Nabeyama,[1] Atsushi Toyofuku,[1] Nobuhide Matsuoka,[1] Junko Ono,[2] Masaru Taniguchi,[3] Seiyo Ikeda.[1] *[1]Surg 1, Fukuoka Univ, Fukuoka, Fukuoka, Japan; [2]Lab Med, Fukuoka Univ, Fukuoka, Fukuoka, Japan; [3]Allergy and Immunology, RIKEN Research Center, Yokohama, Kanagawa, Japan.*

Currently, the inability of producing successful islet transplantation from one donor to one recipient is a major obstacle facing clinical islet transplantation (tx). In the present study, we hypothesize that IFN-γ might be induced immediately after islet tx in the liver, which is the site of clinical islet tx, and responsible for primary non-function of islets resulting in the requirement of multiple donors to cure a single recipient. To prove this, IFN-γ-deficient (KO) mice as well as anti-IFN-γ antibody were used for the experiments.

When 200 islets, equivalent to those isolated from a single mouse pancreas, or 400 syngeneic islets were grafted into the liver of STZ-induced diabetic wild-type mice (C57BL/6), 0/10 or 10/10 recipients, respectively, became normoglycemic (<200 mg/dl) after tx. Morphologically, de-granulated or well-granulated β cells of islets were seen in the liver of mice receiving 200 or 400 islets, respectively, at 60 days after tx. In marked contrast to wild-type mice, IFN-γ KO diabetic mice (n=5) became normoglycemic by 14 days after tx when 200 islets were grafted into the liver. IFN-γ KO diabetic mice receiving 200 islets in conjunction with exogenous IFN-γ (human recombinant, 50000 U, ip, day 0-6) remained hyperglycemic at 60 days. Four out of 5 or 0/5 diabetic wild-type mice receiving 200 islets and treated with anti-IFN-γ antibody (ip, 100 μg/injection, day 0, 2, 4) or control antibody, respectively, became normoglycemic. IPGTT at 60 days after tx revealed that the plasma glucose levels of mice receiving 200 islets and treated with anti-IFN-γ antibody were 70.0±8.0, 324.3±6.5 and 188.0±10.6 mg/dl at 0, 30 and 120 minutes after the glucose injection (1g/kg), respectively, and those of mice receiving 400 islets without treatment were 66.5±4.1, 413.8±18.5 and 259.8±18.7 mg/dl, respectively. When diabetic wild-type C57BL/6 mice (n=5) receiving 200 BALB/c islet allografts and treated with anti-IFN-γ antibody in conjunction with anti-CD4 antibody (ip, 200 μg, day 0) to prevent rejection remained normoglycemic at 90 days after tx. These findings clearly show that IFN-γ is responsible for primary non-function of islet isografts and allografts in the liver of mice and suggest that the treatment targeted at IFN-γ may facilitate to produce the success of clinical islet transplantation from one donor to one recipient.

Abstract# 1140 Poster Board #-Session: P70-III

PROTEIN BIOMARKERS OF METABOLIC STRESS IN ISLET TRANSPLANTATION.

Mark R. Nicolls,[1] Michelle M. Kerklo,[1] Nanette Gomez,[1] Joshua Beilke,[1] Mark W. Duncan,[2] Ronald G. Gill.[1] *[1]Medicine, Barbara Davis Center for Childhood Diabetes; [2]Pharmacy, University of Colorado Health Sciences Center, Denver, CO.*

Islet transplants fail due to immune and non-immune insults. Metabolic stress attributable to an inadequate islet cell mass may be an important non-immune contributor to islet graft dysfunction. Characteristic protein biomarkers may be useful for identifying stressed islet grafts. To isolate the effects of metabolic stress on islet graft protein expression, syngeneic islet transplants were used to limit graft infiltration by alloreactive lymphocytes.

Methods: To induce metabolic stress, a marginal number of C57Bl/6 (B6) islets were transplanted into streptozotocin-induced diabetic B6 mice. Specifically, 250 islets (i.e. marginal mass) consistently reduced blood glucose levels but did not restore euglycemia (i.e. ≤10mM) as was achieved with 450 islets (i.e. optimal mass). Islet protein expression was assessed by Western gel or two dimensional gel electrophoresis (2DE) coupled with mass spectrometry. Non-transplanted islet protein expression was compared with day 7 islet transplant protein expression. 'Stressed' (250 islet) transplants were compared with 'non-stressed' (450 islet) transplants for relative protein expression. For Western blots, relative band chemiluminescence was used to assess differential protein expression. For 2DE, Melanie 3.0 Image Analysis Software and Amersham Difference in Gel Electrophoresis were used to detect changes in islet protein constituents.

Results: Differential protein expression was readily demonstrated by both Western and 2DE. Sixteen proteins were upregulated and 29 proteins were downregulated with metabolic stress. Protein biomarkers of islet stress can be grouped into two functional categories based on their conserved amino acid sequence domains: (1) proteins containing stress-responsive heat shock element domains (e.g. heme oxygenase-1, GRP78, HSP70) that confer chaperone activity essential in protein quality control such as folding and translocation to appropriate cellular compartments; and (2) proteins containing thioredoxin-type domains that are responsive to redox homeostasis (e.g. protein disulfide isomerase-related protein, GRP58). 2DE analysis revealed post-translational modifications of islet actin, GRP78, and tumor rejection antigen gp96 under conditions of metabolic stress.

Conclusions: In vivo protein biomarkers of transplanted islets under metabolic stress have been identified and demonstrate that complex differential protein expression during metabolic stress can be meaningfully interpreted. Biomarkers to predict and monitor islet function may prove a useful clinical adjunct.

Abstract# 1141 Poster Board #-Session: P71-III

MESENCHYMAL STEM CELLS CAN REDUCE CONDITIONING REQUIREMENTS FOR ALLOGENEIC ENGRAFTMENT. Phyllis E. Napoles,[1] David Polchert,[1] Ada Moadsiri,[1] Edmund Ho,[1] Julius Turian,[1] Zheng Yu,[2] Pamela Witte,[2] Amelia M. Bartholomew.[1] *[1]University of Illinois at Chicago, Chicago, IL; [2]Department of Cell Biology, Neurobiology, and Anatomy, Loyola University Medical Center, Maywood, IL.*

In allogeneic bone marrow transplantation, permanent engraftment across disparate MHC barriers can be achieved experimentally by reducing host anti-donor immune responses with rigorous host conditioning regimens. Clinically, this approach is limited due to regimen related toxicities, graft failure and the high incidence of graft versus host disease. The use of a bioengineered stem cell graft might aid in reducing the requirements of conditioning while retaining the ability to achieve long-term engraftment. Since mesenchymal stem cells (MSC) are known to suppress T cell responses, we hypothesized that co-administration of these cells with an allogeneic hematopoietic stem cell graft would aid in overcoming the allogeneic barrier and permit a reduction in the intensity of the host conditioning regimen. To test this hypothesis, 8 groups of B6 (H2b, n=6 per group) mice were conditioned with 800, 600, 400 or 200 cGy of sublethal radiation and given MHC disparate Balb/c (H2d) hematopoietic grafts alone or grafts supplemented with Balb/c MSC. To test for durable engraftment, peripheral blood of recipients was analyzed for the presence of donor cells at 120 days via flow cytometric detection of $H2d^+$ cells. MSC-treated recipients had significantly higher levels of donor engraftment when compared to non-MSC treated recipients at conditioning doses of 600, (6.9 ± 3.6 vs. 2.5 ± 1.3 %, $p<0.01$), 400, (23.4 ± 14.7 vs. 2.4 ± 1.8 %, $p<0.01$) and 200 cGy, (17.2 ± 4.9 vs. 7.1 ± 2.6%, $p<0.001$). Phenotype analysis of engrafted donor cells in recipients treated with 200cGy revealed that there was a trend for higher engraftment of $B220^+$ cells,(36±6 vs 19±6%, p=n.s.),$CD4^+$(11±6 vs 3±1%, p=n.s.), and $CD11b^+$(8±2 vs 2±1%, p=n.s) in MSC treated animals, with changes in the $CD8^+$ sup-population being signficant (15±4 vs 7±3%, $p<0.05$). We conclude that the addition of MSC enables successful allogeneic engraftment with minimal host conditioning. The combination of MSC with hematopoietic stem cell grafts may permit the use of less intensive host conditioning regimens and thereby reduce regimen related toxicities without compromising levels of allogeneic engraftment.

Abstract# 1142 Poster Board #-Session: P72-III

TRANSDUCTION OF HUMAN PANCREATIC ISLETS USING A BICISTRONIC FELINE IMMUNODEFICIENCY VIRAL VECTOR; IMPLICATIONS FOR ISLET TRANSPLANTATION. Elizabeth S. Fenjves,[1] Maria S. Ochoa,[1] Carlota Gay-Rabinstein,[1] Camillo Ricordi,[1] Michael A. Curran.[2] *[1]Diabetes Research Institute, University of Miami, Miami, FL; [2]Department of Molecular Biology, Berkeley Universiy, Stanford, CA.*

Background: Type 1 diabetes is an autoimmune disease resulting in the destruction of the insulin-producing beta cells of pancreatic islets. Islet transplantation offers the potential to reestablish regulated insulin secretion in patients with type 1 diabetes. Encouraging clinical results have been hampered by the fact that large numbers of transplanted islets are functionally impaired due to apoptosis. Genetic engineering of islets with cytoprotective genes may augment islet survival following transplantation. We have shown that islets can be efficiently transduced using a feline immunodeficiency viral (FIV) vector. However, the delivery of more than one cytoprotective gene, which would be highly advantageous for the protection of islets, has never been demonstrated with this vector. The goal of this research was to determine whether a bicistronic FIV could deliver two genes to human pancreatic islets.

Methods: Human pancreatic islets were isolated and transduced using a bicistronic FIV vector expressing an anti-apoptotic gene (cFLIP) driven by a cytomegalovirus (CMV) promoter, and a marker gene (YFP) on a viral internal ribosome entry site (IRES). This system allows for translation of two cDNAs from a single transcript. Control islets were transduced with an FIV vector expressing only YFP. Apoptosis was induced by adding 0.05mM doxorubicin to the islet culture media for 24 hours. Islets were disassociated and examined for Annexin PE and 7AAD to determine cell death. YFP fluorescence was used to determine transduction in these cells.

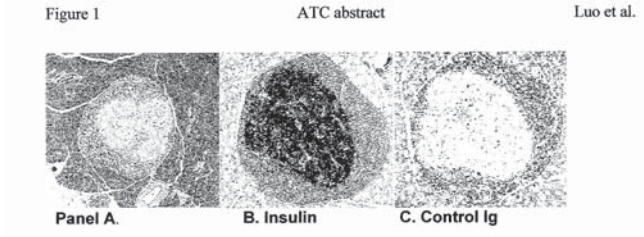
Results: All transduced islets were positive for YFP fluorescence. cFLIP-transduced islets induced with doxorubicin showed apoptosis levels of 15.5±0.7 whereas induced islets transduced only with YFP showed significantly higher apoptosis levels (33.5±3.5). These results show that both genes were expressed and that cFLIP-transduced islets were significantly protected from doxorubicin-induced apoptosis.

Discussion: In this study we show the successful transduction of human islets with a bicistronic FIV. This is the first report of the concomitant expression of two genes by an FIV vector in pancreatic islets. The capacity of an FIV vector to express two genes has important implications in terms of strategies aimed at cytoprotection of these cells in the transplant setting. FIV vectors may be useful in transducing islets with more than one cytoprotective, anti-lytic and/or immuno-modulating molecule.

Abstract# 1143 Poster Board #-Session: P73-III

A NEW TREATMENT FOR TYPE 1 DIABETES: SYSTEMIC TGF-BETA1 GENE THERAPY RESTORES SELF-TOLERANCE AND FACILITATES ISLET REGENERATION. X.-R. Luo,[1] H. Yang,[1] F. Sainte-Hilaire,[1] D. Thomas,[1] I.-S. Kim,[1] B. De,[1] W. W. Hancock,[2] R. G. Crystal,[1] M. Suthanthiran.[1] *[1]Medicine, Weill Medical Coll. of Cornell Univ., New York, NY; [2]Pathology, The Children's Hosp. of Philadelphia, PA.*

We designed and constructed a replication deficient adenovirus (Ad) vector encoding the active form of human TGF-β_1 and tested the hypothesis that systemic TGF-β_1 gene therapy restores self-tolerance and facilitates islet regeneration. Diabetic female NOD mice received intravenous injection of $5x10^{10}$ particle units of Ad.AH.TGF-β_1 and 500-600 syngeneic islets one week later. Hyperglycemia was promptly corrected by the islet grafts but reoccurred by day 17 in all untreated NOD mice. In striking contrast, long-term functioning of syngeneic grafts was found in 7 of 12 NOD mice treated with Ad.AH.TGF-β_1. Analysis of islet grafts retrieved from Ad.AH.TGF-β_1 treated NOD mice showed well-preserved islets with a peri-islet infiltrate primarily of CD4+ T cells and expression of CD25 and Foxp3. Staining for IFN-γ was negative whereas TGF-β_1 and IL-10, and PD-L1 and PD-L2 all showed heightened expression. Systemic TGF-β_1 gene therapy was also associated with functional restoration of endogenous islets (islet regeneration) in the native pancreas. Not only the Ad.AH.TGF-β_1 treated NOD mice remained normoglycemic after removal of the syngeneic islet grafts, examination of the native pancreas revealed scattered but intact islets, among pancreatic exocrine tissues (panel A). There were few intra-islet cellular infiltrates. However, similar to what was found in the syngeneic islet grafts, there were well-demarcated lymphocytic infiltrates on the periphery of the islets without invasion of the islets themselves (peri-insulitis). Moreover, the intact islets stained strongly for insulin (panel B), providing definitive evidence of functional restoration of endogenous beta cells.



Our studies demonstrating that systemic TGF-beta1 gene therapy restores self-tolerance and facilitates islet regeneration suggest a new treatment for type 1 diabetes.

Abstract# 1144 Poster Board #-Session: P74-III

THE ROLE OF NEUTRAL PROTEASE DURING ISLET ISOLATION EVALUATED WITH A NEW ENZYME PREPARATION. Pascal Bucher,[1] Domenico Bosco,[1] Philippe Morel,[1] Zoltan Mathe,[1] Manfred Kurfuest,[2] Leo Buhler,[1] Thierry Berney.[1] *[1]Department of Surgery, Geneva University Hospital, Geneva, Geneva, Switzerland; [2]Chemical, Nordmark Arzeinmittel, Uetersen, Germany, Germany.*

Aim: Several enzyme preparations are available for islet isolation. One of them is a highly purified product with separate components which allow adaptation of neutral protease concentration. Study aim was to evaluate the role of neutral protease for islet isolation.

Methods: Seven groups of SD rats (18 rats/group) were designed for standardized islet isolation. We used in group I, collagenase type XI at 2mg/ml (Sigma Chemical), in group II Liberase at 0.6mg/ml (Roche) and in group III to VII collagenase NB1 0.6mg/ml (Serva Electrophoresis). In group III, pure collagenase NB1 was used. In group IV, V, VI and VII concentration of 7.5, 15, 22 and 30 mg/ml neutral protease (NP) were added, respectively. Results were analyzed in term of islet equivalent (IE) yields, apoptosis, Cytokine (IL-1b, IL-6, TNFa, IFNg) secretions, insulin secretion (static incubation) and islet morphology.

Results: Endotoxin contents of enzyme solution were for group I and II of 170 and 120 EU/ml. Among group III to VII it gradually increased, according to the NP concentration, from 0.4 to 58 EU/ml ($p<0.05$). IE yields per rat were of 1367 ±522 and 1755 ±110 for group I and II, respectively. Among group III to VII, IE yields per rat followed a Gaussian distribution according to NP concentration with higher yield for group V, 1712 ±245, and lower for group III (597 ±277) and VII (905 ±297) ($p<0.05$). Among group III to VII, islet morphology was influenced by the NP concentration with decreasing number of trapped islet and increasing number of fragmented islet as the NP content increased. Increase in NP concentration was associated with progressive damage to the islet, which was similar in group VII to the damage observed in group I and II. Cytokines release were lower in group III to VII in comparison to group I and II, but were not influenced by NP concentration. Islet cell apoptosis was statistically significantly lower in group III to VII in comparison to group I and II, but was not influenced by NP concentration. Mean insulin secretion stimulation indexes were of 2.96 for group I, 5.17 for group II and between 5.25 and 5.43 for group III to VII.

Conclusion: Neutral protease is a crucial additive to collagenase for islet isolation in terms of islet yields. Collagenase NB1 with appropriate neutral protease adjunct is comparable to liberase in terms of islet yields and function. However, it is associated with lower islet cells apoptosis, and improved islet morphology.

Abstract# 1145 Poster Board #-Session: P75-III

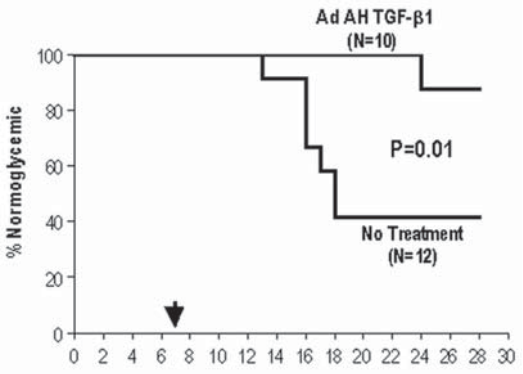
EARLY MOLECULAR MECHANISMS IN APOPTOSIS OF ISOLATED ISLETS (ANOIKIS). Francis T. Thomas,[1] Jianguo Wu,[1] Jin He,[1] Stacie Jenkins,[1] Clement Asiedu,[1] Juan L. Contreras,[1] Anne Hutchings,[1] Judith M. Thomas.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL.*

The mechanisms involved in early loss of functional islet mass remain uncertain, although immunologic factors are probably not the primary cause. Evidence indicates that early islet death is largely due to apoptosis, in part related to dissociation of islets from the extracellular matrix (*anoikis*). Most studies of islet apoptosis are in murine and human models and provide little information about the apoptosis mechanisms in isolated nonhuman primate (NHP) islets. We examined molecular mechanisms of NHP islet apoptosis. Normal pancreata were harvested after cold perfusion in UW and digested with Liberase using a standardized semi-automatic procedure. Islets were isolated by density centrifugation using a Cobe processor. Beginning at 5 minutes after islet isolation and at serial time points thereafter, we examined caspase activation, cellular expression and distribution/translocation of Bax, Bmf, and GSK3b, with and without specific inhibitors. Caspase activation was determined by FLICA. Western blots and immunhistochemistry was used to examine protein expression. Apoptosis was measured using annexin V staining. Activation of effector caspases 3, 6, and 7 and also caspase 9 was observed within 5 minutes after islet isolation. In contrast, caspase 8 was activated 2 hrs later, and caspases 1, 2, 4, and 10 were not activated in the first 24 hrs. Inhibitory peptides, specific for caspase 3,6, 8, or 9, added alone or in combination, yielded a maximum 35% protection, suggesting involvement of a caspase-independent pathway. Bax activation, by nuclear and mitochondrial translocation, was observed within 5 minutes after islet isolation, whereas there was negligible Bax translocation *in situ* in non-isolated pancreatic islets. Bax activation correlated with Bmf relocation from the myosin V motor complex, an event common to anoikis-type apoptosis in other cell types. The active form of GSK3β, an integral component of the intergrin signaling pathway and an upstream activator of Bax, became increasingly prominent in the nucleus. Addition of the GSK3β inhibitor, $LiCl_2$ blocked Bmf and Bax nuclear translocation, decreased the active form of GSK3β, and blocked apoptosis. In summary, we postulate that the break down of integrin survival signals leads to sequential activation of GSK3β, Bmf and Bax, triggering the caspase cascade to rapidly initiate apoptosis in isolated islets.

Abstract# 1146 Poster Board #-Session: P76-III

SYSTEMIC TGF-BETA $_1$ GENE THERAPY ELICITS T-REGULATORY CELLS, INDUCES FOXP3 EXPRESSION AND RETARDS THE DEVELOPMENT OF TYPE 1 DIABETES. H. Yang,[1] F. Saint Hilaire,[1] X.-R. Luo,[1] D. Thomas,[1] B. De,[1] R. Ding,[1] T. Muthukumar,[1] V. K. Sharma,[1] M. He,[1] W. W. Hancock,[2] R. G. Crystal,[1] M. Suthanthiran.[1] *[1]Medicine, Weill Medical College of Cornell Univ., New York, NY; [2]Pathology, Children's Hosp. of Philadelphia, PA.*

We designed and constructed a replication deficient adenovirus (Ad) vector encoding the active form of human TGF-β_1 (Ad.AH.TGF-β_1) and tested the hypothesis that systemic TGF-β_1 gene therapy prevents the development of type 1 diabetes. Ad.AH.TGF-β_1 was constructed on an Ad 5 backbone and was deleted of E1 and E3 genes. TGF-β_1 was rendered active by mutating the codons encoding cysteines 224 and 226 into serine codons. In order to test the efficacy of systemic TGF-β_1 gene therapy under stringent conditions, we administered Ad.AH.TGF-β_1 to 7-week old female NOD mice with overt autoimmunity and examined whether the onset of diabetes (hyperglycemia) is delayed. Our data demonstrating the striking impact of systemic TGF-β_1 gene therapy on the development of overt diabetes is shown below.



Immunohistologic analysis of pancreatic samples harvested at 13 weeks from the untreated diabetic NOD mice showed insulitis and destruction of most islets. Infiltrates consisted predominantly of CD4+ T cells that expressed CD25 and showed staining for IFN-g but not for TGF-β_1 or IL-10. By comparison, analysis at 19 weeks of the pancreas of a female NOD mouse that had received Ad.AH.TGF-β_1, showed well-preserved islets and small peri-islet mononuclear cell aggregates. As with the control diabetic mice, peri-islet cells in Ad.AH.TGF-β_1-treated mice consisted largely of CD4+ T cells that showed expression of CD25, but in contrast to the control group, lacked IFN-γ and showed staining for Foxp3 and TGF-β_1. Our observations demonstrating that TGF-β_1 gene therapy induces the emergence of T-regulatory cells capable of constraining overt autoimmunity and retarding the development of type 1 diabetes advances systemic TGF-β_1 gene therapy as a potential treatment for type 1 diabetes.

Abstract# 1147 Poster Board #-Session: P77-III

$CD8^+$ TCR^- FACILITATING CELLS HAVE A DIRECT EFFECT TO ENHANCE HEMATOPOIETIC STEM CELL FUNCTION IN VIVO AND IN VITRO : A NOVEL IN VITRO ASSAY FOR FACILITATING CELLS. Francine Rezzoug,[1] Yiming Huang,[1] Michael K. Tanner,[1] Isabelle J. Fugier-Vivier,[1] Suzanne T. Ildstad.[1] *[1]Institute for Cellular Therapeutics, University of Louisville, Louisville, KY.*

Background: Strategies to engineer a bone marrow cell transplant (BMT) to optimize efficiency but avoid graft versus host disease (GVHD) will significantly expand the use of BMT to chronic disease states including tolerance induction. We, and others, have described a subpopulation of BM cells ($CD8^+TCR^-$) that facilitates long-term engraftment of purified HSC in allogeneic recipients without causing GVHD. Facilitating cells (FC) also, significantly enhance the engraftment of limiting numbers of HSC in syngeneic recipients. We therefore hypothesized that FC may act directly on HSC to improve engraftment.

Methods: In the current study, we evaluated the activity of FC on HSC biology *in vitro* using: 1) colony formation in semi-solid media (CFC) to enumerate progenitors and 2) cobblestone area forming cell (CAFC) assay to quantify early hematopoietic progenitors, reflecting long-term repopulating cells (LTRC). We also assessed the effect of FC on HSC death/apoptosis using AnnexinV and 7-AAD uptake by flow cytometry. Sorted HSC ($Sca\text{-}1^+c\text{-}Kit^+lin^-$) were used fresh, or after 18 or 40 hours incubation in the presence or absence of sorted FC or control T cells (all from C57BL/6 mice).

Results: We found that the FC increased the ability of fresh HSC to generate colonies for both assays CFC, and CAFC suggesting a direct effect of FC on HSC quiescence/differentiation. FC alone do not produce colonies. Additionally, unlike FC, T cells did not augment the number of CFC produced by the HSC. We determined that co-incubation with FC for 18 or 40 hours significantly decreased the percentage of apoptotic HSC (from 31.2 % for HSC alone to 22.1 % for HSC + FC at 18 hours and 16.2 % to 8.8 % at 40 hours; ($P<0.01$). Moreover, FC increase the ability of HSC to generate CFC at these same time points.

Conclusion: These data demonstrate a direct effect of FC on HSC maintenance in long-term culture, balance between quiescence/differentiation, and survival. These functions correlate with *in vivo* facilitating activity as control T cells did not facilitate HSC engraftment or result in the same *in vitro* effects. These assays provide effective tools for studies of human facilitating cells. Through their effects on HSC, FC are a powerful tool in therapeutic applications of stem cell transplantation from curing of disease to regeneration of damaged tissue.

Abstract# 1148 Poster Board #-Session: P78-III

PRESERVATION OF PANCREAS IN PERFLUROCARBON INHIBITS APOPTOSIS IN ISOLATED HUMAN ISLETS. S. Ramachandran,[1] K. Narayanan,[1] N. Benshoff,[1] N. Steward,[1] B. Olack,[1] N. Desai,[1] T. Mohanakumar.[1,2] *[1]Department of Surgery, Washington University School of Medicine, St Louis, MO; [2]Department of Pathology & Immunology, Washington University School of Medicine, St louis, MO.*

Introduction: Islet transplantation is a viable option for the treatment of type I diabetes. Preservation of pancreas in two layer UW/PFC (perfluorochemical) immediately after procurement or after cold storage has been shown to increase the quantity and quality of islets isolated. Apoptosis is the major pathway by which isolated islets undergo cell death after isolation. The objective was to study the effect of PFC on apoptosis induction in isolated islets.

Methods: Islets were isolated from three groups of pancreata with varying preserving conditions. Organss were preserved immediately in UW/PFC (group A), or stored 12 hours in UW followed by storage in UW/PFC (group B) or in UW solution alone (group C). Expression profile of pro-apoptotic and anti-apoptotic genes in isolated islets were analyzed by Apoptotic gene array. The levels of caspase activity were analyzed by BD ApoAlert Caspase profiling plates. Expression of Bcl2, BAD, pSurvivin, cytochrome B, cytochrome C, Apaf1 and AIF1 were analyzed by western blot using specific antibodies.

Results: Pro-apoptotic genes including bad, bax, caspases and the TRAF were down regulated by 2-3 fold in islets isolated from pancreas immediately stored in PFC. Preservation in PFC also induced a 2 fold increased expression of survivin (anti-apoptotic). The levels of activated caspase 2 (2 fold), caspase 3 (13.5 fold) and caspase 8 (complete inhibition) were significantly decreased in islets from pancreas immediately preserved in PFC. Similarly an increased expression of Bcl2 (2.8 fold) and survivin (3.1 fold) was observed in islets stored in PFC. Preservation in PFC even after 12 hours of CIT down regulated the pro-apoptotic genes and inhibited caspase activities in the isolated islets.

Conclusion: Preservation of the pancreas in UW/PFC results in significant up-regulation of anti-apoptotic genes and inhibition of pro-apoptotic genes. Therefore, the reported improvement in islet function following PFC preservation could be attributed to the increased expression of anti-apoptotic genes Bcl2, survivin and decreased expression of proapoptotic genes Bad, bax and caspases.

Abstract# 1149 Poster Board #-Session: P79-III

LOSS OF DONOR ISLET GRAFTS IN DIABETIC NOD MICE WITH A LOW LEVEL OF DONOR CHIMERISM IS MEDIATED BY AUTOIMMUNITY. Baolin Liu,[1] Yisheng Pan,[1] Neal Heuss,[1] David E. R. Sutherland,[1] Bernhard J. Hering,[1] Zhiguang Guo.[1] *[1]Department of Surgery, University of Minnesota, Minneapolis, MN.*

A substantial level of donor lymphocyte chimerism is required to protect donor-specific islet grafts in the mouse model with autoimmune diabetes. Donor islet grafts can be rejected in diabetic NOD mice with a low level of donor chimerism. In this study, we investigated whether islet graft rejection in chimeric NOD mice is mediated by autoimmune or alloimmune response.

Allogeneic BALB/c splenocytes were injected into diabetic NOD mice at day -3, followed by cyclophosphamide at day -1. BALB/c bone marrow and islets were transplanted simultaneously at day 0. Anti-CD154 mAb and rapamycin were given from day 0 to day 14. Mixed chimerism was measured by flow cytometric analysis. Autoimmune response was determined by adoptive transferring splenocytes from chimeric NOD mice that have rejected donor-specific islet grafts into NOD.scid mice. Alloimmune response was tested by MLR and ELISPOT for INF-γ-producing cells in vitro.

Mixed chimerism was achieved in all recipient mice. At 8 weeks post-BMT, the level of donor-derived $CD3^+$ T cells was 11.4±7.9%. After donor specific islet grafts were rejected, $5x10^6$ splenocytes from these chimeric NOD mice with at least over 1% donor $CD3^+$ T cells were adoptively transferred into each NOD.scid mouse. Twelve of 15 NOD.scid mice developed diabetes 12 weeks after adoptive transfer. All 8 NOD.scid mice that received splenocytes from control diabetic NOD mice also developed diabetes. Proliferation of splenocytes from these chimeric NOD mice against donor BALB/c stimulators was markedly reduced, as compared with proliferation of splenocytes from naïve NOD mice ($P<0.01$). However, the response of chimeric mice to third-party C3H/He stimulators was not significantly different from the response of naïve NOD mice. The number of IFN-γ-secreting splenocytes significantly increased in the splenocytes of control NOD mice under the stimulation with BALB/c splenocytes. In contrast, the level of IFN-γ-secreting splenocytes in the chimeric NOD mice did not increase and it was the same as the background level under the stimulation of BALB/c splenocytes ($P<0.01$). The number of IFN-γ-secreting splenocytes in both the chimeric NOD mice and control NOD mice significantly increased when third-party (C3H/He) splenocytes were used as the stimulators, as compared with the naïve splenocytes as the stimulators. These data demonstrate that islet antigen-specific autoreactive T cells exist in chimeric NOD mice and they are responsible for islet graft loss.

Abstract# 1150 Poster Board #-Session: P80-III

RECENT PRECLINICAL STUDIES FOR "PROSPECTIVE PHASE I/II SAFETY STUDY OF ENCAPSULATED ISOLATED PRIMARY HUMAN PANCREATIC ISLET TRANSPLANTATION WITH LOW DOSE SHORT TERM IMMUNE SUPPRESSION IN PATIENTS WITH TYPE I DIABETES MELLITUS". Wen-Ghih Tsang,[1] Carolyn C. Huntenburg,[1] Tianli Zheng,[1] Andrew Schaeffer,[1] Yan Ping Wang,[1] Kermit Lim.[1] *[1]AmCyte Inc., Santa Monica, CA.*

To advance islet transplantation the shortage of organs and the toxicity of chronic immune suppression need to be addressed. While we approach the islet shortage by islet regeneration, islet encapsulation has been applied to eliminate chronic immune suppression. Encapsulation also provides islet stability which allows safety and functional determination prior to their transplantation. It allows accumulation of islets for clinical use. This increases the percentage of organs that benefit patients. An approved clinical trial utilizing encapsulated islets without chronic immune supression has been initiated.

Method: Cultured encapsulated islets were analyzed for up to 12 months. Sterility and mycoplasma were tested for safety. Viability (AO/PI), purity (DTZ staining), SGS (SI) and perifusion were tested for function. To evaluate *in vivo* function and immune protection, encapsulated islets, cultured for 3 months, were transplanted into STZ induced diabetic C57/bl mice (n=9) at 18K IEQ/kg. The BG level and other indications were compared with that of the cohort (n=8) which received empty capsules.

Results: Safety and function of cultured encapsulated islets could be maintained for up to 12 months. Encapsulated islets were able to normalize BG levels of diabetic mice for 3 months without immune suppression. Grafts recovered from hosts (n=4) at 8 weeks post transplantation contained insulin, glucagon and somatostatin positive cells. Retrieved grafts were responsive to glucose challenge by perifusion test. Some cellular overcoat on capsules were observed. Necropsy exams were normal. OGTT at week 3 (n=3), 5 (n=2), and 8 (n=4) were normal. At day 107, the BG levels of all remaining mice (n=5) rose above 244 mg/dL (442±151 mg/dL) indicating cessation of graft function. This suggested that prior BG normalization was not caused by recovery of host endocrine function.

Conclusion: Encapsulated islets offer *in vitro* stability allowing for graft testing prior to transplantation. Immunoprotection afforded by the capsules under the xenograft setting for 3 months was remarkable. The protective nature of the capsule affords a potential benefit of eliminating chronic immune suppression in human islet transplantation.

KIDNEY TRANSPLANT OUTCOMES AND PREDICTORS

Abstract# 1151 Poster Board #-Session: P81-III

SERUM CREATININE IN THE FIRST YEAR POST-TRANSPLANT AS A PREDICTOR OF LONG-TERM RENAL TRANSPLANT OUTCOME IN THE UK. Rachel J. Johnson,[1] Christopher J. Rudge,[1] David Collett,[1] John L. R. Forsythe.[1] *[1]on Behalf of the UK Transplant Kidney and Pancreas Advisory Group, UK Transplant, Bristol, United Kingdom.*

Background: As short-term renal transplant survival continues to improve, with known risk factors having a diminishing effect, attention has been increasingly focused on alternative measures of outcome such as renal function. This study explores the relationship between renal function in the first year post-transplant and long-term kidney transplant survival in the UK.

Methods: Cadaveric heartbeating donor kidney only transplants in adult patients in the UK between 1983 and 1987 were analysed. Transplants failing in the first year were excluded. For 75% of the remaining cohort, both 3 and 12-month serum creatinine measures were recorded on the national transplant database. Transplant survival analysis (death with function counted as a failure) considered the influence of 3 and 12 month serum creatinine; the change between 3 and 12 month serum creatinine (Δ creatinine) and other potential risk factors recorded. Data were available for 2766 transplants with 97% 10-year follow-up including 17% regrafts, which were analysed separately.

Results: Analysis of first transplants showed a highly statistically significant association between ten-year transplant survival and 12-month creatinine, Δ creatinine and an interaction of the two ($p<0.0001$). Unadjusted 10-year transplant survival estimates ranged from 62% (95% confidence interval (CI) 58-65%) for transplants with 12-month serum creatinine <120 µmol/l to 13% (95% CI 9-18%) for those with 12-month creatinine >= 265 µmol/l. There was a negative association between outcome and Δ creatinine, which was greatest for those with poor function at 12-months. Other factors adjusted for were recipient age ($p<0.0001$), blood group ($p<0.001$) and HLA match ($p<0.1$). Univariately, donor age and donor-recipient gender match were significant, but these effects disappeared with the introduction of measures of post-transplant renal function. Analysis of regrafts and of graft (death-censored) survival both showed comparable results. The relevance of findings to more recent data was validated by analysis of 3-year outcome of a recent cohort.

Conclusion: For transplants functioning after the first post-operative year, 12-month serum creatinine and Δ creatinine were highly predictive of 10-year transplant survival. There was a 49% survival difference at 10-years post-transplant between the group with the poorest renal function at one year and the group with the best function.

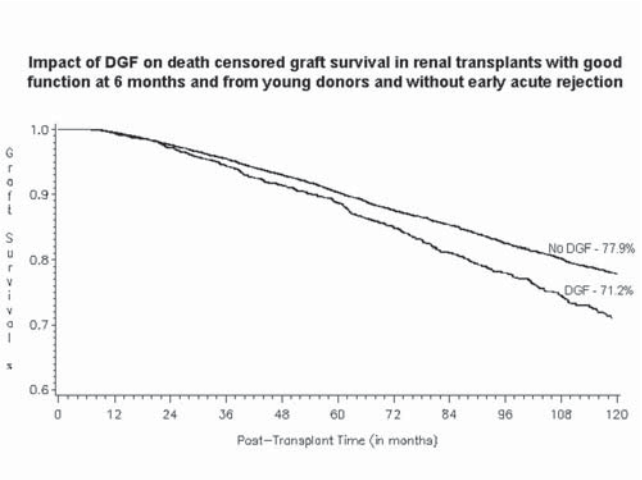
Abstract# 1152 Poster Board #-Session: P82-III

THE IMPACT OF DELAYED GRAFT FUNCTION ON LONG TERM GRAFT SURVIVAL. Herwig-Ulf Meier-Kriesche,[1] Carl J. Cardella,[2] Jesse D. Schold,[1] Alan I. Reed,[1] Ayesha Kaleem,[1] Richard J. Howard,[1] Bruce Kaplan.[1] *[1]University of Florida, Gainesville, FL; [2]University of Toronto, Toronto, ON, Canada.*

The importance of delayed graft function (DGF) kidney transplants (tx) remains controversial. We examined the long-term impact of DGF in recipients with stable function at six months post-tx in the attempt to separate true DGF from those events that are more attributable to technical failures.

We examined 67,725 first solitary deceased donor kidney tx in the SRTR database from 1990-2000. We generated models for overall graft survival and death censored graft survival comparing patients with and without indications for DGF. Subsequently, we examined the impact of DGF in situations in which recipients had stable renal function at 6 months post-tx, with relatively young donor organs, and with no indications of acute rejection during the first week post tx.

Our results indicated that even in rather ideal conditions, for recipients with stable renal function and low-risk donations, incidence of DGF was associated with late graft loss. Death censored graft survival at ten years was 71.2% in the DGF group and 77.9% in tx without DGF (p-value <.0001). This result was confirmed in multivariate analysis correcting for potential confounding variables. On the other hand with shorter follow up, censoring the same cohort at five years, we found no effect of DGF (Relative risk = 1.031 (0.896, 1.185), p-value = 0.6729). Contrariwise if all DGF situations were considered, including early failure, there was a very early detectable difference in graft survival.



In summary, DGF including early failures, most likely secondary to technical problems and rejection, constitutes obviously a strong risk for early graft loss. More importantly even in situations in which technical failures are unlikely, looking at patients who had reasonable renal function at 6 months post tx, DGF was a significant risk factor for graft loss in the long term. 5-year follow up might not be sufficient to elucidate this late effect.

Abstract# 1153 Poster Board #-Session: P83-III

IS EPITOPE MATCHING (EPI M) A BETTER PREDICTOR OF SHORT-TERM RENAL ALLOGRAFT OUTCOME THAN HLA MATCHING (HLA M)? A. Haririan,[1] S. Gruber,[2] H. Daneshvar,[1] K. Morawski,[2] J. ElAmm,[1] D. Granger,[2] M. West,[2] D. Sillix,[1] O. Fagoaga,[3] S. Nehlsen-Cannarella.[3] *[1]Departments of Medicine; [2]Surgery; [3]Pathology, Wayne State University School of Medicine, Detroit, MI.*

HLA molecules have a central role in allorecognition and alloimmune response in organ transplantation (Tx). Although donor/recipient (D/R) HLA M is considered important, it is not uniformly predictive of successful outcome. More specifically, alloresponse depends on recognition of the mismatched EPIs that constitute the donor's class I and II HLA molecules. Thus, we hypothesized that Epi M would correlate better with short-term graft outcome than HLA M. We reviewed the records of 76 patients (Pts) who underwent kidney Tx from 1/02 to 5/03 with follow-up 15±6 mo (all ≥ 6 mo). Data on Pt characteristics, acute rejection (AR), and graft loss (GL) were obtained. HLA typing was performed pre-Tx using molecular methods. D/R Epi M for HLA-A, B, DR, DRw, and DQ was performed using the Duquesnoy HLA MatchMaker program. Linear and logistic regression models were used for statistical analysis. Pt age was 47.7±11.6; 93% were African American (AA); and 59% male. Duration of ESRD was 5.5±3.7 yrs; 71% of grafts were from deceased donors; and 20% were re-Tx. 23 Pts had peak PRA > 10%. 62% received basiliximab and 38% received Thymoglobulin for induction. 34% of Pts had DGF. Maintenance immunosuppression included mycophenolate mofetil, prednisone, and either tacrolimus (FK) (68%) or sirolimus (SRL) (32%). The number of HLA mismatches (MM) was 3.6±1.7. The number of Epi MM for class I, class II, and both combined was 14.8±7.8, 11.2±9.3, and 26.0±13.6, respectively. 19.7% of Pts experienced at least 1 episode of biopsy-proven AR, and GL from death or graft failure occurred in 9.2%. The total number of Epi MM correlated well with the number of HLA MM (r=0.61). We did not find any statistically significant association between the number of Epi MM nor HLA MM and the development of AR or GL. Choice of maintenance agent (FK vs SRL) correlated with AR in some models. Although our results did not reveal a significant association between the number of Epi MM and short-term graft outcome in our high-risk AA kidney Tx population, we cannot refute its potential role as a predictor for long-term graft survival. It has been noted that Epi MM are predictive of the antibody specificities detected after GL and impact on matching with future donors. A larger study with longer follow-up is warranted to explore the influence of humorally-recognized Epi MM on extended graft survival.

Abstract# 1154 Poster Board #-Session: P84-III

POST TRANSPLANT CHANGE IN GFR BETWEEN 6 AND 12 MONTHS ($\Delta GFR_{6\text{-}12}$) CORRELATES WITH LONG-TERM GRAFT FAILURE. Nauman Siddiqi,[1] John S. Gill,[2] Nathan Johnson,[2] Sundaram Hariharan.[1] *[1]Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]Division of Nephrology, University of British Columbia/ St. Paul's Hospital, Vancouver, BC, Canada.*

Background: Improvement in short and long-term graft survival has necessitated the need for early markers of late graft outcome. Post-transplant Serum Creatinine (SCr) at 6 month and 1 year as well as delta SCr has been correlated with long-term graft failure. The current study evaluates the correlation between the change in GFR (calculated using MDRD equation) between 6 and 12 months post transplant ($\Delta GFR_{6\text{-}12}$) and long-term survival.

Methods: A total of 23,522 adult cadaveric renal recipients patients recorded in the USRDS database for 1996-1999 were included for this s tudy. These recipients were categorized according to $\Delta GFR_{6\text{-}12}$: Non-decliners (0), 5, 5.1-10 and > 10ml/min/1.73m^2 respectively. Multivariate regression analysis and Cox proportional hazard modeling were performed after correcting for donor and recipient demographics such as age, gender, and race, recipient cause of ESRD; transplant and post-transplant variables such as HLA match, PRA, CIT, DGF, induction antibody use, type of Calcineurin inhibitor, transplant year and baseline GFR at 6 months.

RESULTS: The projected median graft survival time (half-life) was calculated for various $\Delta GFR_{6\text{-}12}$ are shown in table below.

table 1

ΔGFR6-12	0 (N = 13,819)	1 – 5 (N = 2,316)	5.1 – 10 (N = 3,018)	> 10(N = 4,369)
half-life(95% CI) yrs.	18.8 (16.7, 20.9)	15.3 (13, 17.5)	11.7 (10.2, 13.3)	7.1 (6.3,7.9)

A decline of > 5ml/min/1.73m2 was associated with nearly 40% lower graft survival (p< 0.0001). A similar pattern of lower long-term graft survival was noted when patients were censored for death with a functioning graft.

The hazard ratio for long-term graft failure using Cox proportional hazard model revealed for each 10 ml decline ΔGFR6-12 after correcting for various donor and transplant variables was 1.61 (1.38, 1.67), p < 0.0001

Conclusions: $\Delta GFR_{6\text{-}12}$ correlates with long-term graft failure and a rapid decline in $\Delta GFR_{6\text{-}12}$ would help in separating high-risk patients for further intervention.

Abstract# 1155 Poster Board #-Session: P85-III

LONG-TERM RESULTS OF ELDERLY DONOR KIDNEY TRANSPLANTS. A SINGLE-CENTER EXPERIENCE. Kazunari Tanabe,[1] Tadahiko Tokumoto,[1] Hideki Ishida,[1] Nobuo Ishikawa,[1] Naoshi Miyamoto,[1] Tsunenori Kondo,[1] Hiroaki Shimmura,[1] Kiyoshi Setoguchi,[1] Ichiro Nakajima,[2] Shouhei Fuchinoue,[2] 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: The shortage of cadaveric organs is serious. An expansion of the donor pool not only for cadaveric but also for living donor kidney transplantation is necessary. Marginal donor kidney transplantation using elderly donor or non-heart beating donor has been performed since the beginning of the 1980s. We reviewed the long-term results of elderly donor (more than 60 years old) kidney transplantation and analyzed the risk factors.

Patients and Methods: One thousand, two-hundred and ninety-three adult (age 20<) patients who underwent living renal transplantation between 1983 and 2002 at our institution were included in this study. The patients were divided into two groups according to donor age. Namely, Group 1 included 451 patients more than 60 years old and Group 2 842 patients less than 59 years old. The average age of Groups 1 and 2 was 64.7 and 48.9 years, respectively. Basic immunosuppression was cyclosporine or tacrolimus, azathioprine or mizoribine or mycophenolate mofetil, and steroids.

Results: In Group 1, 5-, 10-, and 15-year patient survival was 95%, 89%, and 81%, respectively and graft survival was 78%, 51%, and 35%, at 5, 10, and 15 years, respectively. In Group 2, 5-, 10-, and 15-year patient survival was 94%, 90%, and 84%, respectively and graft survival 81%, 66%, and 52%, respectively. Both groups showed equivalent patient and graft survival up to 5 years after transplantation. Although Group 1 showed significantly poorer long-term graft survival than Group 2, 10-year graft survival of Group 1 was 51% and was acceptable graft survival compared to that of cadaveric renal transplantation. The time-dependent multivariate non-proportional Cox's hazards model revealed that the hazards ratio of elderly donor grafts was 1.1 (p=0.6), 1.8 (p=0.0003), and 1.4 (p=0.16) within 5 years, 6-10 years, and more than 10 years, respectively.

Conclusion: Although the long-term results of elderly donor renal transplantation were poorer than those of younger donors, there is no difference up to 5 years after transplantation between two groups. Furthermore, 10-year graft survival of elderly donor kidneys was acceptable compared to that of cadaveric renal transplantation. We think that elderly donors can be kidney donors with an acceptable long-term outcome. Obviously, this is a significant advantage compared to dialysis therapy.

Abstract# 1156 Poster Board #-Session: P86-III

KIDNEY TRANSPLANT OUTCOME IN A LARGE SINGLE CENTER HISPANIC POPULATION. Alger Aquino,[1] Sali Aswad,[1] Akshay Mehta,[1] Rafael G. Mendez,[1] Robert Mendez. *[1]National Institute of Transplantation, Los Angeles, CA.*

Introduction:

In recent years, the Hispanic (H) population in the U.S. has increased in that they have now become the largest minority, surpassing African American. Consequently, recent figures show the Hispanic kidney transplant population at now between 29-36%. We report our experience at a large single center with a 43% Hispanic kidney transplant population.

Materials and Methods:

A retrospective review of our kidney transplant population from Jan. 1996- Dec. 2002 shows our experience with transplants in the Hispanic (H) group (n=745) with demographics, graft and patient survival rates compared to Non Hispanic (NH) (n=973) transplant population and recent UNOS published statistics.

Results:

We analyzed 1718 kidney transplant patients from 1996-2002, with the Hispanic population n= 745 (43%) vs. Non Hispanic n= 973 (57%). Of the total transplants in the H group, 95% had kidney transplant alone vs. 89% for NH with 3% simultaneous kidney pancreas for H vs. 9% for NH. (p=<0.01). There was a steady increase in the Hispanic kidney transplant group at 39.49% in 1996 to 50.4% in 2002. We found a significant difference in the greater number of H living donors 34.8% vs. NH 27.4%. (p=0.001). We also found greater prevalence of Diabetes in the H population, as expected, at 30% vs 24% of the NH group. (p=0.0032). The cadaveric graft survival at 5 yrs. for Diabetic H was 68.43% vs. Diabetic NH 61.5%. Living donor graft survival at 5 yrs in the Diabetic H was 78.44% vs. Diabetic NH=61.14% a significant difference. When comparing graft survival, by age group for cadaveric kidney transplants, there was no significant difference between H= 66.65% vs. NH= 66.87%, but slightly greater than that reported by UNOS 63.3%. The greater difference in graft survival rates was seen in the live donor kidney transplants with H population at 82.32% survival vs. NH=72.03%, and also greater than the UNOS graft survival at 76.3%.

Conclusion:

In our experience, Hispanics appear more willing than other race groups to donate a kidney to a family or friend. A far greater transplant survival outcome is seen in the living donor kidney transplants for H diabetics with 78.44% vs NH diabetics with 61.14%. It is our conclusion that not only are Hispanics increasing in numbers as kidney transplant recipients, but also there is an increase in patient and graft survival correlated to this fact.

Abstract# 1157 Poster Board #-Session: P87-III

PREVIOUS TRANSPLANT CHARACTERISTICS PREDICT ALLOGRAFT AND RECIPIENT SURVIVAL. Alex S. Goldfarb-Rumyantzev,[1] Gregory Stoddard,[2] Bradley Baird,[1] Sukil Kim,[3] Lev Barenbaum,[4] Sergei Krikov,[4] Alfred K. Cheung.[1,2] *[1]Medicine/Nephrology, University of Utah, Salt Lake City, UT; [2]VA Salt Lake City Health Care System, Salt Lake City, UT; [3]Medical Informatics, University of Utah, Salt Lake City, UT; [4]RenalService.com, Inc., Salt Lake City, UT.*

The role of parameters of prior transplant (Tx) in the allograft and recipient survival is not well established. Using Cox proportional hazard model we analyzed USRDS records from all patients receiving kidney or kidney-pancreas Tx between 1/1/90 through 12/31/99 with the follow-up period through 12/31/00 (n=92,844, 60% males, 70% white, 23% black) and a subsample of all patients with a history of more than one Tx (n=11,714, 59% males, 78% white, mean=32.7 months between previous graft failure and current Tx). Separate Cox models were fit for time from Tx to graft failure and time from Tx to recipient death. Cox models were adjusted for donor and recipient characteristics (e.g. demographic data, comorbidities) and Tx procedure characteristics. In the dataset of all patients Cox models demonstrated that history of prior Tx increased the risk of graft failure (HR 1.24, p<0.001), but was not significant for recipient death. Total number of Txs increased the risk of graft failure (HR 1.35, p<0.001), but was not significant for recipient death. In the subsample of patients with history of more than one Tx, the total number of Txs increased both the risk of graft failure (HR 1.25, p<0.001) and recipient death (HR 1.09, p=0.026). The time period between the last graft failure and the most recent Tx had no significant association with the graft outcome, but slightly worsened the recipient outcome (HR 1.04, p<0.001). Having Tx as a renal replacement therapy modality immediately prior to the last Tx without dialysis in between posed a greater risk of graft failure (relative to hemodialyis, HR 1.99, p<0.001), but had protective effect on recipient survival (HR 0.74, p=0.005). Previous donor type did not significantly affect the graft or the patient outcome. In conclusion, the history of prior Tx and the number of Txs increased the risk of graft failure, but not the recipient death (except in the subsample of patients with >1 Txs, where total number of Txs increased the risk of mortality); the time period between the last graft failure and the most recent Tx was not associated with the graft outcome, but worsened the recipient outcome. While preemptive first Tx seems to increase graft and recipient survival, consecutive Tx without dialysis in between appears to increase the risk of allograft failure.

Abstract# 1158 Poster Board #-Session: P88-III

OUTCOME OF RENAL TRANSPLANTATION IN ELDERLY RECIPIENTS. Jeffrey Rogers,[1] Sylvia Odom,[1] Muriel Labonte,[1] Greg Gilbert,[1] Angello Lin,[1] Kenneth Chavin,[1] Prabhakar Baliga,[1] Osemwegie Emovon,[1] Fuad Afzal,[1] Elizabeth Ashcraft,[2] G. Mark Baillie,[2] David Taber,[2] Ruy Marques,[1] P. R. Rajagopalan.[1] *[1]Division of Transplant Surgery, Medical University of South Carolina, Charleston, SC; [2]Department of Pharmacy Services, Medical University of South Carolina, Charleston, SC.*

Background: The aging population has resulted in an increasing number of elderly kidney transplant (KT) recipients. Elucidating the outcome of KT in the elderly will be essential to optimize recipient selection and management as well as choice of donor organ. Aim: To determine outcome of KT in elderly patients. **Methods**: Retrospective review of 269 cadaver (CAD) and live donor (LD) kidney transplants performed at our institution 7/1/99 – 10/15/01. **Results**: Recipients were divided into 2 groups: patients ≥60 years old (yo) and patients < 60 yo. Mean age of patients ≥ 60 yo and < 60 yo was 65 ± 4 years and 42 ± 11 years, respectively, p<0.0001. Groups were well matched for recipient age, gender, ethnicity, HLA mismatch, PRA, maintenance immunosuppression regimen, use of antibody induction, LD or CAD, and donor age. Patients ≥ 60 yo had more diabetic nephropathy (40% ≥ 60 yo vs. 19% < 60 yo, p=0.007). 1-and 4-year cumulative rejection (AR) incidence was 28%, 35% in ≥ 60 yo and 43%, 53% in < 60 yo, respectively, p=0.02. Actuarial patient and death-censored graft survival appear below.

Kaplan-Meier Patient and Death-Censored Graft Survival

	Patient		Graft	
	1-year	4-years	1-year	4-years
≥ 60 yo	87%	72%	95%	95%
< 60 yo	96%	87%	91%	78%
	p=0.0018		p=0.07	

Grade of rejection episodes tended to be less severe in patients ≥ 60 yo. The most common cause of graft failure in patients ≥ 60 yo was death with functioning graft (DWFG, 85%), whereas the most common cause of graft failure in patients < 60 yo was chronic allograft nephropathy (39%). Distribution of the causes of recipient death was similar between age groups. **Conclusions:** Elderly KT recipients have less rejection and similar long-term graft survival compared to younger patients, possibly reflecting inherently lower immune reactivity in these patients. DWFG accounts for the vast majority of graft losses in this group of patients. Development of comprehensive algorithms for recipient risk stratification, appropriate selection of type and source of donor organ, and immunosuppression strategies in the elderly patient is necessary, particularly given the limited availability of cadaver kidneys.

Abstract# 1159 Poster Board #-Session: P89-III

ARE PRE-TRANSPLANT SENSITISED KIDNEY RECIPIENTS AT HIGHER RISK OF TRANSPLANT FAILURE? Iren Szeki, Susanne Mills, Faieza Qasim, Phil Dyer. *Manchester Institute of Nephrology and Transplantation, Royal Infirmary, Manchester, United Kingdom.*

There is a general presumption that highly sensitised renal transplant recipients are at increased risk of transplant failure. In our centre we practice efficient pre-transplant antibody screening and allocate deceased donor kidneys to minimise HLA mismatches. As a result, we hypothesise that transplant survival should not differ between highly sensitised and non-sensitised recipients. This cohort study aimed to investigate patient and graft survival, acute rejection and renal function in highly sensitised recipients of deceased donor kidneys.

Methods: Between 1998 and 2002, 15.7% of 535 adult recipients were sensitised pre-transplant. Four recipients were transplanted twice and 18 had a pre-transplant HLA specific antibody reaction frequency of >85% in CDC, FC and ELISA screening assays thus fulfilling the UK Transplant definition of highly sensitised (HSP) recipients. All transplants were done in the absence of donor reactive, clinically relevant antibodies (negative crossmatches). Patient records and histology reports were retrospectively reviewed. The follow-up period ranged from 14-60 months. Kidneys were allocated acoording to UK Transplant criteria, which aims to minimimse HLA mismatches. 64% of patients were initiated on triple therapy [csa/tac+aza/mmf+pred], 23% on double therapy [csa/tac+pred/mmf] and 12% received monotherapy with csa or tac.

Results: Data was complete on 94% of 84 patients. Partial data was available on 4 of the remaining 5 patients. The 1 year graft survival was 83.6% (expected 87-92%). The mean serum creatinine at 1 year was 163 µmol/l (see table 1). Over the five year study period 21.5% of grafts were lost (see table 2). 37 patients had rejection episodes. 34 patients had 1 rejection episode and 3 patients had 2 rejection episodes. Of these we found 16 vascular, 10 cellular, 8 combined vascular and cellular and 3 clinical (biopsy not undertaken or biopsy inadequate for diagnosis) rejections. Delayed function, defined as the need for dialysis within the first post transplant week, occurred in 30 (37.9%) transplants (expected 34%).

Conclusion: We find that efficient, clinically relevant antibody screening and allocation to minimise HLA mismatching lead to graft loss and rejection rates which are similar to those observed in non-sensitised recipients.

table 2

Death with function	Renal vein thrombosis	Rejection
5	3	9 (2 due to non compliance)

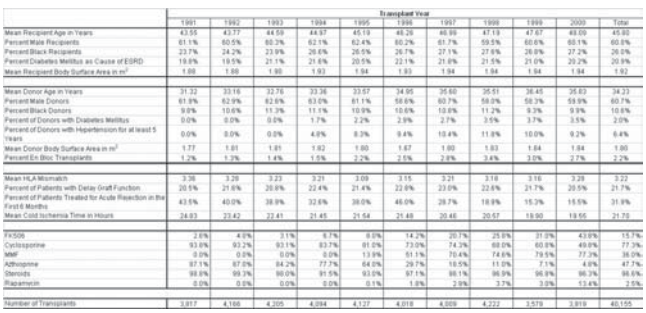
table 1

serum creatinine µmols/l at 1 year	Died	Failed	<100	101-150	151-200	>201
% recipients	2.4	14	2.4	40	30.6	10.6

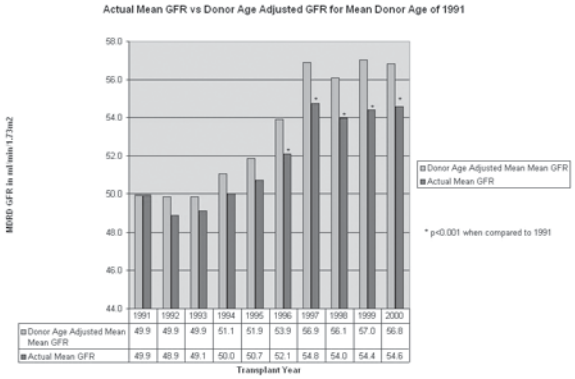
Abstract# 1160 Poster Board #-Session: P90-III

FACTORS RESPONSIBLE FOR IMPROVEMENT IN GRAFT FUNCTION SEEN IN THE 1990S, ANALYSIS OF OPTN DATA. Douglas S. Keith,[1] Angelo deMattos,[1] Muralikrishna Golconda,[1] Jonathan Prather,[1] Douglas Norman.[1] *[1]Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, OR.*

Previous analysis of OPTN data has shown that cadaveric renal transplant function at six months post-transplant has improved over decade of the 1990's (figure 2). Using OPTN data, 40,155 cadaveric renal transplants between 1991 and 2000 with graft survival of at least six months were idenfied that had complete data for HLA mismatch, donor and recipient age, sex and race, delayed graft function, clinical rejection in the first six months, and creatinine data at six months. Figure 1 shows the trends in donors and recipient characteristics as well as trends in maintenence immunosuppression, HLA mismatch, acute rejection and delay graft function over the ten year span. Linear regression analysis of six month post-transplant MDRD GFR was preformed using variables shown in figure 1 to determine the impact of each variable on graft function. The reduction in the clinical acute rejection rate accounted for only 19.8% of the improvement in mean GFR over the decade. MMF use accounted for 24.4% and FK506 use accounted for15.1% of the improvement while 37.2% of improvement was due to unidentified factors. The improvement in GFR associated with MMF and FK506 was seen after accounting for clinical rejection suggesting other benefits such as a reduction in subclinical rejection or nephrotoxicity accounts for their beneficial effect on graft function.



	Transplant Year										
	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	Total
Mean Recipient Age in Years	43.55	43.77	44.59	44.97	45.19	46.26	46.99	47.19	47.67	48.00	45.80
Percent Male Recipients	61.1%	60.5%	60.3%	62.1%	62.4%	60.2%	61.7%	59.5%	60.6%	60.1%	60.8%
Percent Black Recipients	23.7%	24.2%	23.9%	26.6%	26.5%	26.7%	27.1%	27.6%	26.0%	27.2%	26.0%
Percent Diabetes Mellitus as Cause of ESRD	19.8%	19.5%	21.1%	21.6%	20.5%	22.1%	21.6%	21.5%	21.0%	20.2%	20.9%
Mean Recipient Body Surface Area in m^2	1.88	1.88	1.90	1.93	1.94	1.93	1.94	1.94	1.94	1.94	1.92
Mean Donor Age in Years	31.32	33.16	32.76	33.36	33.57	34.95	35.60	35.51	36.45	35.63	34.23
Percent Male Donors	61.8%	62.9%	62.6%	63.0%	61.1%	58.6%	60.7%	58.0%	58.2%	59.9%	60.7%
Percent Black Donors	9.8%	10.6%	11.3%	11.1%	10.9%	10.6%	10.8%	11.2%	9.3%	9.9%	10.6%
Percent of Donors with Diabetes Mellitus	0.0%	0.0%	0.0%	1.7%	2.2%	2.9%	2.7%	3.5%	3.7%	3.5%	2.0%
Percent of Donors with Hypertension for at least 5 Years	0.0%	0.0%	0.0%	4.0%	8.3%	9.4%	10.4%	11.8%	10.0%	9.2%	6.4%
Mean Donor Body Surface Area in m^2	1.77	1.81	1.81	1.82	1.80	1.87	1.90	1.93	1.84	1.84	1.80
Percent En Bloc Transplants	1.2%	1.3%	1.4%	1.5%	2.2%	2.5%	2.8%	3.4%	3.0%	2.7%	2.2%
Mean HLA Mismatch	3.38	3.20	3.23	3.21	3.09	3.15	3.21	3.18	3.16	3.28	3.22
Percent of Patients with Delay Graft Function	20.5%	21.6%	20.8%	22.4%	21.4%	22.8%	23.0%	22.8%	21.7%	20.5%	21.7%
Percent of Patients Treated for Acute Rejection in the First 6 Months	43.5%	40.0%	38.9%	32.6%	36.0%	46.0%	39.7%	18.9%	15.2%	15.5%	31.8%
Mean Cold Ischemia Time in Hours	24.03	23.42	22.41	21.45	21.54	21.48	20.46	20.67	19.90	19.55	21.70
FK506	2.6%	4.0%	3.1%	6.7%	8.0%	14.2%	20.7%	25.8%	31.0%	43.0%	15.7%
Cyclosporine	93.0%	93.2%	93.1%	83.7%	81.0%	73.0%	74.3%	69.0%	60.8%	49.9%	77.3%
MMF	0.0%	0.0%	0.0%	0.0%	13.9%	51.1%	70.4%	74.6%	79.5%	77.3%	36.9%
Azthioprine	87.1%	87.0%	84.2%	77.7%	64.0%	29.7%	10.5%	11.0%	7.1%	4.8%	47.7%
Steroids	98.8%	99.3%	99.0%	91.5%	93.0%	97.1%	98.1%	96.9%	96.9%	96.3%	96.6%
Rapamycin	0.0%	0.0%	0.0%	0.0%	0.1%	1.9%	2.9%	3.7%	3.0%	13.4%	2.5%
Number of Transplants	3,817	4,166	4,205	4,094	4,127	4,018	4,009	4,222	3,579	3,819	40,155



Abstract# 1161 Poster Board #-Session: P91-III

DETERMINANTS OF KIDNEY GRAFT FUNCTION DURING THE FIRST TWO YEARS POST-TRANSPLANT. Fernando G. Cosio,[1] Matthew D. Griffin,[1] Thomas R. Schwab,[1] James M. Gloor,[1] Jorge A. Velosa,[1] Stephen Textor,[1] Mark S. Stegall,[2] Timothy S. Larson.[1] *[1]Division of Nephrology, Mayo Clinic, Rochester, MN; [2]Division of Transplant Surgery, Mayo Clinic, Rochester, MN.*

Kidney function is a significant correlate of long term graft survival. In this study we sought to determine the variables that relate to the glomerular filtration rate (GFR) post-transplant. GFR was measured by iothalamate clearance and was not corrected for body surface area in an attempt to minimize the contribution of changes in BMI, early post-transplant, on GFR. GFR was measured at 3 weeks, 1 and 2 years post-transplant in 407 patients transplanted between 1/98 and 9/01 and including 337 living (LD) and 70 deceased (DD) donors. Patients with delayed graft function were excluded from the analysis. At 3 weeks post-transplant, GFR was significantly higher in LD than in DD recipients (Table); and also in younger donors (p=.001); and younger recipients (p=.04, all multivariate). The difference between LD and DD in GFR_{3wks} increased with increasing donor age: donor <30 years: GFR_{LD} 69±21 and GFR_{DD} 61±13, p=.06; donor >45 years: GFR_{LD} 59±20 and GFR_{DD} 48±18, p=.01. At 1 year post-transplant, GFR was still significantly related to donor age (r=.354, p<.0001) but the difference between GFR_{LD} and GFR_{DD} was no longer significant (Table). This was due to an average increase in GFR_{DD} (p<.001) during year 1 with no significant change in GFR_{LD}. During this period grafts from younger donors loss less GFR than those from older donors (p=.001). Other variables tested such as acute rejection and BK nephropathy did not correlate with the loss of GFR. At year 2, GFR was still significantly related to donor age (r=.416, p<.0001) and GFR was not significantly different in LD and DD (Table). Among patients without rejection or BK nephropathy (N=323) 20% loss GFR (>20%) during year 1 and these patients had reduced graft survival (Log rank, p=.003). During year 2, 18% of patients lost GFR (>20%) and had reduced graft survival (p=.0001). Conclusions: 1) donor age relates to GFR throughout the first two years post-transplant; 2) compared to younger donors, older donors lose more GFR during year 1 post-transplant; 3) relatively small declines in GFR, measured precisely, relate with poor graft survival; 4) losses in GFR during years 1 and/or 2 are most often unrelated to clinically recognized events such as acute rejection.

Time	LD	DD	p
3 wks	63+20	55+16	.003
1 year	62+22	65+25	NS
2 years	61+24	63+26	NS

Abstract# 1162 Poster Board #-Session: P92-III

FUNCTIONAL CAPACITY CORRELATES WITH QUALITY OF LIFE IN RECENT AND LONG-TERM TRANSPLANT RECIPIENTS. Patricia A. Cowan,[1,2] Donna K. Hathaway,[1,2] Ann K. Cashion,[1] Gayle Gower,[1] A. Osama Gaber.[2] *[1]College of Nursing, University of TN Health Science Center, Memphis, TN; [2]Transplant Surgery, University of TN Health Science Center, Memphis, TN.*

Purpose: Good short-term quality of life (QoL) has been reported in transplant recipients and has been linked to functional capacity. It is unclear whether long-term transplant recipients continue to experience good QoL and how QoL compares to the general population. This study examined the relationship between functional capaity and QoL in recent and long-term kidney (K) and kidney-pancreas (KP) recipients.

Methods: Transplant recipients(n=61, 48% female, 69% African-American, 13% KP, 0.25 to 19 yrs post-transplant) completed a maximal treadmill exercise test with measured (VO_2peak) and calculated (METS) functional capacity and the SF-36 QoL questionnaire. SF-36 subscale and composite scores were standardized to a 0-100 scale and normalized for comparison to the US general population. Higher values reflect better QoL. Data were analyzed using Pearson correlations and ANOVA.

Results: VO_2peak was 55% of age-predicted values. QoL scores on the SF-36 were within 1 standard deviation of values reported by the general US population. Recipient age and time post-transplant did not correlate with QoL or functional capacity measures. Functional capacity was significantly associated with better SF-36 subscales of physical functioning (r=0.60), physical role (r=0.63), general health (r=0.32), vitality (r=0.42), social functioning (r=0.41), emotional role (r=0.48), mental health (r=0.35), and composite physical (r=0.53) and mental (r=0.38) scales (p<0.05). K and KP recipients had similar functional capacity (measured VO_2peak: 16.95 vs. 16.99, calculated METS: 6.5 vs. 6.6; p=NS) and QoL scores (P=NS). Men had higher functional capacity then women and reported less body pain (70.9 vs. 55.9, P<0.01) and better physical QoL (47 vs. 42, p<0.05).

Conclusion/Implications: Functional capacity remains below age-predicted values regardless of time post-transplant. Interventions aimed at improving functional capacity are needed in both recent and long-term transplant recipients regardless of transplant type. Efforts to improve functional capacity may aid in improvements in post-transplant QoL.

Abstract# 1163 Poster Board #-Session: P93-III

RENAL ALLOGRAFT OUTCOME FOR MAGHREBIAN VERSUS CAUCASIAN RECIPIENTS. Marc Hazzan,[1] Maxime Hoffmann,[1] Francois Provot,[1] Francois Glowacki,[1] Francois-Rene Pruvot,[2] Christian Noel.[1] *[1]Clinique de Nephrologie, CHRU de Lille, Lille, 59 (Nord), France; [2]Unite de Transplantation, CHRU de Lille, Lille, 59 (Nord), France.*

The effect of race on kidney graft outcome remains debated. However, while a large number of studies deals with black recipients, only 2 reports are published about Maghrebian recipients (MR) showing poor graft survival rates compared to Caucasian recipients (CR).

We retrospectively compared 95 consecutive MR to 1013 CR, who received a kidney transplant between 1986 and 2002, in our center.

The pre-transplant characteristics were similar in these 2 groups except for the number of mismatches HLA-B (MR : 1.4±0.7 versus CR: 1.2±0.7, p=0.029) and the causes of end stage renal failure, especially for diabetes (MR: 10.5% versus CR: 2.9%) and polykystosis (MR: 1.1% versus CR: 13.2%, p<0.001).

In 1986, all these patients received the same sequential immunosuppressive regimen: ATG induction, steroids, azathioprine and delayed introduction of cyclosporine. After 1997 new immunosuppressive drugs were introduced in our protocol: mycophenolate mofetil replaced azathioprine, anti-IL2R monoclonal antibodies replaced ATG and tacrolimus replaced cyclosporine. However, the incidence of MR did not change significantly between 1986 and 2002.

The incidence of delayed graft function, CMV diseases and acute rejection were comparable for MR and CR (respectively 21.1% versus 19.2%, 21.1% versus 29.1% and 28.4% versus 38.4%, NS).

At 5 years, the graft survival rates were 65% and 68% (NS) and the patient survival rates were 91% and 92% (NS) for MR and CR respectively. Actuarial survival curves for the recent era (1997-2002) showed 5 years graft survival rates at 73% and 76% (NS) and patient survival rates at 95 and 96% (NS) for MR and CR respectively.

Conversely to the previous reports, this retrospective study, with a larger number of patients, concludes that the results of kidney transplantation are equivalent for MR and CR.

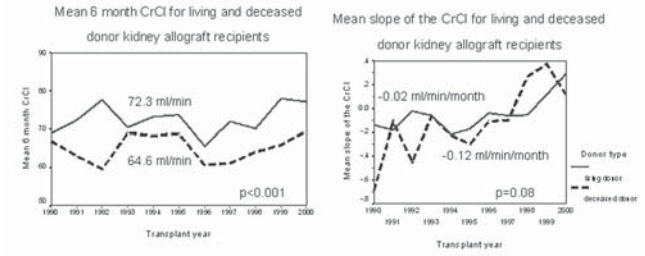
Abstract# 1164

Abstract has been withdrawn.

Abstract# 1165 Poster Board #-Session: P95-III

THE LIVING DONOR ADVANTAGE: BETTER GLOMERULAR FILTRATION RATE BUT NOT BETTER LONG TERM STABILITY (SLOPE). Sita Gourishankar,[1] Lawrence G. Hunsicker,[2] Philip F. Halloran.[1] *[1]Medicine, University of Alberta, Edmonton, AB, Canada; [2]Medicine, University of Iowa, Iowa City, IA.*

We have previously shown that the slope of the estimated creatinine clearance (GFR) in deceased donor kidney transplants (DDT) improved in recent years (JASN 14:2387,2003). In the present study we compared function in 191 live donor transplants (LDT) to 429 DDT transplanted between 1990 and 2000. **Results:** 6mGFR (ml/min) was 72.3 ± 1.4 in LDT vs 64.6 ± 1.1 in DDT (p<.001). Slope (ml/min/m) was -0.02 ± .04 in LDT vs -0.12 ± .04 in DDT (p=0.08). Rejection rates were similar in LDT vs DDT: proven or suspected 30% vs 33%; severe rejection 9% vs 13%. In multivariate analysis, higher 6mGFR in LDT correlated with younger donor age and male recipients. In DDT, higher 6mGFR correlated with younger donor and recipient age, male recipients or donors, no rejection, and no previous grafts. The slope of GFR for LDT improved (less negative) only with more recent transplant year (p=0.002). The slope of GFR for DDT was better in male recipients, lower blood pressure, absence of rejection and more recent transplant year. Multivariate analysis of the entire population (n=620) showed that better 6mGFR correlated with LDT vs DDT, no rejection, younger donor and recipient age, male donor or recipient, higher donor GFR, no previous grafts, and immediate function. A less negative slope was determined by male recipient, absence of severe rejection, older recipient age, later transplant year, and lower blood pressure, but was not better in LDT. **Conclusions:** 1. A major advantage of LDT is higher GFR post-transplant, perhaps due to lack of brain death. The similar rates of rejection/suspect rejection in LDT and DDT suggest that the advantage of LDT is primarily non-immunologic; 2. The slope (stability) of GFR is similar for LDT and DDT, and has improved for both in recent years; 3. Rejection strongly determines 6m GFR and increases the slope of GFR in DDT and in the combined population



Abstract# 1166 Poster Board #-Session: P96-III

CHARACTERISTICS OF RENAL TRANSPLANT RECIPIENTS WITH GOOD LONGTERM RENAL FUNCTION. Jeremy Chapman,[1] Andreas Bock,[2] Bertrand Dussol, Lutz Fritsche, John Jeffery, Volker Kliem, Yvon Lebranchu, Federico Oppenheimer, Erich Pohanka, Maurizio Salvadori, Gunnar Tufveson. *[1]Renal Medicine, Westmead Hospital, Westmead, NSW 2145, Australia; [2]for the MOST Study Group.*

Background: Excellent graft function for many years is a desirable goal for renal transplant recipients (RTR). The aim of this analysis was to describe characteristics of RTR who preserved excellent graft function 5 or 10 years post transplantation (Tx).

Methods: MOST is a global observational study in transplant recipients with functioning grafts, receiving cyclosporine microemulsion (CsA-ME) based regimens. The actual data export included 19,499 RTR in 38 countries. Amongst these, 951 evaluable patients had a serum creatinine (SCr) < 130umol/L 5 years post transplantation (5y-group), and 324 at 10 years post Tx (10y-group). Their demographics were compared to 552 patients with a five-year SCr of >= 170umol/L (high-SCr-group) and 1362 patients transplanted 2000-2003 (recent Tx) from the same regions (Europe, Australia, Middle East, Canada). IS-regimen and CsA-ME dose were assessed in the 5y-group and the high-Scr-group only. Results are expressed as median, or as %.

Results: Patients in the 10y-group and 5y-group received grafts from younger donors compared to the high SCr- and recent Tx-patients (donor age 31 or 36 vs. 50 or 46 years), were more likely to be females (43 or 46 vs. 23 or 35%), and showed a lower serum creatinine and higher GFR at any timepoint post Tx. Other parameters, such as recipient age, second grafts, immunized patients, living donation, sex of donor, or CIT were similar or even less favorable in the 5- and 10y-groups compared to both the high SCr- and recent Tx-patients.

The immunosuppressive regimen used at one year post Tx by the 5y-group and by the high SCr-group was a triple regimen for 67% vs 69% (37% and 38% with MMF), a dual regimen (CsA-ME and steroids) in 23% vs 23% and a steroid-free regimen in 10% vs

7%. Five years post Tx, 46% vs 52% still received a triple regimen with either Aza or MMF, 25% vs 25% a dual regimen and 27% vs 19% a steroidfree regimen. The CsA-ME dosages were similar in both groups at one year (3.3 vs 3.3 mg/kg/d) and at 5 years post Tx (2.6 vs 2.6mg/kg/d).
Conclusion: Excellent long-term graft function was achieved with various CsA-ME-based immunosuppressive regimen. The comparison to high-SCr or recent-Tx patients showed marked differences in donor age and recipient gender. Interestingly, other factors such as living donation, CIT or Neoral dosage appeared not to contribute to good long-term graft function.

Abstract# 1167 Poster Board #-Session: P97-III

LIMITATIONS OF CREATININE CLEARANCE EQUATIONS IN RENAL TRANSPLANT PATIENTS. Ahmed Shoker,[1] Dharmapaul L. Raju,[1] Vaneeta K. Grover.[2] *[1]Medicine, University of Saskatchewan, Saskatoon, SK, Canada; [2]Community Health & Epidemiology, University of Saskatchewan, Saskatoon, SK, Canada.*

Background: Cockcroft-Gault Formula, MDRD-1 and 2, Jellife-1 and 2, Mawer, Bjornsson, Gates and Salazar are nine equations available to measure Glomerular Filtration Rate (GFR) in patients with native renal dysfunction.
Aim: To determine the validity of the above equations in renal transplant recipients with different GFR values measured by standard DPDT isotope scans as a reference test for GFR and in patients with different weight.
Methods: Ninety GFRs were performed on 65 renal transplant patients with a range of GFR measured by isotope scan between 11 mL/min-129 mL/min using three point concentration of the isotope. Accuracy of each method was measured at different percentiles. The bias and precision of all the methods were then compared. A paired t-test was used to compare the performance of each calculation to the respective GFR measured by isotope study done on the same day. SPSS was used for the statistical analysis. Significance was measured at 5%.
Results: For patients with GFR less than 50 mL/min, Jellife-1 and 2, Gates and MDRD-2 were not statistically significant from the isotope GFR. All other methods were statistically different from the isotope result. For patients with GFR more than 50 mL/min, Salazar, Cockcroft-Gault Formula and 24-hour urinary creatinine clearance were not statistically different from the isotope GFR. All of the other calculated methods were different. For patients with body mass index (BMI) less than 25 kg/m², Bjornsson, Salazar, Cockcroft-Gault Formula and Mawer were not statistically different from the isotope GFR. In patients with BMI from 25-30 kg/m², Bjornsson, Salazar, 24-hour urinary creatinine clearance and Cockcroft-Gault Formula were not statistically different from the isotope GFR. In patients with BMI of 30 kg/m², Salazar and 24-hour urinary creatinine clearance were not statistically different from the isotope GFR. The rest of the equations were statistically different, however.
Conclusion: The Salazar Formula and Cockcroft-Gault Formula had least bias (1.8 and 2.26). Our results demonstrate the inherited limitation in the currently available equations to calculate creatinine clearance in patients with different weights or GFRs. Cockcroft-Gault formula is the best available method except for patients with BMI above 30 kg/min². The Salazar method is suitable for patients with different BMI provided GFR is > 50 mL/min.

Abstract# 1168 Poster Board #-Session: P98-III

RECURRENCE OF IgA NEPHROPATHY AND HENOCH – SCHÖNLEIN PURPURA NEPHRITIS AFTER KIDNEY TRANSPLANTATION: MAIN RISK FACTORS. Mª Josep Soler,[1] Josep Mª Puig,[1] Marisa Mir,[1] M. Antonia Orfila,[1] Eva Rodriguez,[1] Mª Asuncion Munne,[2] Susana Vazquez,[1] Josep Lloveras.[1] *[1]Nephrology Department, Hospital del Mar, Barcelona, Spain; [2]Pathology Department, Hospital del Mar, Barcelona, Spain.*

Recurrence of IgA nephropathy (IgA) and Schönlein-Henoch purpura nephritis (H-SP) is frequent after renal transplantation (RT). **Purpose:** To identify the possible risk factors for developing recurrence of IgA and S-HP after RT. **Patients and methods:** Between January 1980 and April 2003, 518 RT were performed at our center. 31 RT in 29 patients who had IgA as original disease and 6 RT in 4 patients who had H-SP. 32 grafts (86.5%) were of cadaveric origin and 5(13.5%) were living-related donors. 8(21,6%) patients showed recurrence of their original renal disease. We analysed the following variables: mean time from diagnosis of disease to onset of dialysis program, mean time on dialysis, cigarette smoking, recipient age at RT, gender, donor age, and type (cadaveric or living-related), HLA (A1, B35, DR4) in donor and recipient, number of match, cold ischemia time, creatinine clearance, proteinuria, dislipemia, hypertension and the number of antihypertensive drugs after RT, CMV infection, HCV status, acute rejection episodes, withdrawal of steroids and the mean time from RT to withdrawal, graft function and the mean time from RT to loss of graft function. Statistical analyses: Univariate analysis: "U" Mann-Whitney for quantitative parameters and $\chi 2$ test for qualitative parameters. **Results:** Analyzed variables with significative risk for recurrence:

Variables	Recurrence	Non recurrence	P
Male	4 (12.9%)	27 (87.1%)	0,013
Female	4 (66.7%)	2 (33.3%)	0,013
Nº of Match	3 (2-6)	2 (0-5)	0,029

Conclusions: In our experience, females are significantly more prone to develop recurrence of the IgA and S-HP disease. Also a good match could enhance the likelihood of IgA and S-HP relapse in the graft. We didn't found any association between recurrence and living-related donors nor between recurrence and donor or recipient HLA.

Abstract# 1169 Poster Board #-Session: P99-III

OUTCOME OF RENAL TRANSPLANTATION IN PATIENTS WITH AN ILEAL CONDUIT. Molly M. P. Eng,[1] Richard E. Power,[1] Saad Al-Dousari,[1] David P. Hickey,[1] Denis M. Murphy,[1] Dilly M. Little.[1] *[1]Transplantation and Urology, Beaumont Hospital, Dublin, Dublin, Ireland.*

Introduction and Objective: Controversy exists regarding the efficacy of renal transplantation into recipients with ileal conduits. We retrospectively reviewed our experience with renal transplantation into ileal conduits over a 17-year period. **Methods:** Between Janueary 1986 and December 2002, we performed a total of 2055 renal transplants. Thirteen grafts were implanted into 13 recipients who had an ileal conduit diversion. There were 7 male and 6 female patients, mean age 35.8 years (range 4-63 years). The indications for ileal loop diversion were: neuropathic bladder associated with spina bifida (n=%); total tractectomy for tuberculosis (n=3); atrophic bladder (n=2); prune belly syndrome (n=1); bladder extrophy (n=1); radical cystectomy for transitional cell carcinoma (n=1). All ureters were implanted into the ileal loop using a Bricker technique over an 8F infant feeding tube. Twelve patients were on haemodialysis at the time of surgery and 1 patient received pre-emptive graft. All patients were immunosupressed with triple induction therapy with the exception of the paediatric patients (n=3) who received quadruple induction immunosupression with ATG. Results: Three patients have died at 5, 5 and 39 months post transplant. The cause of death was fungal sepsis, miliary TB and myocardial infarction, respectively. One patient died with a functioning graft. Of the 10 surviving patients all have a functioning graft with a mean follow up of 7 years (range 17-198 months). The mean serum creatinine of the 10 functioning grafts is 111.8mmol/l (+/- 25.9mmol/l). **Conclusion:** Given improvements in immunosupression, surgical technique, infection treatment and selection criteria we believe that renal transplantation in the patient with an ileal conduit yields excellent graft and patient survival.

Abstract# 1170 Poster Board #-Session: P100-III

PERFORMANCE OF TWO SERUM CREATININE BASED FORMULAS AND 24 HOUR CREATININE CLEARANCE IN KIDNEY TRANSPLANT RECIPIENTS WITH EXCELLENT GRAFT FUNCTION. Hassan Ibrahim,[1] Ziad Zoghby,[1] Furqan Raja,[1] Richard Spong,[1] Will Thomas,[1] Arthur Matas,[1] Michael Mauer.[1] *[1]Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]Department of Surgery, University of Minnesota, Minneapolis, MN; [3]Department of Pediatrics, University of Minnesota, Minneapolis, MN.*

Background: Serum creatinine and formulas based on it continue to be the most used measures of kidney function. The limitations of serum creatinine and the formulas are well recognized but the alternative; measuring glomerular filtration rate(GFR) is both time consuming and expensive. Creatinine based formulas have been validated in many populations. Their use in transplant recipients is common and clinical decisions are often made based on the estimate of GFR yielded from these formulas.
Purpose: To assess the performance of the Cockcroft-Gault, the MDRD number 7 and the 24 hour creatinine clearance methods in renal transplant recipients with excellent kidney function in the early post transplant period.
Methods: 33 renal transplant recipients underwent iothalamate GFR measurements at our General Clinical Research Center one month after their transplant. Four timed urine and plasma collections were done. GFR was the average of at least 3 periods.
Results: 64% were males, 30% received cadaveric grafts, 42% were diabetics and the mean blood pressure was 131±72. The mean GFR was 55±14ml/min, the estimated GFR from CG was 64±16, 59±13 from the MDRD formula and 59±22 from the 24 hour CrCl. The bias and accuracy of these methods is shown in table.
Conclusion: The MDRD formula performed best in this population. The largest bias was observed with the utilization of the Cockcroft-Gault formula.

Bias and Accuracy of creatinine based formulas

Method	Bias	% within 10mls/min of iothalamate GFR
Cockcroft-Gault	9.1	57%
MDRD formula 7	4.2	70%
24 h CrCl	3.7	45%

n=33, CV for iothalamate GFR < 10%

Abstract# 1171 Poster Board #-Session: P101-III

CALCIUM LEVELS AS A RISK FACTOR FOR DELAYED GRAFT FUNCTION. Henk Boom,[1] Marko J. K. Mallat,[1] Johan W. de Fijter,[1] Jan A. Bruyn,[2] Leendert A. van Es,[1] Leendert C. Paul.[1] *[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Background: Delayed graft function (DGF) occurs in upto 50% of renal transplants. Hyper-calcemia and hyperparathyroidism are associated with impaired renal function. Little is known on the effects of serum calcium levels on delayed graft function. This issue was addressed in the current study.

Methods: Patients receiving a cadaveric renal transplant between 1986 -1996, were studied. Data on calcium metabolism and histological characteristics of nephrocalcinosis, acute tubular necrosis (ATN) and acute rejection in biopsies taken within the first week, were related to the occurrence of DGF.

Results: The incidence of DGF in a cohort of 585 cadaveric transplants was 31%. DGF correlated independently with serum calcium levels (OR 1.14 (95% CI 1.04-1.26) per 0.1 mmol/l). The use of calcium channel blockers before transplantation protected against DGF (OR 0.5 (95% CI 0.29 – 0.87). In this selected group we found an association with histological signs of ATN and DGF. However, most of the biopsies also had features of acute rejection or nephrocalcinosis. Nephrocalcinosis was found in 12 of 71 biopsies and was not associated with serum calcium levels or the occurrence of DGF.

Conclusions: In this study, serum calcium levels were independently associated with DGF. This could not be explained by the presence of microscopic nephrocalcinosis. Therefore DGF is attributed to high intracellular calcium levels. As calcium supplementation and vitamin D analogues are commonly used in dialysis practice, hypercalcemia influences long-term graft outcome by its effect on DGF. The pretransplant use of calcium channel blockers has a protective effect on the occurrence of DGF.

Abstract# 1172 Poster Board #-Session: P102-III

SEQUENTIAL LIVING DONOR KIDNEY AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR AL ("PRIMARY") AMYLOIDOSIS WITH PREDOMINANT RENAL INVOLVEMENT. Matthew D. Griffin,[1] Nelson Leung,[1] Angela Dispenzieri,[2] James M. Gloor,[1] Thomas R. Schwab,[1] Stephen C. Textor,[1] Martha Q. Lacy,[2] Mark R. Litzow,[2] Timothy S. Larson,[1] Morie A. Gertz,[2] Mark D. Stegall.[3] *[1]Dept. Medicine, Division of Nephrology, Mayo Clinic and Foundation, Rochester, MN; [2]Dept. Medicine, Division of Hematology, Mayo Clinic and Foundation, Rochester, MN; [3]Dept. Surgery, Division of Transplantation Surgery, Mayo Clinic and Foundation, Rochester, MN.*

Background and Aims: Patients with ESRD due to AL amyloidosis have poor clinical outcomes from chronic dialysis and from autologous stem cell transplantation (SCT) while kidney transplantation (KTx) alone is likely to result in recurrent disease. To overcome this dilemma, we have pursued a strategy of living donor KTx followed by SCT for patients with advanced renal AL amyloidosis. **Methods:** Clinical and laboratory outcomes were examined for 7 patients who were accepted for sequential transplantation by the kidney and bone marrow transplant programs at a single center between Dec 1999 and Feb 2003. **Results:** All 7 patients received living donor KTx with good initial graft function. Immunosuppression consisted of tacrolimus (n = 4), cyclosporine (n = 2), or sirolimus (n = 1) combined with mycophenolate mofetil and prednisone. Five of the 7 were on dialysis prior to transplantation. No patient had acute rejection of the renal graft. Four have subsequently had successful SCT (see table):

Of the other 3 patients, one, for whom SCT was not recommended due to worsening liver amyloid, died following SCT at another institution. A second died of demyelinating disease which occurred 4 weeks following the KTx. A third has thus far elected not to undergo SCT and has minimal recurrent disease with stable renal graft function 3 years post-KTx. **Conclusions:** (a) Living donor kidney transplantation followed by autologous SCT is a feasible approach for AL amyloidosis with advanced renal impairment. (b) Stem cell harvest prior to renal transplantation is recommended. (c) Stem cell engraftment was not severely impaired in renal transplant recipients on triple-drug immunosuppression.

Clinical and laboratory data on 4 patients receiving KTx followed by SCT for AL amyloidosis

	Case 1	**Case 2**	**Case 3**	**Case 4**
Timing of Stem Cell Harvest	Post-KTx	Pre-KTx	Pre-KTx	Pre-KTx
Time from KTx to SCT (Days)	374	130	242	138
Yield of CD34+ Cells (10^6/kg)	0.7 / 4.3 *	10.1	5.3	5.9
Time to neutrophils > 0.5 x 10^9/L (Days)	17	14	15	16
Time to Platelet > 50 x 10^9/L (Days)	39	23	20	16
Total episodes of infection	1	1	1	1
Duration of follow-up from SCT (Days)	570	476	205	145
Serum creatinine at last follow-up (mg/dl)	1.3	2.0	1.3	2.2
Current GFR (iothalamate, ml/min/SA)	54	26	53	57

* Two mobilizations were necessary.

Abstract# 1173 Poster Board #-Session: P103-III

URINARY TRANSFORMING GROWTH FACTOR-BETA1 (TGF-β_1) EXCRETION IS NORMAL IN RENAL TRANSPLANT RECIPIENTS WITH STABLE ALLOGRAFT FUNCTION. Geeta G. Gyamlani,[1] Catherine E. Gray,[2] Mark D. Stegall,[3] Joseph P. Grande,[2] Timothy S. Larson.[1] *[1]Nephrology, Mayo Clinic, Rochester, MN; [2]Pathology, Mayo Clinic, Rochester, MN; [3]Transplant Surgery, Mayo Clinic, Rochester, MN.*

Background: Up-regulation of TGF-β_1 is pivotal in extracellular matrix deposition. Although urine TGF-β_1 excretion has been proposed as a surrogate marker of fibrosis, no detailed studies of urine TGF-β_1 excretion in patients with well functioning renal allografts have been performed. We tested the hypothesis that both tissue deposition and urinary excretion of TGF-β_1 are normal in renal transplant recipients with stable allograft function.

Methods: Fifty-six adult kidney transplant recipients followed at our institution and 49 healthy controls were studied. Random urine samples were analyzed for TGF-β_1, creatinine, albumin and protein from controls and from recipients at the time of their 4-month post transplant visit. Renal function was assessed by measurement of glomerular filtration rate (GFR) in all transplant recipients. TGF-β_1 was determined using an ELISA assay. All transplant recipients had a protocol biopsy obtained 4 months after renal transplantation, which was used for semiquantitative assessment of chronic histopathologic alterations and for assessment of renal deposition of TGF-β_1 by immunohistochemistry.

Results: The mean GFR was 51.7 ± 13.8 ml/min in the transplant recipients. At 4 months post-transplant, none of the recipients had chronic changes on biopsy (chronic tubular or interstitial scores of >1, as assessed by Banff '97). Although there was a trend for higher total urinary TGF-β_1 /creatinine ratios in the recipients vs healthy controls, this difference was not significant (26.64 ± 39.55 vs 18.26 ± 17.19 pg/mg, p= 0.117). The low TGF-β_1 excretion in the recipients was associated with relatively low intrarenal TGF-β_1 deposition. Urinary TGF-β_1 correlated with proteinuria (r=0.31, P<0.05). However, there was no correlation between TGF-β_1 levels and the degree of fibrosis, sub-clinical rejection, or GFR

Conclusion: In kidney transplant recipients with stable allograft function, urine excretion of TGF-β_1 is not significantly greater than that observed in healthy control subjects who did not receive a renal allograft. We propose that the similar urine TGF-β_1 levels in transplant recipients and healthy controls reflects the lack of fibrosis in recipients with stable, well functioning allografts 4 months after transplantation. These studies provide important baseline information for interpretation of urine TGF-β_1 excretion in transplant recipients who develop significant fibrosis following transplantation.

Abstract# 1174 Poster Board #-Session: P104-III

MARKERS OF INFLAMMATION BEFORE AND AFTER RENAL TRANSPLANTATION. Alfonso Cueto-Manzano,[1] Luis E. Morales-Buenrostro,[2] Liliana Gonzalez,[1] Norma Gonzalez-Tableros,[2] Isela Valera,[1] Ricardo Correa-Rotter,[2] Josefina Alberu.[2] *[1]Clin. Epidemiol.Research Unit, Hospital de Especialidades, CMNO, Guadalajara, Jal., Mexico; [2]Nephrology and Transplantation, Inst. Nal. de Ciencias Medicas y Nutricion S.Z., Mexico City, Mexico.*

Inflammation is a phenomenon frequently present in ESRD patients (pts) even before dialysis, and it has been associated with cardiovascular mortality. Data regarding the behavior of inflammation markers after kidney transplantation (KT) are limited. The aim of this study is to report serum concentrations of C-reactive protein (CRP), TNFα, and IL-6 in ESRD pts before and after receiving a KT. **Patients & Methods**: Serum samples from 37 ESRD pts (24 male, age 34±13 yrs) were collected the day *before* KT (baseline value) and 13 (8-15) months *afterward* (last value). Serum samples from 31 kidney donors (as controls) were obtained the day before nephrectomy. All serum samples were frozen at -20°C until tested. Serum CRP concentrations were measured using Nephelometry (Dade Behring); TNFα and IL-6 were measured by ELISA (Amersham Pharmacia Biotech). Clinical and biochemical relevant information before and after KT was obtained from the clinical records of the patients. **Results**: Values of inflammation markers are shown on the Table.

No significant correlation was observed between inflammation markers and: pre KT dialytic method, time on dialysis, gender, immunosuppressive therapy, anti-IL2R use, acute rejection or chronic allograft nephropathy. Before KT, CRP correlated with age (r 0.45, p=0.006), and serum albumin (r -0.36, p=0.04). At the end of the study, CRP correlated with serum albumin (r -0.54, p=0.003), and with IL-6 (r 0.33, p=0.05), while TNFα correlated with body weight (r 0.35, p=0.04), and with IL-6 (r 0.55, p=0.001), and IL-6 correlated with serum creatinine (r 0.34, p=0.04). **Conclusions**: CRP decreased significantly *after* KT, whereas an *increase* in TNFα and IL-6 was observed. These results deserve further investigation to determine the impact on graft and patient survival, as TNFα and IL-6 have been associated with vascular damage progression.

	Patients (n=37) Baseline	Patients (n=37) Last	Controls (n=31)
CRP (mg/L)	5.05±3.55	3.61±2.63*	3.65±1.52
TNFα(pg/mL)	0.10 (0.03-0.24)	0.26 (0.10-0.41)*	0 (0-0.15)*, **
IL-6 (pg/mL)	1.91 (1.24-7.07)	2.64 (1.33-3.64)	1.07 (0.59-1.88)*, **
	*p<0.05 vs. baseline	**p<0.05 vs. last	

Abstract# 1175 Poster Board #-Session: P105-III

RENAL ALLOGRAFT AND PATIENT SURVIVAL IN PATIENTS WITH AND WITHOUT A GENETIC HYPERCOAGULABLE STATE WHO UNDERGO REPEAT RENAL TRANSPLANTATION AFTER AN INITIAL PRIMARY RENAL ALLOGRAFT THROMBOSIS.

Ashley B. Irish,[1] Fiona R. Green,[2] Derek W. R. Gray.[3] *[1]Department of Nephrology, Royal Perth Hospital, Perth, WA, Australia; [2]Department of Cardiovascular Medicine, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; [3]Nuffield Department of Surgery, Oxford Transplant Unit, Oxford, United Kingdom.*

Introduction: Primary Renal allograft thrombosis (PRAT) occurs in 1-3%of kidney transplants. Causal factors include a primary hypercoagulable state. The short and long term outcome of patients subsequently maintained on dialysis or re-transplanted after a renal allograft thrombosis is unknown.

Methods: We retrospectively identified 40 patients with a primary graft thrombosis of whom 30 were subsequently re-transplanted (TG) and evaluated outcomes after a median follow-up of 15 years. 33 patients who had lost 1 or more previous grafts to non-thrombotic causes were used for a control group (CG). Patients were also evaluated for the Factor V Leiden (FVL) and Prothrombin G20210A genotype.

Results: Patients were largely drawn from the triple therapy era. PRAT patients who were re-transplanted were younger (median 43 vs 53 years) and had less prevalent cardiovascular disease than patients not retransplanted. The TGs were re-transplanted a median of 1.5 years after their first graft, around 70% received some form of anticoagulation (aspirin, heparin, warfarin). Graft survival in TG vs CG regrafts was significantly worse with 11/30 vs 2/33 graft losses (P=0.004 Fishers) with a median survival of 16 vs 24 years (P=0.003). 2 patients suffered a second immediate graft thrombosis. There was a significantly higher risk of vascular rejection in the TRG (6/30 vs CG 0/33 P<0.01) however patient survival after re-transplant did not differ. 9/10 of the patients who were not re-transplanted died within 5 years post PRAT. Inheritance of FVL (n=4) or the Prothrombin Gene mutation (n=3) did not influence cumulative graft or patient survival.

Conclusions: Patients with PRAT who undergo repeat renal transplantation are at significantly greater risk of graft failure and vascular rejection than those re-transplanted for non-thrombotic causes. This suggests genetic and environmental causes of a chronic hypercoagulable state could contribute to allograft attrition and be amenable to therapeutic interventions.

Abstract# 1176 Poster Board #-Session: P106-III

HIGH-RISK FACTORS (HRF) IN RENAL TRANSPLANTATION HAVE CHANGED WITH THE ADVENT OF NEW IMMUNOSUPPRESSIVE AGENTS.

Carlton J. Young,[1] Clifton Kew,[2] Sharon Hudson,[1] Arun Chandrakantan,[2] Michael Gallichio,[1] 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.*

HRF for renal transplantation negatively impact graft survival (GS). We wanted to know if new immunosuppressive agents have changed the composition of HRF. **Methods:** Between 1/1/87 and 8/30/03, 4,476 Living Donor (LD) and Cadaveric (CD) renal transplants were performed at our institution. Two ERAS were retrospectively reviewed: ERA 1 (E1) (1987-1995; n=2297, LD=654, CD 1638) had induction therapy with either MALG or OKT3 and maintenance immunosuppression with Cyclosporine/Azathioprine/Prednisone. ERA 2 (E2) (1996-2003; n=2179, LD=934, CD=1214) patients were treated with induction therapy consisting of either OKT3 or Daclizumab. Maintenance immunosuppression consisted of a combination of either Neoral or Tacrolimus plus Mycophenolate mofetil/Prednisone. No demographic differences between the eras with respect to cause of renal failure, age, sex, or ethnic composition were noted. Actuarial survival was by Kaplan-Meier. **Results:** Overall, CD and LD graft survival improved significantly from E1⇒E2 (p<0.0001). Some HRF persisted from E1⇒E2.

TABLE 1: 3 Yr GS HRF (persistent)

	ERA1	p (E1v. E1)	ERA2	p (E2 v. E2)
Weight <90kg	75%		86%	
Weight ≥ 90kg	68%	0.001	84%	0.05
Donor Age <60	71%		86%	
Donor Age ≥ 60	59%	<0.0001	72%	<0.0001
AA (CD)	62%		78%	
C (CD)	69%	<0.0001	87%	0.0004
AA (LD)	74%		87%	
C (LD)	84%	<0.0001	91%	0.04

Disappointingly, African-American (AA) (LD) GS was commensurate only with Caucasian (C) (CD) GS in E1 (p=0.68) and E2 (p=0.77). Diabetic patients had significanlty improved overall GS from E1⇒E2 (p<0.0001), and did as well as non-diabetics in both eras, E1 (p=0.95); E2 (p=0.79). Other HRF no longer had an impact in E2.

TABLE 2: 3 Yr GS By HRF (improved)

	ERA1	p (E1 v. E1)	ERA2	p (E2 v. E2)
Primary Tx	71%		85%	
Re Tx	63%	0.0002	86%	0.79
PRA < 20	71%		85%	
PRA ≥ 20	56%	<0.0001	87%	0.94
Recipeint Age < 60	85%		93%	
Recipient Age ≥ 60	76%	0.01	90%	0.36

Death censored data used for Recipeint Age calculations

Conclusions: Some HRF no longer have the same impact in E2 as they did in E1, while others persist. Optimizing individual immunosuppressive regimens based on HRF combinations is the next challenge facing renal transplantation.

Abstract# 1177 Poster Board #-Session: P107-III

MISMATCHES IN AFRICAN-AMERICAN VERSUS NON-AFRICAN AMERICAN RECIPIENTS OF CADAVERIC RENAL TRANSPLANTS BASED ON LOW RESOLUTION (LR) DNA TYPING AND AMINO-ACID SEQUENCE AT HLA LOCI.

Ajay K. Israni,[1,2] Christina Gaughan,[2] Tracey Hoy,[1,2] Marshall M. Joffe,[2] Kevin C. Mange,[1,2] Malek Kamoun,[3] Melissa Newman,[2] Jane Kearns,[3] Noah Goodman,[3] David Feldman,[3] Sylvia E. Rosas,[1] Rene J. Duquesnoy,[4] Harold I. Feldman.[1,2] *[1]Medicine, University of Pennsylvania, Philadelphia, PA; [2]Center for Clinical Epidemiology & Biostatistics, University of Pennsylvania, Philadelphia, PA; [3]Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; [4]University of Pittsburg School of Medicine, Pittsburg, PA.*

African-American (AA) recipients of cadaveric renal transplants (CRT) have shortened allograft survival and higher rates of acute rejection than non-African-Americans (nonAA). Matching of donors and recipients to amino acid triplets from exposed portions of the protein sequences of HLA molecules exposed to alloantibodies has been associated with allograft survival overall, but has not been examined by race. We conducted a prospective cohort study of 550 donor-recipient CRT pairs to evaluate the extent of HLA mismatching among AA vs. nonAA using amino-acid triplet analysis and compared this to traditional HLA analysis using LR DNA typing employing SSOP or SSP methods. Amino acid triplet analysis was performed utilizing the computer program HLA Matchmaker (Duquesnoy, Human Immunology 63, 339-352, 2002).

Results: Traditional HLA analysis revealed that, AA (n=163) had higher % of mismatching than nonAA (n=396) at HLA-A (10 vs. 25, 23 vs. 33, 67 vs. 42 for 0, 1 and 2 mismatches respectively, p<0.0001) and HLA-B (12 vs. 27, 41 vs. 43, 48 vs. 30 for 0,1 and 2 mismatches respectively, p<0.0001). By amino acid triplet analysis, AA had higher mean number of triplet mismatching than nonAA on HLA-A and B (14.4 ± 6.8 vs. 11.6 ± 8.0 mismatches, p=0.0001). Among AA, the most frequent triplet mismatches occurred at amino acid a66rKv (polymorphic amino acid lysine at position 66 with arginine in position 65 and valine in position 67 on HLA-A), a107W, and a74H with 55 mismatches at each of these positions (34% of AA). Among nonAA, the most frequent mismatch occurred at amino acid a70aQs with 88 mismatches at this position (22% of nonAA). These mismatches are on the HLA-A loci. In addition most of these polymorphic amino acid residues are located at the exposed portions of HLA molecule and participate in peptide binding.

Conclusion: Like traditional HLA analysis, amino acid triplet analysis of HLA-A and B loci reveals a greater degree of mismatching among AA than nonAA recipients. The role of this higher level of amino acid mismatching in the observed worse allograft outcomes among AA needs further exploration.

Abstract# 1178 Poster Board #-Session: P108-III

AFRICAN AMERICANS HAVE A LOWER RATE OF CONVERSION FROM TRANSPLANT EVALUATION TO PLACEMENT ON THE ACTIVE WAITING LIST. Erica L. Hartmann,[1] Aimee K. Sundberg,[2] Julie A. Roskopf,[2] Alan C. Farney,[3] Michael S. Rohr,[3] Patricia L. Adams,[1] Robert J. Stratta.[3] *[1]Medicine, Wake Forest University, Winston-Salem, NC; [2]Pharmacy, Wake Forest University, Winston-Salem, NC; [3]Surgery, Wake Forest University, Winston-Salem, NC.*

Introduction: The increase in referrals for renal transplantation (tx) reflects not only an increased acceptance of transplanting higher risk and older patients (pts), but also the steep rise in the number of pts with end-stage renal disease. The purpose of this study was to characterize referrals evaluated at our center as well as to define barriers to the waiting list.

Methods: Our cohort was composed of pts presenting for an initial evaluation to determine eligibility for renal or combined renal/pancreas tx. A retrospective review of pts who completed their evaluation over the past twelve months was performed.

Results: A total of 225 candidates were evaluated. Of these pts, 120 (53%) were male. Caucasians (N=114, 51%) slightly outnumbered African-Americans (AA, N=99, 44%). The mean age was 48.4 years (range 22-73); 27 (12%) were >65 years. The mean body mass index (BMI) was 27 kg/m² (15.3-42.3). Although one third of pts (N=68) were evaluated prior to starting dialysis, the remainder were on dialysis for an average of 25 months (0-128) prior to referral. A total of 141 pts (62%) were placed on the active waiting list. Of the non-wait listed (NWL) pts, 63 were deemed unsuitable for tx and 21 continue to undergo further evaluation. Reasons for not listing included: psychosocial concerns (N=23), inadequate financial coverage for medications (N=12), prohibitive cardiovascular disease (N=7), center not in tx network (N=6), malignancy (N=4), pt death (N=3), obesity (N=3), pt reluctance to listing (N=3), and chronic infection (N=2). There were no differences between the WL and NWL groups regarding age, gender, BMI, or duration of dialysis prior to referral. However, 50% of AA pts evaluated were placed on the active WL compared to 65% of non-AA pts (P<0.001). The major exclusion criterion for AA pts was psychosocial (N=18), as evidenced by ongoing non-adherence to medical regimens, inadequate social support, or active substance abuse. Financial problems were also common (N=7). In contrast, the reasons for not listing pts in the non-AA group were more evenly distributed.

Conclusion: Of all patients evaluated at our center, AA pts have a significantly decreased chance of being placed on the active waiting list because of psychosocial and financial issues. It is imperative to delineate socio-economic barriers to tx in otherwise medically acceptable candidates.

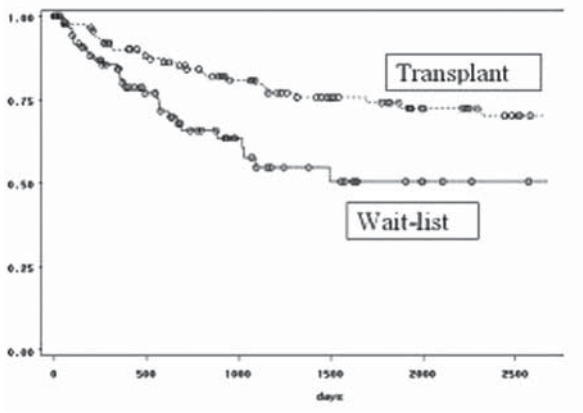
Abstract# 1179 Poster Board #-Session: P109-III

IMPROVEMENT IN DISEASE ACTIVITY AND MORTALITY FOLLOWING RENAL TRANSPLANT FOR SYSTEMIC SCLEROSIS. Eric M. Gibney,[1] Alkesh Jani,[1] Michael J. Fischer,[1] Chirag R. Parikh,[1] David Collier,[2] Alex C. Wiseman.[1] *[1]Renal Diseases and Hypertension, University of Colorado Health Sciences Center, Denver, CO; [2]Rheumatology, University of Colorado Health Sciences Center, Denver, CO.*

Introduction: Systemic sclerosis (SS) is an unusual cause of ESRD with historically poor outcomes on hemodialysis. Our experience suggested that renal transplantation in SS was marked by an improvement in nonrenal disease activity and comparable mortality benefit compared to SS patients on dialysis. We thus decided to take a systematic approach to understanding disease activity and outcomes in SS after renal transplant.

Methods: A query of the UNOS registry on transplants provided information on all U.S. systemic sclerosis patients listed for renal transplants between 1985 and 2002. We compared 1yr and 3 yr survival in patients who received cadaveric transplants with patients who remained on the waiting list. Graft survival was also analyzed. Survival data was assessed by Kaplan-Meier analysis. Also, local charts were reviewed for pre and post-transplant Modified Rodnan skin scores, a validated measure of disease activity.

Results: 258 patients with SS were listed in the UNOS database with 78% female and a mean age of 51 years. Of these 258 patients, 115 received cadaveric transplants, 64 were still waiting and 29 died on the waiting list. Survival was significantly prolonged in patients receiving transplants as demonstrated by Kaplan-Meier analysis. (**Figure 1, p=0.0045**). Graft survival at 1 and 3 yrs was 68% and 60%. Skin scores declined in all 4 subjects at our center, with a mean difference of 20. (**p=0.0235**). Of note, graft survival has not improved in the post-MMF era (data not shown).



Survival after listing
p=0.0045

Summary: Renal transplantation in SS is associated with improved long-term survival when compared to wait-listed patients who remain on dialysis, a finding not previously described. Transplantation may be associated with an improvement in systemic manifestations of disease as suggested by data at our center. Renal transplant should be considered the treatment of choice in ESRD due to SS.

KIDNEY: CARDIOVASCULAR RISKS, CHRONIC ALLOGRAFT NEPHROPATHY AND DONOR ISSUES

Abstract# 1180 Poster Board #-Session: P110-III

ASSESSING RISK FOR POSTOPERATIVE MYOCARDIAL INFARCTION AFTER RENAL TRANSPLANTATION. Anup Manoharan,[1] Anita Patel.[2] *[1]Internal Medicine, Henry Ford Hospital, Detroit, MI; [2]Nephrology, Henry Ford Hospital, Detroit, MI.*

Background. Myocardial infarction is a frequent complication after renal transplantation. Pre-transplant screening for coronary artery disease in patients undergoing renal transplantation may improve outcome in post transplant period. The aim of the study was to determine if dobutamine stress echocardiography (DSE) is a good predictor of myocardial infarction in first year after transplantation.

Methods. We did a chart review of 800 patients that had undergone renal transplantation at Henry Ford Hospital between 1990-2001. Of the 800, only 179 had stress testing at our institution or were followed at our institution after their transplantation. We divided the patients into those who underwent dobutamine stress echo, nuclear stress test or other cardiac testing. The groups were then followed for one year. Any patient who had a troponin >3 ng/ml were considered to have had a myocardial infarction.

Results. 179 patients underwent stress testing and were followed at our institution. Of these, 67 had DSE, 55 had nuclear stress testing, 41 had other testing done. From the DSE group, 11 were lost to follow up, 4 had a positive stress test, and 52 had a negative stress test. Among the nuclear stress test group, 10 were lost to follow up, 13 had a positive stress test and 32 had a negative test. A total of 8 patients had a myocardial infarction, 5 were from the group who had a negative DSE and 1 was from the group of 4 patients with positive DSE. 1 patient had myocardial infarction from the group who had a negative nuclear stress test and 1 out of 32 patients who tested negative with nuclear stress test had an ischemic cardiac event. The positive predictive value for DSE was 25% and for nuclear stress test was 7.8%. The negative predictive values were 90.1% and 96.9%, respectfully.

Conclusion. Both DSE and nuclear stress tests seem to have excellent negative predictive value, with regards to cardiac events in the first post transplant year. Nuclear stress test may be superior but the small number of patients studied makes it difficult to make this claim with certainty.

Abstract# 1181 Poster Board #-Session: P111-III

PURE LAPAROSCOPIC RIGHT DONOR NEPHRECTOMY: EQUIVALENT DONOR AND RECIPIENT OUTCOMES AS COMPARED WITH LAPAROSCOPIC LEFT DONOR NEPHRECTOMY. Harish D. Mahanty,[1] Jonathan T. Carter,[1] Sang-Mo Kang,[1] John P. Roberts,[1] Chris E. Freise,[1] Andrew M. Posselt.[1] *[1]Department of Surgery, University of California, San Francisco, San Francisco, CA.*

Purpose: Laparoscopic donor nephrectomy has become the preferred method for procuring left kidneys from living donors. In contrast, experience with laparoscopic procurement of right donor kidneys is limited and some series report increased donor and recipient complication rates with this approach. Consequently, many centers continue to employ the traditional open approach to procure these organs. In the present study, we compared donor and recipient outcomes after pure (not hand-assisted) laparoscopic right or left donor nephrectomies performed at our institution during a 4 year period.

Methods: From 11/99 to 11/2003, 361 laparoscopic donor nephrectomies were performed at UCSF, of which 49 (14%) were right-sided. All procedures were performed without the use of hand-assist devices. Four ports were used for dissection and kidneys were removed through transverse suprapubic (Pfannenstiel) incisions. Indications for selecting the right kidney included: multiple left renal arteries (57%), abnormal right renal arteries (36%), renal stones (5%), ureteropelvic junction obstruction (2%), cyst (2%), ptosis (2%), left retroaortic renal vein (2%). Renal vein lengths were sufficient for standard graft implantation in all right kidney recipients. Donor and recipient data were acquired prospectively from each study group and are depicted in the table below as means ± SD.

Results:

	Right (n=49)	Left (n=312)	P value
Age (yr)	40.9 ± 13.4	39.7 ± 11.4	ns
Gender			
Male	23 (47%)	129 (41%)	ns
Female	26 (53%)	183 (59%)	ns
Operative time (min)	166 ± 36	208 ± 47	<0.0001
Estimated blood loss (ml)	61 ± 20	78 ± 64	ns
Extraction time (sec)	257 ± 96	260 ± 90	ns
Multiple Arteries	6 (12%)	40 (13%)	
Multiple Veins	10 (20%)	5 (2%)	
Length of stay (days)	3.42 ± 1.37	3.22 ± 1.1	ns
Complications:			
Conversion to open	0	1	
Postoperative transfusion	0	3	
Bowel injury	0	2	
Wound infection	1	3	
Recipient:			
Warm ischemic time (min)	30.4 ± 10.3	29.4 ± 6.7	ns
Cr change over 1st 24h (%)	-39 ± 28	-46 ± 31	ns
Cr at one month	1.3 ± 0.5	1.5 ± 0.4	ns

Conclusions: We demonstrate that pure laparoscopic right donor nephrectomy can be performed safely and with success rates comparable to those of laparoscopic left donor nephrectomy. Furthermore, operative times for the right sided approach are consistently shorter than for the left side, possibly due to the absence of adrenal and gonadal veins originating from the renal vein. These results have led us to perform essentially all of our donor nephrectomies laparoscopically, irrespective of which side is to be procured.

Abstract# 1182 Poster Board #-Session: P112-III

NORMAL DIPYRIDAMOLE-STRESS TECHNETIUM-TETROFOSMIN SPECT STUDIES PREDICT LOW RISK OF CARDIAC MORTALITY FOLLOWING KIDNEY TRANSPLANTATION. Pary Sivaraman,[1] Kheng-Thye Ho.[2] *[1]Medicine, National University Hospital, Singapore; [2]Cardiology, National University Hospital, Singapore.*

Cardiac mortality following kidney transplantation has been a major cause of graft and patient loss in most centers. In our center, cardiac death accounts for about 22% of patient mortality. Prior to kidney transplantation numerous strategies have been evaluated to stratify cardiac risks and prognosis. However, following transplantation there is no consensus as to what is the optimal way to screen and stratify patients at risk of cardiac morbidity and mortality risks.

The aim of our study was to determine the usefulness of a normal dipyridamole-stress technetium-tetrofosmin SPECT study in predicting low risk of cardiac morbidity and mortality following kidney transplantation.

Due to the high cardiac mortality at our center all kidney transplant patients on follow-up underwent routine dipyridamole technetium tetrofosmin SPECT study since October 2000. A total of 156 patients had undergone imaging till April 2003 with analysis done at end October 2003 giving at least 6 month follow-up data on all patients.

Of these156 patients, 136 (87%) had a normal dipyridamole-stress technetium-tetrofosmin SPECT study with respect to myocardial ischemia. Follow-up was complete in 100 % of patients with mean follow-up of 2.03 ± 0.86 years from the stress study. The mean time from transplantation to the time of dipyridamole stress-testing was 6.88 ± 3.95 years. None of the patients had a history of coronary artery disease prior to kidney transplantation.

Mean age 48 years (range 24 – 68 years). 80 (59%) males and 56 (41%) females. Cause of ESRF: chronic glomerular disease 66 (48%), hypertension 31 (22%), diabetic nephropathy 8(6%) and others 31 (22%). On follow-up 110 (81%) had hypertension, 92 (68%) had an LDL-cholesterol level of more than 2.6mmol/L, 24 (18%) had diabetes mellitus (8 pre transplant & 16 post transplant). All patients were on calcineurin inhibitors (either cyclosporin (77%) or tacrolimus (23%)) and 114 (84%) were on long-term steroid therapy.

Two patients (1.47%) with a normal study had myocardial infarctions during the follow-up period. No patients experienced cardiac-death. The annualized cardiac event rate was 0.7% for patients with a normal study.

Our study has demonstrated that a normal dipyridamole-stress technetium-tetrofosmin SPECT study predicts a low risk of cardiac events following kidney transplantation. We would recommend it as the non-invasive screening method of choice to evaluate cardiac mortality and morbidity risks following kidney transplantation.

Abstract# 1183 Poster Board #-Session: P113-III

RESULTS USING A RISK-WEIGHTED ALGORITHM FOR CARDIAC SCREENING IN RENAL TRANSPLANT CANDIDATES. Ayman Iskander,[1] Beth Bouthot,[2] Edward Walshe,[2] Christopher Ying,[2] Samir Kassissieh,[2] Hannah Gilligan,[2] Marti Hoar,[2] Mary Ann Simpson,[2] Arsalan Shahzad,[1] Kan Fang,[1] Richard W. Nesto.[1] *[1]Cardiovascular Department, Lahey Clinic Medical Center, Burlington, MA; [2]Nephrology Department, Lahey Clinic Medical Center, Burlington, MA.*

Background: Coronary artery disease (CAD) is a major determinant of survival after renal transplantation (RT). Practices vary as to which patient needs CAD screening and which screening modality to use. Non-invasive testing for CAD in this population may lack adequate sensitivity and specificity.

Methods: We devised a CAD risk-weighted algorithm that stratified RT candidates into high-risk (DM or history of CAD), intermediate risk (≥ 2 CAD risk factors other than DM), or low risk (≤ 40 years and ≤ one risk factor) for CAD. High-risk patients were evaluated by coronary angiography (CA), and intermediate risk patients by stress (exercise or Dobutamine) Echo. Low risk group did not require CAD screening.

Results: From July 2001 to October 2003, 50 RT candidates were evaluated with the screening algorithm. Mean age was 56 ± 12 years and 72% were male. Etiology of renal failure was DM in 61%, hypertension in 10%, chronic glomerulonephritis in 12%, and miscellaneous in 16%. According to the algorithm, 36 patients were classified as high risk requiring CA and 14 patients were intermediate risk of whom 4 required CA on basis of abnormal stress test. Overall CA was mandated in 40/50 patients (80%) and performed in 34 patients. CA revealed no CAD in 5 (15%), non-obstructive CAD [≤ 50% diameter stenosis] in 4 (12%), one vessel CAD in 10 (30%), 2 vessels CAD in 7 (20%), and 3 vessels CAD in 8 (23%). Revascularization was performed in 14 patients (5 had CAB surgery and 9 had percutaneous stenting). Based on poor coronary anatomy, 3 patients were removed from transplant list. From this cohort, 10 patients underwent renal transplant surgery. No peritransplant cardiac events were encountered. Of the 30 diabetic patients (7 type 1, 24 type 2), 27 underwent CA, 20 (74%) had evidence of obstructive CAD of whom 14 (70%) were asymptomatic. Revascularization was indicated in 14 (51%) and performed in 10 (37%) patients. Among all patients with documented obstructive CAD, 79% were free of cardiac symptoms.

Conclusion: The prevalence of CAD in renal transplant candidates is high and usually silent in most patients. As the accuracy of non-invasive testing of CAD in this particular population is suboptimal, a risk-weighted screening algorithm is a valuable and efficient tool to evaluate CAD in RT candidates.

Abstract# 1184 Poster Board #-Session: P114-III

INFLAMMATION, ENDOTHELIAL DYSFUNCTION AND INHERITANCE PREDICT PREVALENT AND INCIDENT CARDIOVASCULAR DISEASE IN RENAL TRANSPLANT RECIPIENTS: RESULTS OF THE *OxSPIRes* COHORT. (Oxford Study of Procoagulant factors In Renal Transplant Recipients).

Ashley B. Irish,[1] Fiona R. Green,[2] Derek W. R. Gray.[3] *[1]Department of Nephrology, Royal Perth Hospital, Perth, WA, Australia; [2]Department of Cardiovascular Genetics, Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; [3]Nuffield Department of Surgery, Oxford Transplant Centre, Oxford, United Kingdom.*

Introduction: Cardiovascular disease (CVD) is the major long-term risk of RTR. Familial predisposition, inflammation and endothelial dysfunction is associated with CVD in the non-transplant population but is of unknown relevance in RTR.

Methods: We measured a mediator of inflammation Interleukin-6 (IL6), fibrinogen, endothelial function by Von Willebrand Factor Antigen (VWF)) and family history of CVD (FHCVD) in 231 RTR (141 male; median age 50 years) a median of 6 years post transplant and followed them for a median of 9 years for CVD endpoints.

Results: 48 patients died (42 with a functioning graft) and 36 grafts failed. 62 patients had new CVD (CVD+) and were compared with the remaining patients (CVD-). 28% of patients had a positive FHCVD. Prevalent and incident CVD were significantly associated with a FHCVD (P<0.005).

Results for parameters of interest

	Age (yrs)	Albumin g/l	WCC 10^9/l	IL6 pg/ml	VWF %	Fibrinogen mg/l
CVD+	49 (1.3)*	40.7 (0.4)*	7.1 (0.2)*	4.0 (0.5)*	173 (7.5)*	384 (11)
CVD-	39 (1.1)	42.6 (0.3)	6.4 (0.2)	2.6 (0.2)	137 (3.9)	380 (8)

Results mean (SEM). IL6 and VWF were log transformed. *=P<0.05

IL6 was correlated with VWF (0.23 P=0.001), fibrinogen (0.31 P<0.001) and inversely with albumin (0.27 P<0.001). At entry, VWF was independently predicted by age, blood group (O vs Non-O) and IL6 (R^2 0.25 F=17.8, P=0.001). Independent predictors of incident CVD in a multiple logistic regression model were prevalent CVD, VWF, age at transplant and a positive FHCVD while IL6, smoking history, total-cholesterol and albumin were not associated with CVD (P=0.001). Patients with venous thrombosis at any time also had a statistically significant increased VWF (176 vs 142% P =0.007).

Conclusion: These data suggest that despite immunosuppression, evidence of endothelial dysfunction (VWF) associated with persistent inflammation (IL6), and a family history of CVD are powerful determinants of prevalent and incident all cause CVD in RTR.

Abstract# 1185 Poster Board #-Session: P115-III

ANGIOTENSIN GENE POLYMORPHISM IS ASSOCIATED WITH DEVELOPMENT OF POST RENAL TRANSPLANT HYPERTENSION. Kamal Sud,[1] Madhu Khullar,[2] Deepak Sharma,[1] Vinay Sakhuja.[1] *[1]Department of Nephrology, Postgraduate Institute of Medical Education and Research, Chandigarh, UT, India; [2]Department of Experimental Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, UT, India.*

Development of hypertension after kidney transplantation depends upon allograft function, type and doses of immunosuppression, presence of native kidneys, recurrent glomerulonephritis and transplant renal artery stenosis. Presence of hypertension pre-transplant is the most important risk factor for persistence of hypertension post transplant in patients receiving a kidney from living related donors. Polymorphism of the angiotensin converting enzyme (ACE) gene has been implicated in the development of essential hypertension in the general population and the DD genotype of the ACE gene is associated with poorer allograft functions in renal transplant recipients. The association of ACE gene polymorphism with post renal transplant hypertension is far from clear. We studied the ACE gene polymorphisms in 42 patients with post transplant hypertension on maintenance doses of immunosuppression, normal allograft functions with no proteinuria and no evidence of renal artery stenosis. The DD genotype was seen in 93%, ID genotype in 5% and II genotype in 2% as compared to 40%, 52% and 8% respectively in patients with essential hypertension and 31%, 47% and 22% respectively in normotensive control Indian population (p<0.05). We conclude that the DD genotype of the ACE gene is associated with occurrence of post transplant hypertension in patients with normal allograft functions on maintenance doses of immunosuppression. This genotype may be an important factor predicting persistence of hypertension after transplant.

Abstract# 1186 Poster Board #-Session: P116-III

QUALITY ASSURANCE OF HYPERTENSION AND LIPID TARGETS IN RENAL TRANSPLANT RECIPIENTS. Jeffrey S. Zaltzman,[1] Sumeet Suneja,[1] Michelle Nash,[1] G. V. Ramesh Prasad.[1] *[1]Medicine, Division of Nephrology and Transplant, St.Michael's Hospital, University of Toronto, Toronto, ON, Canada.*

Background. Cardiovascular disease (CVD) is the major cause of death with a functioning graft in renal transplant recipients (RTR). Hypertension and hyperlipidemia are highly prevalent in RTR and constitute potentially modifiable risk factors for CVD.

Methods. To determine whether our management of post-transplant hypertension and dyslipidemia meet current guidelines, we reviewed charts of 737 stable RTRs. Cross sectional data were obtained on CVD risks including age, gender, diabetes and immunosuppressive medications along with the donor source and age of the transplant. Blood pressure measurements were averaged over the most recent 3 clinic visits and the patients' antihypertensive medication profile was recorded. Optimal blood pressure was defined as <130/80 in patients without proteinuria and <125/75 in patients with proteinuria according to the recently published clinical practice guidelines (J AM Soc Nephrol, 2000). Significant proteinuria was defined as protein excretion of greater than 1g/24h. Adequacy of lipid control was measured by examining the most recent lipid profile from patients' charts. All lipid-lowering medications were recorded as well.

Results. The population was 63% male and 37% female transplanted between 1976-2002. Mean systolic and diastolic BP was 130.05+12.72 and 79.40+7.41. 8.3% patients had significant proteinuria. 33% and 23% of patients failed to meet the systolic and diastolic BP guidelines respectively. Dyslipidemias were common; mean cholesterol was 5.09+ 1.1 mM/L, LDL_C 2.87+ 0.94 mM/L, TG 1.89+1.28 mM/L and TC:HDL 4.05+1.42. The lipid targets were not met in 33% for TG, 8.5% for LDL and 7.6% for TC:HDL. Subgroup analysis for patients with diabetes showed that only 37% met guidelines for LDL_C. Tacrolimus use was associated with a better cardiovascular risk profile when compared to cylosporine. In that 88% of patients on Tacrolimus met TC:HDL guidelines vs. 79% on Cyclosporine.

Conclusion. Current management of post-transplant hypertension and dyslipidemia can be improved to meet published recommendations. Considering the increased risk of premature CVD in RTRs, modifiable risk factors should be carefully addressed on a consistent basis.

Abstract# 1187 Poster Board #-Session: P117-III

THE ROLE OF HOME BLOOD PRESSURE MONITORING (HBPM) IN THE CONTROL OF HYPERTENSION IN RENAL TRANSPLANT RECIPIENTS. Bangi Awosika,[1] Gail Makos,[1] Susan Steigerwalt,[1] Mohamed EL-Ghoroury,[1] Robert Provenzano.[1] *[1]Nephrology, Hypertension, Transplantation, St.John Hospital and Medical Center, Detroit, MI.*

The prevalence of hypertension in renal transplant recipients (RTR) is 70-85% , a value that actually exceeds that of chronic kidney disease patients with comparable level of renal function. The effectivness and feasibilty of home blood pressure monitoring (HBPM) as a practical tool for directing antihypertensive managment in hemodialysis patients was confirmed by Svetkey et al (ASN-2002). No studies to date have investigated the use of HBPM in RTR. In the present pilot study a cohort of 10 stable RTR followed for a period of 3 month. Each patient underwent a 24-hour ambulatory BP monitoring (ABPM) at baseline and at the end of the three month study period. Patients were trained to use the OMRON IC monitor and instructed to measure their BP twice daily, after breakfast and before bedtime and these readings were downloaded once per month. Antihypertensive drugs were adjusted to achieve a goal home BP of 130/80 mm Hg. Serum creatinine (S.Cr) as well as number of antihypertensive medications were documnted at baseline and after the three month study period. All patients were female; 6 African American, 4 Caucasian , average age 45. Average duration of transplantation 15 month , average BMI 28.2 (Range 27.1 to 31.8) , 3 CRA, 2 LRA, 5LURA.

	Results	
	Baseline	3 Months
Clinic BP (Omron IC)	136.2 ±4.4/77±7.8	137.1±8.7/78.9±3.7
24 hr ABPM	129.7±3.8/77±4.1	131±5.4/75±2.7
S.Cr.	1.42±.16	1.49±.13
Number BP meds	2.4±.45	3±.47

4/10 patients had worsening of renal function over three month period and this correlated with poorer BP control (average rise in S.cr. 0.4 mg/dl , ABPM 134/77 mm Hg to 140/76 mm Hg, ns) where as in those with stable renal function , BP control improved (125/77 mm Hg to 122/75 mm Hg, ns).

We conclude: 1) BP control is suboptimal in this cohort of RTR. 2) HBPM is feasible in RTR. 3) Confounding factors such as deteriorating renal function complicate analysis of the usefulness of this technique. 4) A larger study with telephone or home computer transmission of BP data is necessary to clarify the role of HBPM in this group

Abstract# 1188 Poster Board #-Session: P118-III

THE RENIN ALDOSTERONE AXIS IN STABLE RENAL TRANSPLANT RECIPIENTS. Hassan Ibrahim,[1] Arthur Matas,[1] Richard Spong,[1] Michael Mauer.[1] *[1]Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]Department of Surgery, University of Minnesota, Minneapolis, MN; [3]Department of Pediatrics, University of Minnesota, Minneapolis, MN.*

Background :ACE inhibitors have been shown to have especially beneficial effect on the course of several clinical and many experimental renal diseases. Interruption of the renin angiotensin aldosterone system(RAAS) has yielded greater protective effect than other antihypertensive strategies. At least three relatively short-term clinical trials have demonstrated a reduction of proteinuria with converting enzyme inhibition in hypertensive patients with long-standing grafts Whether this system is overly activated in the stable renal transplant recipient is not well established. Insertion/deletion (I/D) polymorphisms of the ACE gene are associated with variation in plasma ACE levels. The homozygous DD phenotype has higher ACE levels and has been associated with more rapid progression of IgA and other nephropathies and with altered response to ACE inhibition. The impact of the ACE gene polymorphisms on graft outcome has not been uniform.

Purpose: To construct the profile of the renin-aldosterone hormonal axis and to describe the prevalence of the different ACE gene polymorphisms in stable renal transplant recipients.

Methods : Forty renal transplant recipients with a mean iothalamate GFR of 55ml/min underwent PRA and aldosterone measurement after 2 hour of being in the supine position at our General Clinical Research Center. Most of these subjects received living donor transplants and diabetic nephropathy was the most common underlying kidney disease. 18% were on diuretics and all received a calcineurin inhibitor in combination with MMF or Rapamycin for maintenance immunosuppression.The main results are shown in table.

Conclusion: This data suggests that there is no demonstrable augmented systemic activity of the renin aldosterone system; a finding akin to native kidney disease where PRA is paradoxically in the normal range considering that these subjects are usually volume expanded. Whether the immunosuppressive strategy affects this system is unclear from this relatively small number of observations.

The Renin- Aldosterone Axis in stable Renal Transplant Recipients

Plasma Renin Activity(ngAI/ml/h)	1.29±1.05
Plasma aldosterone(pg/ml)	169±101
D/D genotype	20%
D/I genotype	45%
I/I genotype	35%

n=40, studies performed 1 month post transplant

Abstract# 1189 Poster Board #-Session: P119-III

PATHOGENIC MECHANISMS OF POST-TRANSPLANT ERYTHROCYTOSIS. A NEW ROLE FOR CALCIUM. Luisa Jimeno,[1] Raquel Rodado,[1] Alfredo Minguela,[2] Matilde Campos,[3] Francisco Nicolas,[4] Luis Marin.[2] *[1]Nefrology Service, Virgen de la Arrixaca Hospital, Murcia, Spain; [2]Immunology Service, Virgen de la Arrixaca Hospital, Murcia, Spain; [3]Statistics Department, Murcia University, Murcia, Spain; [4]Nuclear Medicine, Virgen de la Arrixaca Hospital, Murcia, Spain.*

The pathogenesis of posttransplant erythrocytosis (PTE) is not yet well-known. Erythropoietin (Ep), androgens, insuline-like growth factor-1 (IGF-1) and other stimulators of erythropoiesis have been involved. However, some other factors related to erythropoiesis such as ferritine, transferrin saturation index (TSI), folate, interleukins (IL) or calcium have not been sufficiently studied.

The aim of this study was to investigate the pathogenic role of these factors in PTE.

Methods:

In this study 41 kidney graft recipients with creatinine levels less than 2 mg/dl were included. According to the hematocrit (Ht) levels they were divided into 2 groups: group 1 (21 patients) with PTE (defined by Ht>51%) and group 2 (20 patients) with normal Ht.

The following parameters were determined in both groups: IGF-1, IGF-BP3, Ep, dehydroepiandrosterona (DHEA), PTH, Ca, P, ferritine, TSI, folate, B12 vitamin, TNFa, g-IF, TGFb, IL3, IL6 and GM-CSF.

The results were compared between the two groups and correlated to Ht levels in each group.

Statistical analysis was performed by using the Student´s t test when comparing both groups and the correlation coefficient of Pearson when relating each parameter to the Ht level.

Results:

Patients with PTE showed significantly greater DHEA concentrations compared with patients without PTE, whereas PTH levels were greater in group 2 (table 1). No differences in the other parameters studied were found.

Ht levels correlated significantly with IGF-1 ($p<0.04$), IGF-BP3 ($p<0.01$), GH ($p<0.05$) and Ca ($p<0.003$)levels in group 1, whereas Ht level only correlated with Ca level ($p<0.025$) in group 2. Neither Ep, interleukins nor the other parameters showed correlation to Ht level.

Conclusions:

1.- Calcium is related to Ht levels in both groups. Patients with hypercalcemia are in risk of developing PTE and must be strictly controlled.

2.- Androgens seem to play an important role in the development of PTE.

3.- IGF-1 and its major binding protein BP3 are involved in the pathogenesis of PTE.

4.- In this study Ep and the other factors studied do not seem to be involved in the pathogenesis of PTE.

Serum DHEA and PTH. Differences between group 1 and group 2.

	group 1	group 2	p
DHEA	78.5 ± 42.8	45.1 ± 37.1	< 0.02
PTH	62.0 ± 44.1	97.8 ± 66.5	< 0.05

table 1

Abstract# 1190 Poster Board #-Session: P120-III

RECOVERY OF ANTI-RECOMBINANT-HUMAN-ERYTHROPOIETIN ASSOCIATED PURE RED CELL APLSIA IN END STAGE RENAL DISEASE PATIENTS AFTER RENAL TRANSPLANTATION. Kearkiat Praditpornsilpa,[1] Yingyos Avihingsanon,[1] Somphon Buranasot,[1] Narudee Bhokaisuwan,[2] Talerngsak Kansanabuch,[1] Somchai Eiam-Ong,[1] Sauwaluck Chusil,[1] Kriang Tungsanga.[1] *[1]Division of Nephrology, Thailand; [2]Division of Laboratory Medicine, Chulalongkorn University Hospital, Bangkok, Thailand.*

Introduction: Anti-Recombinant-Human-Erythropoietin-Associated pure red cell aplasia (anti-r-HuEPO associated PRCA) has been reported in CRF patients who are using r-HuEPO and develop an unexplained sudden decrease in their hemoglobin level. Currently, there are no recommendations for the management of anti-r-HuEPO. This unfortunate situation of resistance to r-HuEPO provides a model to access the impact of delivery of the native molecule by renal transplantation on antibody production.

Methods: Sera from four ESRD patients who had hemodialysis with anti-r-HuEPO-associated PRCA as documented by anemia resistance to r-HuEPO, reticulocytopenia, exclusion of gastrointestinal blood loss by endoscopy, and bone marrow biopsy were assayed for antibody by ELIZA and for inhibition of erythroid precusors prior to and after renal transplantation under a standard immunosuppression; anti CD25, cyclosporin, azathioprin, and prednisolone.

Results: The mean pre-transplant hemoglobin was 5.5 g/dl (range 5.1-6.2 g/dl) and the mean maximum r-HuEPO dose was 267.25 unit/kg/week subcutaneously (range 206-338 unit/kg/week) The mean duration of anti-r-HuEPO associated PRCA before renal transplantation was 8.25 months (range 3-11 months). The patients needed average PRC transfusion of 3 units per month (range 2.5-3.4 unit/month) to alleviate anemic symptom. Their pre-transplantation sera documented a 1: 40 mean titer (range 1:16-1:96) in ELIZA and inhibited erythroid precursor generation by mean ratio of 1:40 titer (range 1:16- 1:96) Following renal transplantation, r-HuEPO antibody activity in these in vitro assays disappeared at a mean time of 4 weeks (range 3-5 weeks) and they no longer required any transfusion. At 4 and 8 weeks post-transplantation, the mean hemoglobin was 7.6 g/dl and 9.9 g/dl respectively. Furthermore, a pre-transplant dialysis patient with anti-r-HuEPO associated PRCA and treated with cyclosporin and MMF failed at 6 weeks to show a reduction in anti-r-HuEPO titer, suggesting that it was the renal transplant and not the immunosuppression that lead to the remission.

Conclusion: Anti-r-HuEPO associated PRCA was recovery after renal transplantation and these findinds suggest that a natural molecule erythropoietin can terminate the humoral antibody response to its recombinant form.

Abstract# 1191 Poster Board #-Session: P121-III

ANEMIA MANAGEMENT PRIOR TO KIDNEY TRANSPLANTATION AND THE RISK OF POST-TRANSPLANT BLOOD TRANSFUSION. 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; [3]Nephrology Analytical Services, Minneapolis, MN.*

Aggressive management of chronic kidney disease complications prior to transplantation (tx) may improve post tx outcomes. We determined the incidence and risk factors for blood transfusion in the first 30 days after kidney tx among adult, cadaveric, first, kidney only transplant recipients between 1995-2000 who were Medicare eligible for at least 12 months prior to tx. Pre tx hemoglobin (HB) levels, erythropoietin (EPO) use within 6 mos pre- tx and post tx blood transfusion were determined in each transplant year. The association of pre-tx HB level with post tx blood transfusion was determined in a multivariate logistic regression model that included patients who received pre-tx EPO (n=15,453). The model included variables associated with post-tx blood transfusion in univariate analyses: gender, race, DGF, and comorbid conditions.The table shows that between 1995 - 2000, the percentage of patients who received EPO within 6 mos pre- tx increased from 81% to 89%. Among patients who received EPO pre-transplant, the percentage of patients with HB within K-DOQI target level of 11-12g.dL increased from 27% to 50%. During this period there was a significant decrease in post tx blood transfusion from 13% to 9%, p <.0001. Blood transfusions were rarely associated with major bleeding episodes (< 1%). In the multivariate analysis, patients with pre-tx HB less than the K-DOQI target of 11-12 g/dl, had increased odds of blood transfusion after tx: Patients with HB 10-11 g/dl had OR = 1.2, 95% confidence interval (CI)1.06-1.36, p = .004 and patients with HB <10 g/dl had OR = 1.5, 95% CI 1.3-1.8, p <.0001. Patients with HB > 12 did not have a significantly reduced risk of blood transfusion. We conclude that the K-DOQI target of 11-12 g/dl is associated with a decreased risk of blood transfusion after kidney tx. However in 2000, 20% of patients still fell below this target. More aggressive treatment of anemia in transplant candidates may decrease the need for post tx blood transfusion and improve post transplant outcomes.

Year	1995	1996	1997	1998	1999	2000
N	2784	2907	3056	3197	3028	3146
Received EPO pre transplant N (%)	2248 (81%)	2340 (81%)	2615 (86%)	2789 (87%)	2664 (88%)	2797 (89%)
Pretransplant Hemoglobin and Post transplant blood transfusion (EPO treated patients only)						
HB<10 %	26	17	11	9	6	5
HB 10-11 %	44	42	37	36	21	15
HB 11-12 %	27	36	46	49	50	50
HB >12 %	3	5	6	6	23	30
Post transplant blood transfusion n(%)	299 (13)	297 (13)	287 (11)	281 (10)	236 (9)	244 (9)

Abstract# 1192 Poster Board #-Session: P122-III

A RETROSPECTIVE ANALYSIS OF EZETIMIBE (ZETIA®) TREATMENT IN RENAL TRANSPLANT RECIPIENTS. Christie L. Buchanan,[1] Lonnie D. Smith,[1] Jacke Corbett,[1] Troy Somerville,[1] John Holman,[1] Fuad S. Shihab.[1] *[1]Solid Organ Transplantation, University of Utah Hospitals and Clinics, Salt Lake City, UT.*

Purpose: Hyperlipidemia is a major risk factor for cardiovascular disease, the leading cause of morbidity and mortality in renal transplant recipients. A retrospective review was conducted to determine the safety and efficacy of a new cholesterol agent, ezetimibe (Zetia®), as an alternative treatment option for this patient population.

Methods: We evaluated renal transplant recipients currently receiving ezetimibe 10mg daily as monotherapy or combination therapy. Fasting lipid profiles were obtained at baseline, 1-6, 7-12 months post-ezetimibe initiation. ***Results:*** 21 renal transplant recipients were evaluated on ezetimibe therapy, the majority of patients being Caucasian (20/21). 12 were male and 9 were female with a median age of 53.1 years (range: 20 to 77 years). Eight patients had received a deceased donor and 13 received a live donor renal transplant. Seven patients had a history of CAD (2 MI and 4 CABG) and 16 patients had a history of HTN. Fifteen patients received cya therapy and 6 patients were on tac. Statin therapy was first line therapy for hyperlipidemia in all patients. Criteria for ezetimibe combination therapy was if the fasting lipid profile did not meet the NCEP III guideline goals for high risk patients after 6 weeks on another agent or reaching the maximum dose of that agent before acheiving goal. Monotherapy was initiated with ezetimibe if the patient did not tolerate the first line agent (s). Monotherapy was started in 3 patients, who had all previously failed statins, and combination therapy was utilized in 17 patients. Prior to ezetimibe therapy 5 patients had a TC <200mg/dl, 7 patients had a TG<150mg/dl, 10 patients had an HDL >60mg/dl and 4 patients had an LDL<100mg/dl. However, none of the patients had reached their lipid goal with the first-line agents. After therapy, 11 patients had a TC<200mg/dl, 12 patients had a TG<150mg/dl, 10 patients had an HDL >60mg/dl and 12 patients had an LDL <100mg/dl. The average reduction in TC and LDL was 20.9% and 24.5%, respectively, after 4.5 months of therapy. Two patients were switched from cyclosporine to tac after ezetimibe was initiated due to toxicity. The cya dose was decreased in 1 patient when diltiazem was initiated. There were no other alterations in cya or tacrolimus levels and no adverse events were attributable to ezetimibe treatment.

Conclusions: Ezetimibe as combination or monotherapy is a safe and effective treatment option for hyperlipidemia in renal transplant recipients without changes in CI levels.

Abstract# 1193 Poster Board #-Session: P123-III

TREATMENT OF SIROLIMUS INDUCED POST-TRANSPLANT HYPERLIPIDEMIA WITH ATORVASTATIN AND SLOW RELEASE NIACIN THERAPY. Martin S. Zand,[1] Richard Demme,[1] Jeremy Taylor.[1] *[1]Nephrology Unit, University of Rochester Medical Center, Rochester, NY.*

Hyperlipidemia and hypertryglyceridemia are known side effects of sirolimus immunosuppression therapy. Markedly elevated total and LDL cholesterol levels have often been cited as a reason for discontinuing sirolimus in renal transplant recipients. While most cases of sirolimus induced hyperlipidemia can be treated with single agent statin therapy, some patients require additional measures. We report successful reductions in total and LDL cholesterol in sirolimus treated renal transplant recipients using combination therapy with atorvastatin and slow release niacin. **Methods:** From November 2000 through June 2003 we had 5 renal transplant recipients on sirolimus based immunosuppression with total serum cholesterol levels above 300 mg/dl despite at therapy with atorvastatin (40 mg/day). Patients were started on slow release niacin (Niaspan) 500 mg q.h.s with 325 mg of aspirin, and the dose increased every 4 weeks until they experienced the side effect of flushing. All patients were followed for 6 months. **Results:** Total serum cholesterol levels declined from 334 ± 22 mg/dl to 296 ± 15 mg/dl (3 months) and 274 ± 26 mg/dl (6 months) (p<0.05). LDL cholesterol levels also declined from 153 ± 27 mg/dl to 124 ± 15 mg/dl (3 months) and 113 ± 32 mg/dl (6 months) (p<0.01). Triglyceride levels were not statistically different at 707 ± 105 mg/dl to 685 ± 135 mg/dl (6 months), nor were there significant changes in the HDL cholesterol levels at 40 ± 52 mg/dl and 42 ± 8 mg/dl. The most common side effect was flushing, although no patient had to be discontinued from the niacin. The mean dose at 6 months was 1000 mg per day. One patient had to be briefly withdrawn for acute gouty arthritis. No elevation in creatinine kinase levels was observed. Two patients had transaminase elevations, which improved when the dose of niacin was reduced. Despite improvements, no patient achieved NCEP recommended guidelines. **Conclusions:** The regimen of atorvastatin with slow release niacin can lower total and LDL cholesterol in patients with post-transplant hyperlipidemia on sirolimus based immunosuppression therapy.

Abstract# 1194 Poster Board #-Session: P124-III

ADIPONECTIN AND PROTECTION AGAINST POSTTRASPLANT DIABETES MELLITUS. Beatriz Bayés,[1] Ricardo Lauzurica,[1] Marisa Granada,[2] Asumpta Serra,[1] Nestor Fontseré,[1] Isabel Salinas,[3] Ramón Romero.[1] *[1]Nephrology, Hospital Universitari "Germans Trias i Pujol", 08916 Badalona, Barcelona, Spain; [2]Biochemistry, Hospital Universitari "Germans Trias i Pujol, 08916 Badalona, Barcelona, Spain; [3]Endocrinology, Hospital Universitari "Germans Trias i Pujol, 08916 Badalona, Barcelona, Spain.*

Background: Posttransplant diabetes mellitus (PTDM) is a serious complication of renal transplant (RT) with negative effects on patient and graft survival. Several risk factors for PTDM have been described, but does not exist any early marker that can predict it. Recently it has been demonstrated that adiponectin (APN) levels -an adipocyte-derived peptide with antiinflammatory and insulin-sensitising properties- are protective for future development of type 2 diabetes in healthy individuals.

Objective: To study pretransplant insulin resistance and to analyze pretransplant serum leptin and APN levels as independent risk factors for the development of PTDM.

Patients and methods: 31 RT patients with PTDM (mean age: 48±8 years, 80% males, BMI: 25±3 kg/m², 80% tacrolimus and 100% prednisone) and control group of 37 RT patients without PTDM matched by age, gender, BMI, immunosuppressor treatment and posttransplant time. Familiar history of DM was collected. Homeostasis model assessment for insulin resistance (HOMA-IR) index was measured. Serum leptin and APN concentration were determined by radioimmuno assay.

Results: PTDM patients showed higher pretrasplant HOMA index (PTDM: 5.1±3.4; non-PTDM: 3.6±2.9, p=0.042) and lower pretrasplant serum APN concentration (PTDM: 9.3±3.1 mg/ml; non-PTDM: 11.8±4.2 mg/ml, p=0.011). Pretrasplant serum leptin levels were similar in both groups (PTDM: 25.5±20.4 ; non-PTDM: 20.1±20.5, p=0.23). An inverse correlation between APN and BMI was observed (r=-0.29, p=0.027), APN and HOMA index (r=-0.45, p<0.001) and APN and PTDM (r=-0.31, p=0.011). There was direct correlation between leptin and BMI (r=0.27, p=0.04). Regression analisis showed that patients with lower APN pretransplant levels are those with greater risk for developing PTDM independently of age, gender, familiar history of DM and inmunosupressor treatment (Odds Ratio=0.832 (0.71-0.96), p=0.01).

Conclusions: Serum APN pretransplant level is an independent protective factor for PTDM development in renal patients.

Abstract# 1195 Poster Board #-Session: P125-III

A PROSPECTIVE RANDOMISED TRIAL OF MYCOPHENOLATE MOFETIL AND AZATHIOPRINE AFTER CALCINEURIN REDUCTION IN RENAL ALLOGRAFTS WITH ESTABLISHED CHRONIC ALLOGRAFT NEPHROPATHY. Nicholas R. Brook,[1] Matthew S. Metcalfe,[1] Julian R. Waller,[1] Sunjay Jain,[1] Sarah A. Hosgood,[1] Michael L. Nicholson.[1] *[1]Transplant Surgery, University of Leicester, Leicester, United Kingdom.*

Background

This trial investigated the effects of calcineurin dose reduction plus the introduction of a non-nephrotoxic immunosuppressive agent in renal allografts recipients with established chronic allograft nephropathy (CAN). The additional immunosuppressive agents studied were azathioprine (AZA) and mycophenolate mofetil (MMF), which is both non-nephrotoxic and has anti-proliferative effects that may be of benefit in renal allografts with fibrosis.

Patients and methods

Patients with biopsy proven CAN and receiving cyclosporin (CsA) or tacrolimus (Tac) based immunosuppression underwent a 40% dose reduction of their calcineurin inhibitor and were then randomised to receive either AZA (1 mg/kg/day) or MMF (1g bd). Renal allograft function was assessed by isotope GFR measurements. Allograft fibrosis was measured by computerised histomorphometry of protocol needle core biopsies performed at trial entry and 6 months later.

Results

21 patients receiving cyclosporin (AZA 11, MMF 10) and 22 patients receiving tacrolimus (AZA 10, MMF 12) were recruited into the study. All groups were well matched for potentially confounding variables. The pre-trial decline in GFR was reversed in both Tac groups, the CsA + MMF group but not in patients treated by CsApatients treated with CsA + AZA (Table). Renal allograft fibrosis increased during the 6 months of the trial in patients treated with CsA + MMF but remained stable in the other groups (table).

Conclusion

Tac reduction plus either MMF or AZA improved renal function without changing established allograft fibrosis. For patients receiving CsA, dose reduction plus MMF improved renal function despite significant worsening of allograft fibrosis, whilst AZA did not have this effect

	Δ post trial GFR slope (mean - ml/min/mth)		Δ biopsy fibrosis (mean % area fraction)	
CsA + MMF	+ 0.85	P<0.01	+13.3	P<0.01
CsA + AZA	-0.45	NS	+1.8	NS
Tac + MMF	+0.92	P<0.02	+1.1	NS
Tac + AZA	+1.25	P<0.05	-0.1	NS

Abstract# 1196 Poster Board #-Session: P126-III

CORRELATION OF RENAL RESISTANCE INDEX AND BIOPSY PROVEN CHRONIC ALLOGRAFT NEPHROPATHY: A PART OF THE GENERAL TRANSPLANT PHYSICAL EXAM. Eric A. Elster,[1] Douglas Hale,[1] Linda B. Cendales,[1] David Kleiner,[1] Micheal Ring,[1] Beverly Niles,[1] S. John Swanson,[1] Roslyn B. Mannon,[1] Allan D. Kirk.[1] *[1]Transplantation Branch, NIDDK/NIH, Bethesda, MD.*

Chronic allograft nephropathy (CAN) remains a common cause for renal allograft loss that is minimally responsive to therapy once graft dysfunction is clinically evident. A screening test to identify individuals at high risk for CAN could be a valuable adjunct to patient care, but to be cost effective should be administered during routine evaluations by transplant clinicians. We have compared the resistive index as measured by Doppler ultrasound with subsequent biopsy findings on 91 renal allograft recipients undergoing clinic evaluation who had a subsequent protocol biopsy at least 3 months after renal transplantation. All ultrasounds were performed in the transplant clinic by the transplant surgical staff as part of the general patient evaluation without involving the radiology department or separate appointment time. The ultrasound exam was a directed exam measuring general dimensions, fluid collections, hydronephrosis, and a cortical resistive index and typically lasted less than 10 minutes. The RI was calculated as 100 x [1-(Vmin / Vmax)] and averaged over two measurements. Biopsies were evaluated histologically using the Banff 1997 criteria. Twenty-one patients had a RI > or = 80 (average 621 days post transplant). Among these individuals, the subsequent incidence of CAN (CG1, CG2, or CG3) was 38%. The length of time between initial assessment of an increased RI and biopsy proven CAN averaged 233 days. The remaining 70 patients with a RI < 80 had an incidence of CAN of 11.4% (p=0.018, two tailed Fisher's test). The incidence of subsequent acute rejection was equivalent in both groups (p = 1.0). These results suggest that RI is an early predictor of histologically relevant CAN, possibly a result of burgeoning vasculopathy. The technical expertise required to make this appraisal is well within the capabilities of transplant clinicians and trainees. Early evidence of subsequent chronic injury as determined by ultrasonography may allow for targeted heightened scrutiny for CAN allowing a change in therapy prior to clinically significant injury. Ultrasound should become a routine part of a transplant clinic evaluation.

Abstract# 1197 Poster Board #-Session: P127-III

HYPERPARATHYROIDISM AND BONE TURNOVER IN THE FIRST YEAR FOLLOWING KIDNEY TRANSPLANTATION: A PROSPECTIVE STUDY. Martin Flamant,[1] Magali Ciroldi,[1] Dominique Prié,[2] Eric Thervet,[1] Frank Martinez,[1] Gérard Friedlander,[2] Christophe Legendre.[1] *[1]Service de Néphrologie, Hôpital Saint-Louis, Paris, France; [2]Laboratoire d'Explorations Fonctionnelles, Hôpital Bichat, Paris, France.*

After kidney transplantation, an important loss of bone mineral density is observed. The exact mechanism of such a bone loss is unknown, but there is evidence for a high bone resorption state. Several factors may play a role in this bone loss: use of immunosuppressive treatments like corticosteroids or ciclosporine, disturbances in vitamin D metabolism and persisting hyperparathyroidism. The aim of the present study was to evaluate the role of persisting hyperparathyroidism in the bone turnover in the first year following kidney transplantation.

Patients and methods.

Twenty eight patients who underwent kidney transplantation in our center were studied. Three months (M3) and twelve months (M12) after transplantation, markers of bone formation (serum osteocalcin (OC)) and bone resorption (urinary levels of deoxypyridoline [DPD]) were measured as well as parameters of calcium metabolism, including intact parathyroid hormone (iPTH), 25(OH) and 1,25OH vitamin D. The glomerular filtration rate was assessed using 51CrEDTA urinary clearance.

Results.

Three months after transplantation, elevated levels of iPTH were observed in 86% of patients (mean iPTH = 91,32 ± 9,07 pg/mL), despite normal levels of 25OH, 1,25 OH vitamin D and preserved graft function (mean GFR = 56,51 ± 3,8 ml/mn/1,73m2). A positive correlation between iCa and PTH was found (r = 0,47 p<0.05). Levels of OC and DPD were increased, suggesting a high bone turnover. These markers of bone remodeling were significantly higher in the group of patients who had an elevated iPTH.

At M12, the mean levels of iPTH were not different (84,75 ± 10,36 pg/mL , NS vs M3). There was no longer a correlation between iCa and PTH. Levels of OC significantly increased, whereas the DPD ones decreased, but there remained a high bone turnover state. Interestingly, the markers of bone turnover were not significantly higher in the group of patients who had an hyperparathyroidism.

Conclusion.

In spite of the persistence of a high basal iPTH one year after transplantation in the majority of kidney transplant recipients, there is a significant improvement of the parathyroid function. Taken together, these results suggests that the role of the persisting hyperparathyroidism in the high bone turnover at M12 might be minor when compared with other factors like immunosuppressive drugs.

Abstract# 1198 Poster Board #-Session: P128-III

LAPAROSCOPIC PROCUREMENT OF DONOR KIDNEYS WITH MULTIPLE RENAL ARTERIES DOES NOT COMPROMISE DONOR OUTCOME BUT IS ASSOCIATED WITH INCREASED URETERAL COMPLICATIONS IN THE RECIPIENT. Jonathan T. Carter,[1] Andrew M. Posselt,[1] Ryan A. McTaggart,[1] Harish D. Mahanty,[1] Sang-Mo Kang,[1] John P. Roberts,[1] Chris Freise.[1] *[1]Transplant Division, Univ. of California-San Francisco, San Francisco, CA.*

Purpose: Although laparoscopic nephrectomy is the preferred method for procuring donor kidneys with single arteries, experience in kidneys with multiple arteries is limited. In the present study, we compared donor and recipient outcomes after laparoscopic procurement of kidneys with single or multiple renal arteries.

Methods: From 11/99 to 11/03, 361 laparoscopic donor nephrectomies were performed at UCSF; 46 (13%) involved multiple renal arteries. All procedures were performed using four subcostal ports without hand-assist devices. Kidneys were removed through a transverse suprapubic incision. Accessory arteries were ligated in 14 donors and preserved in 32 donors. Laparoscopic donors with single arteries served as controls.

Results: Of the 32 organs with preserved multiple arteries, 20 were implanted separately, 9 were joined *ex-vivo* and implanted using a single arteriotomy, 2 were implanted end-to-side to the main renal artery, and one combined techniques. Of the 14 organs with ligated arteries, 13 arteries supplied the superior pole and one supplied the inferior pole. Donor and recipient data from each group are depicted in the table below as means±SD. § = p<0.05 versus controls.

Conclusions: Pure laparoscopic procurement of donor kidneys with multiple arteries can be performed with equivalent donor outcomes when compared to single artery kidneys. Although ultimate graft function is similar between the two groups, organs with accessory arteries requiring implantation are associated with longer warm ischemia time and more ureteral complications. Ligation of small-caliber superior pole arteries does not adversely affect graft function, but increases donor operative times. These data refute earlier reports of equivalent recipient outcomes using laparoscopically procured kidneys with multiple arteries.

	Multiple Arteries (n=46)		Controls (n=315)
	Utilized (n=32)	Ligated (n=14)	Single Artery
Donor Age (yr)	42.6 ± 11.9	43.2 ± 5.7	39.6 ± 11.6
Donor Gender			
Male	19 (59%)	4 (29%)	129 (41%)
Female	13 (41%)	10 (71%)	186 (59%)
Donor Side			
Left	27 (84%)	13 (93%)	272 (86%)
Right	5 (16%)	1 (7%)	43 (14%)
Operative time (min)	206 ± 50	229 ± 49§	202 ± 47
Estimated blood loss (ml)	75 ± 34	71 ± 22	76 ± 64
Donor length of stay (days)	3.39 ± 0.95	3.21 ± 0.60	3.23 ± 1.14
Extraction Time (seconds)	303 ± 115§	274 ± 84	255 ± 87
Warm ischemic time (min)	35.8 ± 9.6§	28.0 ± 5.7	28.7 ± 6.9
Donor complications			
Conversion to open	0 (0%)	0 (0%)	1 (0.3%)
Transfusion	1 (3%)	0 (0%)	3 (1%)
Bowel injury	0 (0%)	0 (0%)	2 (0.6%)
Wound infection	0 (0%)	1 (7%)	2 (0.6%)
Recipient			
Ureteral leak or stricture	6§ (19%)	1 (7%)	9 (3%)
% Cr change / initial 24h	-39.7 ± 19.6	-40.2 ± 18.3	-41.1 ± 21.8
Cr at 1 month	1.39 ± 0.62	1.60 ± 0.46	1.41 ± 0.6
Dialysis in first week	1 (3%)	0 (0%)	13 (4%)

Abstract# 1199 Poster Board #-Session: P129-III

PRESERVATION OR SACRIFICE: DETECTION OF SMALL RENAL ARTERIES WITH 3-DIMENSIONAL OR AXIAL COMPUTED TOMOGRAPHY FOR DECISION MAKING. Nobuyuki Fukuzawa,[1] Masayoshi Miura,[2] Hiroshi Harada,[1] Hirokazu Shinojima,[1] Toshimori Seki,[1] Masaki Togashi,[1] Tetsuo Hirano.[2] *[1]Department of Urology, Sapporo City General Hospital, Sapporo, Hokkaido, Japan; [2]Department of Renal transplantation, Sapporo City General Hospital, Sapporo, Hokkaido, Japan.*

OBJECTIVES: The purpose of this retrospective study was to evaluate the limitation of 3-dimensional computed tomography (3DCT) in visualizing small renal arteries and the influence of sacrificing such arteries on posttransplant renal function. METHODS: Forty-nine cases of living-related renal transplants were enrolled in this study. Kidneys were harvested with open donor nephrectomy (OLDN) in 27 cases and with hand-assisted laparoscopic donor nephrectomy (HALDN) in 22 cases. 3D images and axial images of arterial phase of CT were evaluated for diameter of prehilar branches and aberrent arteries. The effect of following factors on renal graft function were compared between the groups divided in terms of sacrificing small renal arteries: pre/post graft function ratio (24h creatinine clearance (24CCr) of the recipient at 1 month post-transplant divided by split 24CCr calculated using DTPA renal scan), short-term graft survival, velocity of serum creatinine (s-Cr) decrease within 7 postoperative days which is expressed as the area under s-Cr curve (sCrAUC0-7). RESULTS: Rate of failure to detect the prehilar branches or aberrent arteries are shown in the Figure. Overall detection rate is 79.6% with 3D image and 95.9% with axial image (p<0.05). There was no difference in rate of detection between OLDN and HALDN. Smaller renal arteries were sacrificed in 7 cases (14.3%) and the average diameter was 1.26 mm (0.87 to 1.90). Sacrificing did not result in decreased graft survival. sCrAUC0-7 was also comparable between the preseved group and the sacrificed group (14.7 vs 17.3). Change in graft function (recipient 1 month 24CCr/donor split 24CCr) was not different between preserved and sacrificed group (1.26+-0.42 vs 1.52+-0.35). CONCLUSIONS: Axial image is superior to 3D image in detection of small renal arteries. However, sacrificing smaller arteries less than 1.9 mm in diameter did not affect the posttransplant graft function.

Failure rate of small renal arteries (%)

types of small artery	prehilar branches			aberrent renal arteries		
	total	OLDN	HALDN	total	OLDN	HALDN
Surgery + / 3DCT -	16.6	33.3	33.3	50.0	50.0	50.0
Surgery + / Axial -	0	0	0	12.5	0	12.5
Axial + / Surgery -	33.3	33.3	33.3	12.5	0	12.5
sacrificed arteries (%)	25	25	0	50	0	50
average of arterial diameter (mm)	2.25	2.04	2.45	1.72	2.38	1.05

Surgery + / - : vessels found during surgery / not found

Abstract# 1200 Poster Board #-Session: P130-III

IMPACT OF KIDNEY WEIGHT-BODY WEIGHT RATIO ON ALLOGRAFT FUNCTION IN LIVING RENAL TRANSPLANTATION. Mukut Minz,[1] Navalkishor Udgiri,[1] Munish K. Heer,[1] M. S. Reddy,[1] Vivekanand Jha,[2] Randeep Kashyap.[1] *[1]Transplant Unit, Deptt of General Surgery, PGIMER, Chandigarh, UT, India; [2]Nephrology, PGIMER, Chandigarh, UT, India.*

Introduction

Kidneys with insufficient functional renal mass for the recipient may be damaged by hyperfiltration injury resulting into proteinuria & allograft loss.

Aim: To study the effect of donor kidney weight and recipient body weight ratio on allograft function at discharge and follow up.

Material and Methods: 77 living renal allograft recipients and their donors were included in this study. Mean age of recipients and donors was 32.87±9.73yrs and 43.81±11.44yrs respectively. Sex Ratio (M:F) among recipients and donors was 62:15 and 33:44 respectively. Donor kidney weight was recorded. Postoperatively, renal allograft function was compared with kidney weight and recipient body weight ratio. For study purposes, patients were divided into two groups based on ratio.

Results: Out of 77 recipients, 39 were in high ratio group (2.73-5.4) and 38 were in low ratio (1.52-2.72). Mean serum creatinine at discharge in the two groups were 1.2 & 1.4mg% (P=0.017) and creatinine clearance were 59.4 and 61.8 ml/min respectively. In both the groups, there was a significant correlation between the ratio to serum Creatinine, creatinine clearance at discharge and serum creatinine at one month (Spearman's correlation, P=0.01 and P=0.04). By linear regression analysis, it was found that donor kidney weight/recipient body weight ratio in both the groups was independently affecting. Sr. Creatinine at discharge and creatinine clearance at discharge (r=0.67, P=0.001 and P=0.003 respectively).

Conclusion - Donor kidney weight/recipient body weight ratio is a good predictor of renal function at discharge and 1 month.

Abstract# 1201 Poster Board #-Session: P131-III

IMPACT OF RENAL ALLOGRAFT IMMUNOGLOBULIN A DEPOSITS ATTRIBUTABLE TO DONOR ON THE OUTCOME OF LIVING-RELATED RENAL TRANSPLANTATION. Kanji Nagahama,[1] Shingo Yamamoto,[1] Noriyuki Ito,[1] Hidehumi Kinoshita,[1] Toshiyuki Kamoto,[1] Hiroshi Okuno,[3] Takahiko Ono,[2] Osamu Ogawa.[1] *[1]Department of Urology & Dialysis, Kyoto University, Kyoto, Japan; [2]Department of Nephrology, Kyoto University, Kyoto, Japan; [3]Department of Urology, Kyoto National Hospital, Kyoto, Japan.*

Introduction and Objectives:

Although mesangial immunoglobulin A (IgA) deposits are occasionally observed in the allograft biopsies, the frequency and impact on renal function of the IgA deposition has not been clearly elucidated in living-related renal transplantation (RTX). Thus, we examined the correlation between the duration of IgA deposition and the outcome of RTX.

Methods:

Out of 41 living-related RTX cases, 15 cases were associated with mesangial IgA deposits in renal allografts, which were revealed by episode as well as sequential protocol biopsies (1 hour, 3 months, 1 year and 3 years after RTX). These cases were classified into 3 groups based on the results of biopsies. Group A (4 cases): IgA deposits were observed in 1-hour biopsies but disappeared within 3 months. Group B (3 cases): IgA deposits were found both in 1-hour and 3-month biopsies. Group C (8 cases): IgA deposits were first discovered more than one month after RTX. The allograft functions were compared among these groups in terms of proteinuria, hematuria and creatinine clearance (CCr) at one year post-transplantation, to determine the relationship between the appearance of IgA deposits and IgA nephropathy after RTX.

Results:
One of 4 cases in Group A, 2 of 3 in Group B, and 5 of 8 in Group C had originally IgA nephropathy. The mean CCr was 56.9 ± 6.9 ml/min in Group A, 33.0 ± 16.1 ml/min in Group B, and 65.7 ± 17.7 ml/min in Group C, respectively ($p < 0.05$; Group A vs. Group B, $p < 0.05$; Group B vs. Group C). In Group A, hematuria and proteinuria were found in 50 % and 25 %, respectively, while both were in all the cases of Group B ($p < 0.05$ in proteinuria). The frequency of hematuria and proteinuria in Group B were also higher than those of group C (87.5 % and 37.5 %, respectively; p=0.063 in proteinuria).
Conclusions:
IgA deposits observed in 1-hour biopsies and maintenance of these deposits, especially in recipients with IgA nephropathy as their original disease, could represent a risk of allograft dysfunction, although allograft functions are rarely impaired only by IgA recurrence. These findings suggest that a combination of certain donors and recipient factors interfere with renal allograft functions.

Abstract# 1202 Poster Board #-Session: P132-III

RENAL FUNCTION AND HORMONE OUTPUT. A PROSPECITVE STUDY COMPARING OPEN, LAPAROSCOPIC AND RETROPERITONEOSCOPIC LIVE DONOR NEPHRECTOMY. Pernilla Sundqvist,[1] Ulla Feuk,[2] Mikael Haggman,[1] A. Erik G. Persson,[3] Mats Stridsberg,[4] Jonas Wadstrom.[1] *[1]Department of Surgical Sciences, Uppsala University Hospital, Uppsala, Sweden; [2]Simrishamn Hospital, Simrishamn, Sweden; [3]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden; [4]Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden.*
Hand-assisted retroperitoneoscopic nephrectomy (HARS) is a new surgical approach for living donor nephrectomy. The HARS technique was, in a prospective study, compared to transperitoneal laparoscopic nephrectomy (LAP) and open nephrectomy (OPEN) with regard to the perioperative renal function and hormonal response.
Methods: 36 donors were included and divided into HARS, LAP and OPEN groups according to the surgical technique applied. During the operation and on postoperative day 1 and 2 renal function and hormone output (aldosterone, plasma active renin, N-ANP, BNP and catecholamines) were measured. Donor renal function and allograft outcome was then followed for one year.
Results: There was an activation of the renin-angiotensin-aldosterone system as demonstrated by elevated levels of aldosterone and active renin in arterial blood during surgery. Increased levels of active renin were maintained in the OPEN group postoperatively. The concentrations of aldosterone were higher in the OPEN group during surgery compared to the LAP group. There was no change in N-ANP and BNP responses during surgery but a marked six-fold increase of these peptides on postoperative days 1 and 2 in all groups. A general output of catecholamines during surgery was seen, though endoscopic techniques had a higher catecholamines release. Urine flow rate decreased in all groups during the operation, though there were no significant differences between the groups. A reduction of sodium concentration in the urine was seen in all groups. GFR decreased from 150 ml/min preoperatively to about 50 ml/min at 60 min into surgery, recovering to 100 ml/min on the first postoperative day, with a similar pattern in all donor groups. Postoperatively, kidney function in donors seems to be less compromised with HARS compared to LAP, demonstrated as a smaller rise in creatinine at time of discharge. HARS recipients have a better creatinine clearance than the other groups in the early post-transplantation period.
Conclusion: Evaluation of a newly developed surgical technique, HARS, shows that the renal function is somewhat favorable for donors and recipients compared to open surgery and transperitoneal laparoscopic approaches. The hormonal response shows only small differences between the different surgical techniques.

Abstract# 1203 Poster Board #-Session: P133-III

HLA MATCHING AND OUTCOME OF LIVING DONOR KIDNEY TRANSPLANTS IN THE UK. Rachel J. Johnson,[1] Rachel A. Hodge,[1] Susan V. Fuggle,[1] Christopher J. Rudge,[1] John L. R. Forsythe.[1] *[1]on Behalf of the UK Transplant Kidney and Pancreas Advisory Group, UK Transplant, Bristol, United Kingdom.*
Background: The number of kidney transplants from living unrelated donors has been increasing rapidly in the UK. These transplants are usually less well matched than those from cadaveric heartbeating donors (HBD) or living related donors (LRD). This study investigates the degree of HLA match in transplants from the different donor types (1995-2002) and the influence of HLA match and other factors on outcome of all living donor transplants in the UK (1998-2002).
Methods: HLA-A, B and DR mismatches of 2075 living related and unrelated kidney donor transplants and 10786 cadaveric HBD transplants were compared. For a subset of 1267 LRD and 302 living unrelated donor transplants from 1998-2002, the transplant survival time (time from transplant to graft failure or patient death) was calculated and analysed.
Results: As expected, transplants from living unrelated donors were significantly less well matched. In particular, there were two HLA-DR mismatches in 41% of living unrelated donor transplants but less than 5% in both LRD and cadaveric HBD transplants. There were no significant differences in one-year transplant survival between the two living donor transplant groups. For all living donor transplants one-year transplant survival was 93% (95% confidence interval (CI) 92-94%), while transplant survival of cadaveric HBD grafts (87%, 95% CI 87-88%) was inferior ($p<0.0001$). There was no difference in one-year transplant survival between the least well matched (2 HLA-DR mismatches) living unrelated donor transplants (90%, 95% CI 85-95%) and 000 mismatched cadaveric HBD transplants (89%, 95% CI 87-91%). Of the factors analysed, Cox regression modelling showed only older donor age ($p<0.01$) and recipient gender female ($p<0.03$) to have a significant adverse effect on one-year transplant survival of living donor transplants. There was evidence of an adverse effect of two HLA-DR mismatches in the living unrelated donor transplants in the early half of the cohort that was not apparent in the later transplants.
Conclusion: Living unrelated donor transplants were significantly less well HLA matched than other donor transplants. One-year transplant survival was comparable for LRD and unrelated donor transplants and superior to survival of cadaveric HBD transplants. Multifactorial analysis of one-year outcome of living donor transplants showed significant effects of donor age and recipient gender. An adverse effect of two HLA-DR mismatches in living unrelated donor transplants was apparent only in the earlier years analysed.

Abstract# 1204 Poster Board #-Session: P134-III

LAPAROSCOPIC RIGHT LIVE DONOR NEPHRECTOMY: REFINING TECHNIQUE. Alun R. Williams,[1] Magdi Shehata.[1] *[1]Nottingham Kidney Transplant Unit, Nottingham City Hospital, Nottingham, United Kingdom.*
Introduction
The left kidney is usually chosen for laparoscopic live donor nephrectomy principally because of the greater length of its vessels. Laparoscopic right nephrectomy is technically easier, although some concern has been expressed because of it relatively shorter vessels. We report our experience with laparoscopic right donor nephrectomy. Initially, we utilized open clamp placement through a right subcostal incision. In 2001, we adopted the technique of purely laparoscopic right donor nephrectomy and describe our experience.
Procedure
Donor position is 45° left decubitus with table tilt available. Pneumoperitoneum is created with Verres needle unless contraindicated. The right colon/hepatic flexure are mobililsed. A Nathanson liver retractor is used to retract the right lobe of liver. The duodenum is Kocherised to display the entire width of inferior vena cava (IVC) exposing confluences with right suprarenal, renal and gonadal veins. Dissection commences lateral to the gonadal vein and proceeeds cephalad, followed by lateral dissection of the ureter to dissect the inferior pole of the kidney. Meticulous dissection of the right posterolateral border of the vena cava displays the renal artery and allows an endoscopic stapler to be deployed flush with the vessel. We retrieve kidneys via a 6cm Pfannenstiel incision.
Results
From 2001–2003, 10 right laparososcopic donor nephrectomies were performed (8 purely laparoscopic). Mean operative time was 156 (range 100–200) minutes. Mean warm ischaemic time was 5 (range 2–9) minutes. Mean postoperative stay was 4 (range 2–8) days. There were no conversions to open. There was one wound infection and one episode of postoperative urinary retention.
No prosthetic/autologous grafts were required to provide additional vessel length. Three of 10 recipients required internal iliac vein ligation to allow comfortable venous anastomosis. No manouevres were required to gain extra arterial length although 3/10 required accessory arterial anastomoses. All grafts functioned immediately with no technical complications.
Conclusions
Laparoscopic right donor nephrectomy is technically easier than left, on account of access and fewer venous tributaries. Complete duodenal mobilisation is crucial to display sufficient IVC for attaining adequate vessel length. In our experience, for anastomosing a short renal vein, mobilisation of the recipient external iliac vein has alone been sufficient. We have not experienced insufficient arterial length and have therefore felt it unnecessary to perform extensive retrocaval dissection or formally measure vessel length

Abstract# 1205 Poster Board #-Session: P135-III

LAPAROSCOPIC DONOR NEPHRECTOMY YIELDS KIDNEYS THAT ARE STRUCTURALLY AND FUNCTIONALLY EQUIVALENT TO THOSE PROCURED BY OPEN SURGERY - RESULTS OF A RANDOMISED TRIAL. Nicholas R. Brook,[1] Gareth R. R. Lewis,[1] Julian R. Waller,[1] Jennifer C. Bains,[1] Peter S. Veitch,[1] Michael L. Nicholson.[1] *[1]Transplant Surgery, University of Leicester, Leicester, United Kingdom.*

Background

Laparosopcic transperitoneal donor nephrectomy (LapDN) may reduce disincentives to donors and thereby increase transplant activity. The technical challenges of the operation have raised concerns over the quality of the procured allografts. This study reports on the anatomical and functional outcomes of kidneys retrieved from live donors entered into a randomised controlled trial of open versus laparoscopic procurement.

Methods

Sixty live renal donors were randomised to open (17 left and 3 right kidney) or transperitoneal laparoscopic (35 left and 5 right kidney) nephrectomy on a 1:2 basis. The same two consultant surgeons performed operations.

Results

The open and laparoscopic groups were well matched for age (P=0.18) and body mass index (P=0.49, two tailed t-test). Operating time was longer for the laparoscopic donors (186 versus 135 minutes, P=0.0001). Procured kidney weight did not differ between groups (P=0.21). First warm ischaemic time was longer for the laparoscopic donors (3.6 vs 2 minutes, P<0.001), but total warm time was no different (32.3 vs 33.1 minutes, P=0.52). Left renal vein length (36 versus 39 mm, P=0.14) and left renal artery length (31 versus 32mm, P=0.38) were similar. No differences in right vessel length were observed. Ureteric length was significantly greater in the laparoscopic group (118 vs 101mm, P=0.011).

Conclusions

This study demonstrates that LapDN yields kidneys that are structurally equivalent to those acquired by the open operation. Furthermore, the longer first warm ischaemic time suffered by laparoscopically-retrieved grafts has no effect on post-transplant renal function. This data may go some way towards allaying concerns over the effect of laparoscopic procurement on live donor kidneys.

Serum Creatinine (micromol/l)	Open	Laparoscopic	P value
2 weeks post -op	133 (±60)	141(±99)	0.61
24 weeks	138 (± 42)	124 (± 28)	0.38
52 weeks	130 (± 43)	130 (± 38)	0.63

KIDNEY: COMPLICATIONS, IMMUNOSUPPRESSION AND CMV

Abstract# 1206 Poster Board #-Session: P136-III

PROPHYLAXIS AGAINST CYTOMEGALOVIRUS(CMV) USING GANCICLOVIR(G) OR VALGANCICLOVIR(V) IN KIDNEY(K) AND KIDNEY-PANCREAS(SPK) TRANSPLANTATION UNDER ANTIBODY PRECONDITIONING. Amit Basu,[1] Kenan Keven,[1] Sunil Sharma,[1] Henkie Tan,[1] Akhtar Khan,[1] Amadeo Marcos,[1] John Fung,[1] Thomas Starzl,[1] Ron Shapiro.[1] *[1]Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

We investigated retrospectively the occurrence of CMV disease and aimed to determine the risk factors for CMV under G(n=75) or V(n=136) prophylaxis (oral G, 1 g tid: V, 450 mg/day) in our kidney (K) and simultaneous pancreas-kidney (SPK) transplant patients operated between 1 July, 2001-February 28, 2003. **Methods:**Two hundred and eleven patients (Mean age 48.5± 14.6 yrs, 82 F, 182 K, 29 SPK, 71 Living donor) using prophylactic oral G or V were included in the study.178 patients received Thymoglobulin and 33 received Campath1H as pre-conditioning agents. Mean duration of prophylaxis against CMV was 4.5± 1.6 months and timing of CMV disease was 4.1± 1.8 months after transplantation. Minimum follow up was 6 months and 176 patients were followed for one year. **Results:**Fifteen of 211(7.1%) and 18 of 176 (10.2%) patients developed CMV infection at 6 months and 1 year follow-up, respectively. No patient developed tissue invasive CMV disease. Risk factors(Table 1) for CMV infection were evaluated using Mantel-Haenszel and Cochran's tests. At 6 months, V prophylaxis resulted in lower incidence of CMV than G (p=0.052).Donor CMV positivity led to a higher incidence of CMV infection at 6(p=0.014) and 12 months (p=0.003). Prophylaxis longer than 3 months resulted in a lower incidence of CMV infection at 6(p=0.04) and 12 months(p=0.009). D+/R- pts. had higher risk of developing CMV infection at 6(p=0.013) and 12 months(p=0.018). Whilst prophylaxis with V is more effective compared to G at 6 months (p=0.052), the two groups were not different for duration of prophylaxis(V:4.3+/-1.3months vs G:4.8+/-2months,p=0.06) and D/R groups(Table 2).**Conclusion:**V appears to be more effective than G for prevention of CMV infection in K and SPK recipients using antibody pre-conditioning, and should be used >3 months in high-risk patients.

Table1: Risk of CMV infection

	Pts with CMV		p
Prophylactic agent	**Ganciclovir**	**Valganciclovir**	
6 months	12%	**4.4%**	0.052
12 months	12.7%	8.6%	0.53
Donor CMV Ab	**positive**	**negative**	
6 months	11.7%	**2.0%**	0.014
12 months	16.0%	2.4%	0.003
Duration of prophylaxis	**>3 months**	**3 months**	
6 months	3.9%	12.2%	0.04
12 months	5.3%	19.9%	0.009

Table2:Donor/Recipient CMV status in the G and V groups

	Ganciclovir(n=75)	Valganciclovir(n=136)	p
D-/R-	24%	25%	0.99
D-/R+	27%	25%	0.22
D+/R-	28%	22%	0.35
D+/R+	31%	28%	0.79

Abstract# 1207 Poster Board #-Session: P137-III

ADVERSE IMPACT OF STEROIDS ON OUTCOME IN THE ALERT STUDY. Alan G. Jardine,[1] Hallvard Holdaas,[2] Bengt Fellstrom,[3] on Behalf of the ALERT Investigators.[1] *[1]University of Glasgow, Glasgow, United Kingdom; [2]Rikshospitalet, Oslo, Norway; [3]University Hospital, Uppsala, Sweden.*

The ALERT study followed 2,102 long-term renal transplant recipients, randomised to Fluvastatin or placebo, for 5-6 years and demonstrated a reduction in myocardial infarction and cardiac death in statin recipients. In addition, the placebo arm provides extensive data on the determinants of cardiovascular outcomes and mortality in this population. In this analysis we have investigated the impact of immunosuppressive agents on outcomes in the placebo arm of the study.

All 1052 patients received cyclosporine. In addition, 660 patients received azathioprine, 49 patients received mycophenolate and 819 patients received prednisolone. During follow-up 134 patients experienced a major adverse cardiac event (MACE - myocardial infarction, cardiac death or cardiac intervention). Prednisolone use was associated with an increased risk of MACE (hazard ratio [95% CI]- 1.55 [0.97,2.47], P=0.07), whereas azathioprine and MMF treatment had no impact on MACE. The impact of prednisolone on definite MI and cardiac death was more significant, hazard ratio - 1.85 [1.05, 3.25], P=0.03. There was an even stronger impact on all cause mortality, hazard ratio - 2.00 [1.20, 3.33], P=0.008. Neither MMF, nor azathioprine use had a significant impact on these end points.

In conclusion, in the placebo arm of the ALERT study, prednisolone use was associated with a significant increase in cardiac events and all-cause mortality. These data support the development of steroid-free regimes for the prevention of cardiovascular complications in renal transplant recipients.

Abstract# 1208 Poster Board #-Session: P138-III

IS IT NECESSARY TO WITHHOLD NEORAL® (CSA) THERAPY DURING DELAYED GRAFT FUNCTION IN RENAL TRANSPLANT RECIPIENTS? Flavio Vincenti,[1] Jimmy Light,[2] Thomas Pearson,[3] You Min Wu,[4] John Curtis,[5] Robert Mendez,[6] Ralph Barbeito.[7] *[1]University of California, San Francisco, CA; [2]Washington Hospital Center, Washington, DC; [3]Emory University, Atlanta, GA; [4]University of Iowa, Iowa City, IA; [5]University of Alabama, Birmingham, AL; [6]National Institute of Transplantation, Los Angeles, CA; [7]Novartis Pharmaceuticals Corp., E Hanover, NJ.*

Purpose: In patients with delayed graft function (DGF), a current practice is to withhold calcineurin inhibitors due to their nephrotoxic propensity, presumably to facilitate recovery of function in the allograft. We examined this practice using results of a six month, multicenter, randomized trial using a quadruple immunosuppression regimen. In this study, CsA dose was adjusted to target levels using CsA concentrations two-hours post dose (C2). DGF was defined as either a reduction from baseline serum creatinine (SrCr) ≤20% or the need for dialysis over the first three days post-transplantation.

Methods: Adult renal transplant recipients were recruited at 14 centers. All patients were to receive a regimen of CsA, basiliximab (20mg, Days 0 and 4), mycophenolate mofetil (1 gm BID), and prednisone (per center protocol). The C2 targets were 1.7 and 1.5 ug/mL for Months 1 and 2, respectively, with progressively lower targets over the following 4 months. The study allowed delay of CsA and/or use of antilymphocyte therapy during DGF.

Results: 33/119 patients met the definition of DGF. The mean (± SD) age was 52 (± 12) years, 82% were male, 21% were African American, and cold ischemia time was 21.4 (± 7.7) hours. 31/33 received both doses of basiliximab with 6 patients receiving ATG. All but 3 patients started Neoral by Day 3 and remained on Neoral while in the study. Biopsy-proven acute rejection occurred in 3 (9%) of the DGF patients, 2/6 in the ATG treated patients and 1/27 in the Simulect® only patients. There was one failed graft and no deaths. 25/33 (76%) completed the study; with 6/8 discontinuations occurring

within the first 18 days of the transplant. Renal function, as measured by SrCr, showed good recovery with values comparable to patients with initial graft function (IGF) from the same study; mean (± SD) values were 6.0 (± 3.2) mg/dL vs. 1.8 (± 0.9) on Day 7, and 1.6 (± 0.7) vs. 1.6 (± 0.7) at study end.

Conclusions: These data suggest that for the great majority of patients with DGF, good recovery of function in the kidney allograft can be achieved by C2-monitored Neoral at reduced levels with basiliximab induction, without an increased risk of acute rejection. These results await corroboration from clinical trials designed to assess this question more directly.

Abstract# 1209 Poster Board #-Session: P139-III

PRE-TRANSPLANT RISK FACTORS FOR UK RENAL TRANSPLANT PATIENT WITH CICLOSPORIN. Xiang He, Atholl Johnston. *Clinical Pharmacology, William Harvey Research Institute, Barts and The London, London, England, United Kingdom.*

Aims: Following the introduction of ciclosporin, 2 year graft survival of transplanted kidneys improved from <60% to >80%. Many studies have investigated the risk factors for graft survival, but little attentions has been paid to pre-transplant factors. This analysis investigates the impact of demographics, co-existing diseases, blood pressure, duration of dialysis undertook, blood transfusion and treatment combinations on long-term graft survival with renal transplant recipients from 39 of 44 transplant centres in UK. Two models have been developed of pre-transplant variables that predict outcomes with the aim of improving the quality of care received by recipients.

Methods: Data were available from the Long Term Efficacy and Safety Surveillance study (LOTESS). A total of 4142 patients (recipient and donor age ≥15) with at least for 5 years follow-up were evaluated. The potential risk factors associated with outcome in renal transplant recipients treated with ciclosporin were explored. The endpoints considered were graft failure (included patient death with a functioning graft) and pure graft loss. A stepwise stratified cox model was used to identify donor, recipient and treatment factors that were related to outcome.

Results: See figure

Conclusion: There were only 1658 kidney transplants during 2002 in the UK but the waiting list stands at around 5000 individuals. It is important therefore to maximise graft survival. The analysis suggest that older donor, older recipient, high systolic blood pressure, cadevaric donor, diabetes, BMI not in the normal range (18-25), HLA_Dr mismatch and choose single or double rather than triple therapy are risk factors for graft failure. For pure graft loss, older donor, younger recipient, high systolic blood pressure, cadevaric donor, BMI not in the normal range (18-25), HLA_Dr mismatch and mono- or double- rather than triple therapy are risk factors.

	Graft failure (included patient death)			Pure graft loss		
Variable Name	Hazard Ratio (HR)	95% HR Confident Limits		Hazard Ratio (HR)	95% HR Confident Limits	
Donor age (year)	1.009	1.004	1.009	1.015	1.008	1.014
Recipient age (year)	1.013	1.007	1.013	0.981	0.974	0.989
Systolic blood pressure (mmHg)	1.006	1.002	1.008	1.009	1.004	1.014
Living donor	0.565	0.373	0.565	0.569	0.360	0.901
BMI (18-25)	0.803	0.677	0.803	0.720	0.568	0.913
HLA_Dr (0)	0.808	0.698	0.808	0.791	0.650	0.963
Diabetes	1.738	1.445	2.091			
Pred-Aza-CsA	0.751	0.649	0.751	0.635	0.522	0.773

Abstract# 1210 Poster Board #-Session: P140-III

COMPARABLE RENAL FUNCTION IN LIVING KIDNEY DONORS AND THEIR RECIPIENTS ON TACROLIMUS-BASED IMMUNOSUPPRESSION. Elly M. van Duijnhoven,[1] Henricus J. A. Dackus,[1] Maarten H. L. Christiaans,[1] Johannes P. van Hooff.[1] *[1]Department of Internal Medicine, University Hospital Maastricht, Maastricht, Netherlands.*

Nephrotoxicity of calcineurin inhibitors is an important cause of renal failure after organ transplantation. Studies in healthy volunteers showed that, in contrast to cyclosporin, a short course of tacrolimus did not influence renal function. Also in cadaveric renal allografts tacrolimus was less nephrotoxic than ciclosporin(1-2). The function of a cadaveric renal allograft is strongly influenced by the quality of the kidney and ischaemic times. The quality of kidneys from living donor is generally good and ischaemic times are short. Therefore in these patients renal function of the donated kidney can be compared with the renal function of the donor. In doing so, the long-term nephrotoxicity of tacrolimus can be assessed.

Methods: All consecutive recipients of living donor kidneys transplanted under tacrolimus-based therapy and with a functioning graft at year 1 were included (n=41). Tacrolimus levels were measured regularly. Target range: 15-20 ng/mL day 0-14, 10-15 ng/mL up to 1 month and thereafter <10 ng/mL. Renal function was calculated by Cockcroft-Gault (3). Median renal function was compared by Wilcoxon matched-pais signed rank sum test at month 3 and year 1.

Results: Median tacrolimus trough levels were 9.9 and 7.8 ng/ml at month 3 and year 1. After donation, in both donors and recipients, median renal function of one kidney was approximately 70% of the original function of both kidneys. Median renal function of the donors and recipients were comparable at 1 year (60-65 ml/min for both donor and recipient,p=0.08).

Renal function in living kidney donors and their recipients

Time	Donors	Recipients	P
Before transplantation	89		
Month 3 after transplantation	61	60	0.016
Year 1 after transplantation	64	63	0.082

median Creatinine clearance (ml/min)

Conclusion: At three months and one year after transplantation, renal function of kidney donors and their recipients was almost equalunder tacrolimus-based therapy.

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Abstract# 1211 Poster Board #-Session: P141-III

SERUM SICKNESS DOES NOT SEEM TO BE DUE TO ANTI-Gal IMMUNIZATION. Joanna Ashton-Chess,[1] Guillaume Meurette,[1] Jeanne Naulet,[1] Maghed Ekklas,[1] Jean-Paul Soulillou,[1] Gilles Blancho.[1] *[1]INSERM U437, ITERT, Nantes, France.*

Serum sickness (SS) is a disease encountered in transplanted patients receiving xenogeneic heterospecific antibody therapy such as antithymocyte globulin (ATG) at the induction phase. Its pathogenesis results from a sensitization to the xenogenic reagent leading to an immune complex disease with symptoms including fever, cutaneous rash and prominent arthralgia. No obvious dominant xenogenic Ag has been identified as being responsible for this disease. It has recently been shown that rabbit as well as horse ATG express the dominant xenogenic Ag, the gala1-3gal disaccharides, against which humans and old world primates have natural Ab. Moreover, it is now well known that this Ag may elicit an induced Ab response.

The aim of our work was to determine whether anti-Gal Ab could be involved in the development of SS.

For this purpose, we studied the kinetic modulation of circulating anti-Gal Ab titers at the day of transplantation, at the onset of the disease and four to five weeks later at a stable state. Twelve patients that received ATG and subsequently developed SS were compared with patients also treated by ATG but without SS (n = 12). Finally a group of patients receiving no ATG served as a control group (n = 8). None of these latter control patients showed any modulation of their anti-Gal Ab. On the contrary, in the SS group, 6 of the 12 patients showed a mean increase of 70% (32-120%) of their anti-Gal IgG titer versus 4 of the 12 patients from the ATG without SS group who had an equal mean increase of 70% (42-100%).

This difference is not significant and suggests that the exposure of patients to gal positive xenogenic reagents may induce an evoked Ab response that does not seem to be responsible for the pathogenesis of the SS. However, the anti-Gal modulation we observed was simply a reflection in the periphery of a potential immunization but did not necessarily reflect the local inflammatory phenomenon, in particular inside joints, involving immune complexes. An additional study will be conducted on these patients to further explore their potential circulating immune complexes.

Abstract# 1212 Poster Board #-Session: P142-III

PHARMACOKINETICS AND PHARMACODYNAMICS OF ISA247 A NEW-GENERATION CALCINEURIN INHIBITOR IN STABLE RENAL TRANSPLANT PATIENTS. Patrick Mayo,[1] Derrick Freitag,[1] Bruno Boutouyrie-Dumont,[2] Mahdi Farhan.[3] *[1]Isotechnika Inc., Edmonton, AB, Canada; [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland; [3]Roche Products Ltd, Welwyn Garden City, Herts, United Kingdom.*

Introduction This study compared the pharmacokinetics (PK) and pharmacodynamics (PD) of ISA247, a new-generation calcineurin inhibitor, with cyclosporine (CsA) using data from a phase II, randomized, multi-centre, open-label study in stable renal transplant patients. **Methods** Stable renal transplant patients (≥6 months post-transplant) on an established dose of CsA were randomized to either continue CsA or switch to ISA247 for a 12 week period. ISA247 was estimated in pre-clinical and phase I studies to be at least 3-fold more potent that CsA and was dosed at one-third of the established CsA dose (mean study dose; 3.0±1.5 vs 1.2±0.6 mg/kg). At weeks 1, 6, and 12, serial whole blood samples were drawn and drug concentrations were determined. PK and PD were evaluated using standard non-compartmental analysis and a calcineurin inhibition assay respectively. A direct PK-PD correlation was performed using a sigmoid E_{max} model. **Results:** A total of 132 patients were recruited and randomized to ISA247 (n=65) and CsA (n=67), respectively. Patient demographics were similar between the groups with the exception of age (47.1±10.5 vs 52.0±11.0 years, p<0.05). Time to maximum concentration (t_{max}) and $t_{1/2}$ were similar between the two drugs. For ISA247, the maximum concentration (C_{max}) and the area under the concentration-time curve from 0-8 hours ($AUC_{(0-8)}$) were approximately one third those of CsA.

Pharmacokinetic parameters

Drug	t_{max} (h)	C_{max} (ng/mL)	$AUC_{(0-8)}$ (ng·h/mL)	$t_{1/2}$ (h)
ISA247	2.0±0.8	191.8±99.6	784.5±379.8	4.0±1.4
CsA	1.6±0.8	668.6±245.0	2357.6±851.7	3.6±1.1

Correlation of combined C1 and C2 concentration data with percentage calcineurin inhibition revealed the following:

Correlation parameters

Parameter	ISA247	CsA
E_{max} (%)	71.0±12.0	78.2±25.5
EC_{50} (ng/mL)	129.3±29.1	374.6±246.7
Sigmoidicity	1.9±0.6	1.1±0.5
Correlation	0.8	0.6
Akaike Information Criteria	401	1042

E_{max}=Maximum percentage calcineurin inhibition; EC_{50}=Concentration of drug that provides 50% of E_{max}

Conclusions ISA247 demonstrated similar pharmacokinetic properties to CsA but consistently demonstrated less variability and greater predictability in all analyses. Despite 3-fold lower mean AUC values for ISA247, percentage calcineurin inhibition was similar for both drugs. A comparison of EC_{50} values confirms that ISA247 is 3-fold more potent than CsA in stable renal transplant patients.

Abstract# 1213 Poster Board #-Session: P143-III

COMBINED USE OF SIROLIMUS AND VORICONAZOLE IN RENAL TRANSPLANTATION. A. Scott Mathis,[1,2] Nita K. Shah,[3] Gary S. Friedman.[4] *[1]Pharmacy Practice, Rutgers University, Piscataway, NJ; [2]Pharmacy, Saint Barnabas Medical Center, Livingston, NJ; [3]Transplantation, Saint Barnabas Medical Center, Livingston, NJ; [4]International Regenerative Medicine, West Orange, NJ.*

PURPOSE: Voriconazole is a new antifungal agent with broad spectrum coverage and an oral dosage form. Voriconazole is currently contraindicated for use in combination with sirolimus. We report our experience with voriconazole/sirolimus combination therapy in two renal transplant recipients.

METHODS: Patients in whom it was deemed that the best treatment included voriconazole/sirolimus combination therapy were identified during usual care. Trough levels and dosages were tracked and sirolimus levels were converted to dose ratio [ng/mL]/(mg/kg). Mann Whitney U test was used to compare dose ratio (DR) and levels, on and off voriconazole.

RESULTS: A 56 year old female had *Candida parapsilosis* in the blood and received caspofungin and fluconazole (4 days), followed by voriconazole (4 days). No calcineurin inhibitor was used in the first patient, and she had short bowel syndrome. During azole antifungal therapy, the sirolimus DR was 844 ± 297.5 [range: 548-1108], which was greater than the DR off the azoles, 88.7 ± 31 [range: 42-160; P<0.001]. Sirolimus levels [ng/mL] ranged from 13.7 to 27.7 during azole therapy, and 2.1 to 12 off azoles (P<0.001). Pre-voriconazole QT/QTc ranged from 290-382/396-401 ms, one rhythm strip during fluconazole demonstrated QT/QTc of 440/490 ms, and no ECG was available during voriconazole. The second patient was a 29 year old male with pulmonary *Aspergillus fumigatus*, who received 19 days of caspofungin with 15 days of amphotericin B lipid complex. Therapy was then switched to voriconazole, and 5 days later sirolimus was added to the regimen of tacrolimus and prednisone. DR was 856.4 ± 493.6 [range: 219.8-2101.1] during combination voriconazole/sirolimus therapy. The patient has been on voriconazole/sirolimus combination therapy for one year with sirolimus trough levels [ng/mL] ranging from 4.1 to 18.7. Pre-voriconazole QT/QTc intervals ranged from 294-342/397-422 ms, and on-voriconazole QT/QTc intervals ranged from 326-330/397-418 ms. Therapy was well tolerated in both patients, but periodic ECG recordings should be monitored for all azoles.

CONCLUSIONS: We have described, to our knowledge, the first experience with voriconazole/sirolimus combination. A 10-fold increase in DR was noted, but goal trough levels and clinical tolerability were achieved with careful monitoring and follow-up. These results do not support the package recommendation to avoid this combination, and should be confirmed with extensive pharmacokinetic studies.

Abstract# 1214 Poster Board #-Session: P144-III

ACCOMMODATION OF ABO INCOMPATIBLE RENAL ALLOGRAFTS IS ASSOCIATED WITH PERSISTENT C4D STAINING. Christopher E. Simpkins,[1] Daniel S. Warren,[1] Christopher J. Sonnenday,[1] Matthew Cooper,[1] Karen E. King,[2] Mark Haas,[2] Robert A. Montgomery.[1] *[1]Department of Surgery; [2]Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: The persistence of circulating isoagglutinins without histologic evidence of allograft injury after ABO incompatible (ABOi) renal transplantation has been described as accommodation. It has been assumed that the mechanism underlying accommodation prevents the activation of complement by isoagglutinins.

Methods: During the study period 11 ABOi transplants were performed with planned protocol biopsies at 1, 3, and 6 months. A total of 23 biopsies were performed on 10 patients. One patient who died of West Nile encephalitis in the early post-transplant period with normal renal function was excluded from this analysis (no protocol biopsies). Biopsies were considered C4d+ when the staining was diffuse and present in the peritubular capillaries.

Results: C4d staining was present in 37%, 71%, and 75% of protocol biopsies performed at 1, 3, and 6 months, respectively. 9 out of 10 patients had at least one C4d+ biopsy. Median isoagglutinin titers at each of these timepoints was comparable between patients with C4d+ and C4d- biopsies (Table 1). Only one patient with a C4d+ biopsy had histologic evidence of antibody-mediated rejection (Banff Grade 2A, Table 2). Mean creatinine clearance for the nine patients with at least one C4d+ biopsy was 62.25 +/- 25.5 ml/min at a mean follow-up of 9.5 months. Graft and patient survival were 100%.

Conclusions: C4d staining is present in the majority ABOi allografts that have undergone accommodation. C4d staining is not an indication of antibody mediated rejection and should not trigger therapeutic intervention in the absence of allograft injury.

Table 1. Isoagglutinin Titer

	Titer -1 month	Titer - 3 month	Titer - 6 month
C4d+	8 (2-16)	12 (2-12)	12 (1-12)
C4d-	8 (2-32)	8 (8)	8 (8)

median isoagglutinin titer (range)

Table 2. Renal function and incidence of antibody-mediated rejection after ABOi transplantation

	1 Months		3 Months		6 Months	
	AMR	CrCL	AMR	CrCL	AMR	CrCL
C4d+	0/3	81.5 (+/-23.2)	0/5	81.6 (+/-48.0)	1/6	84.5 (+/-27.1)
C4d-	0/5	81.2 (+/-30.1)	0/2	70.3 (+/-27.7)	0/3	50.9 (+/-18.8)

Antibody-Mediated Rejection (AMR) shown as # positive/n; CrCL=ml/min (Cockroft-Gault)

Abstract# 1215 Poster Board #-Session: P145-III

SALAVAGE OF COMPROMISED RENAL VESSELS IN KIDNEY TRANSPLANTATION USING THIRD-PARTY CADAVERIC EXTENDERS: IMPACT ON POST TRANSPLANT ANTI-HLA ANTIBODY FORMATION. Mahesh C. Goel,[1] Stuart M. Flechner,[1] M. El-Jack,[1] John Veniro,[1] Charles Modlin,[1] Lynne Klingman,[1] Daniel J. Cook.[1] *[1]Transplant Center/Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, OH.*

Purpose: Kidney transplant surgery can be complicated by short, attenuated, or injured renal vessels, which are now more frequently encountered with laparoscopic donor nephrectomy. We describe a technique to overcome this problem by constructing third party vascular conduits or extenders.

Methods: We report seven kidney transplants (cadaveric-2, live donor-5) where vascular reconstructions were necessary. Third-party cadaveric iliac vessels (veins-5, artery-2) were previously retrieved during organ procurement, and stored at 4C in University of Wisconsin solution. Common or external iliac veins were used to either extend and/or reconstruct the compromised renal veins with 5 or 6-0 prolene sutures; hypogastric artery was used to replace the renal artery. Immunosuppression consisted of sirolimus, mycophenolate mofetil, and steroids. Recipient serum was tested for the development of anti-HLA antibodies against either the donor kidney or the extender vessels using donor lymphocytes and HLA coated beads.

Results: Mean cold storage time was 22 (2.0-56) days. Mean recipient age was 51.1 (42-66) years; mean third party vessel donor age was 45.4 (17-44) years. All venous extenders were recipient ABO compatible, while one arterial extender was ABO incompatible. Prompt reperfusion of all allografts was observed, DGF occurred in one, and there were no surgical complications. Integrity of the extenders was confirmed using serial MAG3 renal scans and magnetic resonance angiography, which demonstrated 100% patency. Mean serum creatinine at 1 month was 1.4 (1.1-1.9); at 6

months 1.45 (1.1-1.8) and 1.41 (.9-1.9) mg/dl at last follow-up of 17.1 (2-31) months. There have been no acute rejections. Each recipient had a negative post transplant donor specific flow cytometry crossmatch to both the kidney donor and the third party vessel donor. Two recipients developed HLA antibody, one to DR7 and another DR13 (mismatched to the vessel donor) at 4 and 11 months post transplant, respectively, without apparent clinical significance.
Conclusion: The use of third-party cadaveric vessel extenders is safe with 100% short and medium-term patency. Development of post transplant HLA antibody is minimal. Having a vessel extenders bank is a valuable adjunct to the transplant surgeon and may be especially helpful during right laparoscopic donor nephrectomy.

Abstract# 1216 Poster Board #-Session: P146-III

IMPACT OF HLA-IDENTITY ON RESULTS OF ABO-INCOMPATIBLE LIVING KIDNEY TRANSPLANTATION. Hiroaki Shimmura,[1] Kazunari Tanabe,[1] Hideki Ishida,[1] Tadahiko Tokumoto,[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 is being carried out. Our previous studies showed that the long-term graft survival in ABO-incompatible living kidney transplantation was comparable to that in ABO-compatible living kidney transplantation. However, it has not been investigated previously the impact on HLA-identity in result of ABO-incompatible living kidney transplantation.
Materials and Methods: One-hundred and twenty-seven recipients underwent ABO-incompatible living kidney transplantation at our institution between January 1989, and December 2000. Of these patients, 5 were grafted from an HLA-identical sibling donor (I). The remaining 122 recipients received grafts from an HLA-non-identical donor (N). Both groups were similar in terms of recipient age, donor age, warm ischemic time and total ischemic time. Three or four sessions of plasmapheresis were carried out prior to transplantation. Cyclosporine or tacrolimus, methylprednisolone and azathioprine or mycophenolate mofetil were used for immunosuppression. Splenectomy was done at the time of kidney transplantation in all patients.
Results: Graft loss was seen in one of the five HLA-identical recipients due to chronic rejection. Five- and 10-year graft survival rates were 80.0% I vs 72.0% N and 80.0% I vs 54.2% N, respectively. The incidence of acute rejection in the HLA-identical recipients was much lower than that in the HLA-non-identical recipients (20% I vs 67.2% N).
Conclusions: Long-term graft survival in ABO-incompatible kidney transplantation from an HLA-identical sibling donor was much better than that from an HLA-non-identical sibling donor and with a lower incidence of acute rejection. These results suggest that ABO-incompatible living kidney transplantation could be performed safety if the donor were an HLA-identical sibling.

Abstract# 1217 Poster Board #-Session: P147-III

COMBINED KIDNEY AND BONE MARROW TRANSPLANTATION FOR INDUCTION OF MIXED CHIMERISM AND RENAL ALLOGRAFT TOLERANCE IN HLA MISMATCHED TRANSPLANTATION. Tatsuo Kawai,[1] David H. Sachs,[2] Thomas Spitzer,[3] Megan Sykes,[2] Nina Tolkoff-Rubin,[1] Francis Delmonico,[1] Susan Saidman,[1] Juanita Shaffer,[2] Bimalangshu Dey,[3] Steven McAfee,[3] Winfred Williams,[1] Dicken Ko,[1] Martin Hertl,[1] Nelson Goes,[1] Waichi Wong,[1] Robert B. Colvin,[1] A. Benedict Cosimi.[1] *[1]Transplant Unit, Massachusetts General Hospital, Boston, MA; [2]Transplant Biology Research Center, Massachusetts General Hospital, Charlestown, MA; [3]Hematology, Massachusetts General Hospital, Boston, MA; [4]Pathology, Massachusetts General Hospital, Boston, MA.*
Purpose: To induce allograft tolerance through mixed chimerism, a non-myeloablative conditioning regimen was tested in three recipients of HLA mismatched kidney transplants. **Methods:** Three patients received combined kidney and bone marrow transplantation from HLA haploidentical donors following conditioning with cyclophosphamide (60mg/kg x2), thymic irradiation (7 Gy), anti-CD2 mAb and a limited course of cyclosporine (CyA). **Results: Case 1;** A 22 y.o. woman developed mixed chimerism (T cell 5.2%, Granulo 90.6%) on day 7 with no GVHD. Chimerism became undetectable by day 14 but there was no evidence of rejection on sequential biopsies and her immunosuppressive medications were slowly tapered and finally discontinued by day 240. Her renal function remained stable, with serum creatinine of 1.2 mg/dl on day 440. Normal biopsy at 1 year was obtained and specific hyporesponsiveness has been demonstrated in MLR and CML. **Case 2:** A 22 y.o. man developed similar transient chimerism (T cell 3%, Granulo 60%) on day 7. After day 10, he developed acute tubular necrosis, believed to be secondary to engraftment syndrome, but the kidney allograft gradually recovered after steroid pulse therapy. On day 45, a kidney biopsy prompted by a moderate creatinine elevation suggested humoral rejection with positive C4d staining. Although no anti-donor alloantibody was detected in his serum, he was treated for humoral rejection and CyA was switched to Prograf, resulting in improved allograft function (creat 1.8) on day 171. His immunosuppression has been steadily tapered to current level of Prograf (trough 4 ng/dl) and prednisone (2.5 mg/day). A kidney biopsy on day 87 revealed no rejection and specific hyporesponsiveness was demonstrated in vitro. **Case 3:** A 39 y.o. man, too recently treated for complete evaluation, developed evidence of humoral rejection 10 days post transplantation. He currently has compromised allograft function, not requiring institution of dialysis. Further results will be available at the time of presentation. **Conclusion:** Induction of allograft tolerance via mixed chimerism appears to be achievable in recipients of HLA mismatched kidneys, but may require intensified suppression of humoral responses.

Abstract# 1218 Poster Board #-Session: P148-III

IMPACT OF PREDNISONE FREE IMMUNOSUPPRESSION ON BONE MINERAL DENSITY IN KIDNEY TRANSPLANT RECIPIENTS. Furqan Raja,[1] Arthur Matas,[1] Hassan Ibrahim.[1] *[1]Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]Surgery, University of Minnesota, Minneapolis, MN.*
Background: Loss of bone mineral density (BMD), fractures, and avascular necrosis are significant problems after solid organ transplantation. Whereas pre-existant bone disease related to hyperparathyroidism, diabetes mellitus, and physical inactivity may, in part, play a role, corticosteroids have been held as the major culprit for this rapid bone loss. Loss of BMD has been associated with duration and cumulative dose of steroids. One advantage of steroid-sparing immunosuppression would be minimizing steroid-related side effects, including the loss of BMD.
Methods: We studied the impact of prednisone-free immunosuppression in 13 kidney transplant recipients. All were treated with polyclonal antibody for 5 days, steroids discontinued on POD 6, calcineurin inhibitor, and either MMF or sirolimus. Bone mass was studied by X-ray absorptiometry (DEXA scan). Recipients were studied at transplant and one year later. Bone density, T and Z-scores were obtained at the femoral neck and lumbosacral spine. Paired t- test analysis of the above was carried out to evaluate for significant differences between baseline and one year post transplant.
Results: The mean age was 45.7 ± 13.7 years, 10 patients were male and 8 were on dialysis prior to transplant. 10 patients received Rapamycin/Tacrolimus for maintenance immunosuppression and 3 received Cyclosporine/MMF. The results of the DEXA scans are shown in table.
Conclusion: Our preliminary data from our experience with prednisone free immunosuppression suggest a favorable outcome with this strategy on bone loss after renal transplantation.

Bone mineral density on prednisone free immunosuppression

	At Transplant	At one year	P-value
AP SPINE			
Z score	0.12±1.6	-0.7±1.2	.35
T score	-0.13±2.2	-0.4±1.8	.21
BMD	1.2±0.3	1.15±0.2	.18
FEMORAL NECK			
Z score	-0.85±1.35	-0.9±1.2	.64
T score	-1.23±1.5	-1.4±1.5	.28
BMD	0.78±.48	0.75±0.5	.19

Abstract# 1219 Poster Board #-Session: P149-III

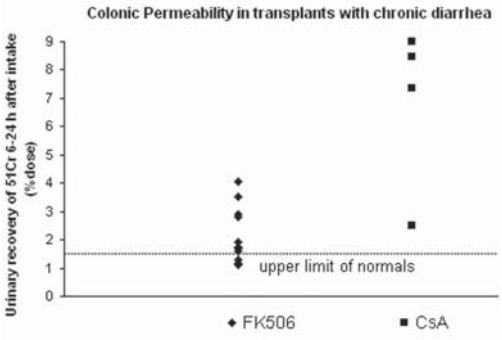
COLONIC PARACELLULAR PERMEABILITY IS INCREASED IN RENAL TRANSPLANT RECIPIENTS WITH CHRONIC DIARRHEA. Wim Lemahieu,[1] Bart Maes,[1] Kirstin Verbeke,[2] Yves Vanrenterghem.[1] [1]*Internal Medicine, Division of Nephrology, University Hospitals Gasthuisberg, Leuven, Belgium;* [2]*Laboratory of Radiopharmaceutical Chemistry, University Hospitals Gasthuisberg, Leuven, Belgium.*

Chronic diarrhea is a frequent adverse event in transplant patients. Inreased paracellular permeability has been demonstrated as a disease enhancing factor in chronic inflammatory bowel disorders. However, it is not known to which extent the mucosal damage caused by chronic diarrhea in transplant patients affects intestinal paracellular permeability.

In the present study, permeability of small and large bowel was assessed in 18 renal graft recipients suffering from chronic diarrhea (faecal output > 200g/day, duration ≥ 2 weeks). Immunosuppressive therapy consisted of low dose corticosteroids and MMF plus either tacrolimus (FK506, n=13) or ciclosporin A (CsA, n=5). Urinary recovery of ^{51}Cr-EDTA during 0-6 h and 6-24 h after oral ingestion was used to assess paracellular permeability of small bowel and colon respectively. The results were compared between the two groups and with the results of normal healthy volunteers using Wilcoxon's Rank Sum Test.

Small intestinal paracellular permeability was not significantly altered. In contrast, colonic paracellular permeability in renal transplant patients affected by chronic diarrhea was clearly increased. In addition, patients on CsA had a significantly higher colonic permeability compared to those on FK506 (figure).

These preliminary results suggest that mucosal damage associated with chronic diarrhea in transplant recipients is more pronounced in the colon. Patients on CsA also seem to be more prone for conditions leading to damage of the tight junctions of the colonic epithelium.



Abstract# 1220 Poster Board #-Session: P150-III

VASCULAR COMPLICATIONS OF ALLOGRAFT NEPHRECTOMY. Molly M. P. Eng,[1] Dilly M. Little,[1] Richard E. Power,[1] John G. Calleary,[1] David P. Hickey.[1] [1]*Transplantation and Urology, Beaumont Hospital, Dublin, Ireland.*

Purpose: Allograft nephrectomy (AN) is a hazardous procedure that carries high mortality and morbidity. The two most common complications identified are significant haemorrhage peri-operatively and infection. Major haemorrhage controlled by iliac vessel ligation is an under reported complication. We retrospectively reviewed our experience of 1407 renal transplants and 126 AN performed in an 11-year period (January1991 to December 2001). We identified 9 patients with significant vascular injury and individual case notes were studied. The aim of this review was to analyze the factors associated with vascular complications post AN and to assess the safety of managing these injuries with early iliac vessel ligation. **Materials and Methods**: 1407 renal transplants were performed in our institution from January 1991 to December 2001. Over this 11-year period, 126 transplant nephrectomies were performend of which 9 patients suffered significant vascular injury. Patient's records were reviewed for demographic data, surgical techniques, indication for AN, patient's risk factors and outcome post emergency iliac vessel ligation. **Results**: Our vascular complication rate post AN is 7.14%. The vascular injuries consisted of 4 patients with pseudoaneurysms of external iliac artery, 2 patients with major haemorrhage of anastomotic site and 3 patients with iatrogenic injury with iliac vessel laceration at the time of nephrectomy. Only 2 of the 9 patients required immediate vascular bypass surgery (2 femoral-femoral cross over), 2 patients at the later study period had successful endovascular stenting of iliac vessel pseudoaneurysms. None of the patient suffered limb loss. Pre-operative local infection rate was high in the vessel injury group and often involve multi-resistant organisms.Three of the 9 patients died. The causes of death were 2 from sepsis at 1 week and 1 month post-operatively and 1 from intra-cerebral haemorrhage 3 months after AN. On follow up, only 1 patient from the study required further revision of their vascular reconstruction 4 years later for symptomatic claudication. **Conclusion**: We recommend that in the event of major bleeding and infection at the transplant vascular anastomotic site, immediate iliac vessel ligation can be performed safely without risk of limb loss for control of haemorrhage. Primary repair of injured vessel is associated with re-bleeding. Immediate vascular bypass reconstruction may not be necessary and patients can be observed in view for delayed vascular procedure if critical ischaemia occurs.

Abstract# 1221 Poster Board #-Session: P151-III

PREDICTIVE MODEL FOR RENAL REPLACEMENT THERAPY (RRT) FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION (OLTX) BASED ON MELD, CREATININE, BUN, ICU LENGTH OF STAY. Edmund Q. Sanchez,[1] Thomas A. Gonwa,[1] Adrian P. Martin,[1] Takehisa Ueno,[1] Nicholas Onaca,[1] Henry B. Randal,[1] Sherfield Dawson,[1] Srinath Chinnakotla,[1] Marlon F. Levi,[1] Robert M. Goldstein,[1] Martin L. Mai,[1] Steven R. Hays,[1] Larry B. Melton,[1] Goran B. Klintmalm.[1] [1]*Tranplant Services, Baylor University Medical Center, Dallas, TX.*

Introduction: Acute renal failure that develops after orthotopic liver transplant (OLTX) and which requires renal replacement therapy (RRT) is a poor prognostic sign. One-year reported mortality ranges from 28% - 85%. Our center has previously reported that patients developing the need for RRT post OLTX had a one-year survival of only 41.8%. This study generated a predictive model to identify patients at risk for RRT.

Materials and Methods: Source of data was a prospectively maintained database. OLTX performed between 1/1/1996 and 12/31/2001 was included. Combined kidney-liver transplants or pre-operative RRT pts. were excluded. 724 OLTX were studied. Patients were divided into Group I: no RRT, n=637; Group II: hemodialysis only post OLTX, n=17; and Group III: Continuous RRT post OLTX, n=70. Pre and peri-operative variables between the groups, which were identified as significant factors in a univariate analysis, were included in a stepwise logistic multivariate analysis to determine odds ratios for the development of the need for RRT. Actuarial patient survival was calculated for all groups and compared between groups.

Results: Actuarial patient survival at one year was 91.0%, 70.6%, and 49.1% for Groups I, II, and III respectively. Variables significant in the multivariate analysis included pre-operative serum creatinine > 1.9 mg/dl (OR 3.57), pre-operative BUN >27 mg/dl (OR 2.68), ICU days >3 (OR 10.23) and MELD score >21 (OR 2.5). A formula including these variables was developed to give a score to be used in a clinical prediction model of developing the need for RRT: PRHD

(Probability of requiring dialysis post transplant) = (-2.4586 + 1.2726 (Creatinine > 1.9) + 0.9858 (BUN >27) + 0.4574 (MELD Score>21) + 1.1625 (ICU days >3).

Patients with a PRHD score of 0.12 or higher were more likely to develop need for RRT post- operatively. When applied to OLTX recipients transplanted in 2002, correct classification occurred in 15 out of 20 patients who received RRT and 111 of 121 who did not.

Conclusions: This model allowed us to identify patients at high risk for developing the need for RRT post-operatively. Strategies for these patients to prevent or ameliorate ARF and reduce the need for RRT post-operatively are needed.

Abstract# 1222 Poster Board #-Session: P152-III

TYPE OF ALLOGRAFT, REQUIREMENT FOR REOPERATION AND NATURE OF INITIAL IMMUNOSUPPRESSION ARE ALL ASSOCIATED WITH WOUND COMPLICATIONS IN RENAL RECIPIENTS. Deborah Verran, Taras Kusyk, Jodi Fisher, Pamela Dilworth, Graham Stewart, Steve Chadban, John Boulas, Josette Eris. *Renal Transplant Service, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia.*

The aim of the study was to document the factors associated with the development of wound complications in renal allograft(RA) recipients in the modern immunosuppressive era.

Methods-Data was obtained from retrospective chart review of 281 RA performed from 1/1998-12/2002. The RA were divided into 2 groups-with/without wound complications. Statistics were performed with SPIDA.

Results- 97/281(34.5%) of RA recipients sustained wound complications of which 66/97(34.5%) were managed conservatively and 31/97(32%) were managed operatively. The nature of the wound complications were-77/97(79%) superficial skin dehiscence, 20/97(21%) wound dehiscence involving fascia. In 62/97(64%) of recipients with wound complications there was associated infection. The median RA recipient age was 47(21-70) years for recipients with wound complications versus 43.5(19-74) years for recipients without wound complications($p<0.065$). Male sex tended to be more common in 59/97(61%) of the recipients with wound complications versus in 90/184(49%) of recipients without wound complications($p<0.057$). Cadaveric allografts were implanted into 82/97(85%) of recipients with wound complications versus 107/184(58%) of recipients without wound complications($p<0.0001$). Delayed graft function occurred in 31/97(32%) of RA recipients with wound complications versus 34/184(18%) of RA recipients without wound complications($P< 0.01$). Immunosuppression with a Sirolimus containing regimen occurred in 36/97(37%) of RA with wound complications versus 26/184(14%) of RA without wound

complications(p<0.0001). A reoperative surgical procedure via the same incision in the first 2 weeks following the initial RA implantation procedure occurred in 31/97(32%) of RA with wound complications versus 13/184(7%) of RA without wound complications(p<0.0001).
Conclusions- Implantation of a cadaveric renal allograft, the presence of delayed graft function, immunosuppression including Sirolimus, and reoperation in the first 14 days are all associated with wound complications in RA recipients.

Abstract# 1223 Poster Board #-Session: P153-III

RENAL TRANSPLANTATION IN THE MORBIDLY OBESE. William M. Bennett,[1] Kevin M. McEvoy,[1] Karen R. Henell,[1] Viken Douzdjian.[1] *[1]Renal Transplant Program, Legacy Good Samaritan Hospital, Portland, OR.*

Renal transplantation is offered to many patients with severe co-morbidities such as diabetes mellitus and pre-existing vascular disease but is routinely denied to the morbidly obese. Since transplantation has a survival advantage compared to dialysis, despite morbid obesity there is no ethical basis for denying transplantation in this population. 47 transplants to patients with body mass indices (BMI) greater than 35 were performed from 2000 through November 2003. Of these 32 were from deceased and 15 from living donors. One-year actuarial graft survival was 95.7% and one-year patient survival was 95.3%. There were three deaths in the series including one death (myocardial infarction) with a functioning graft. Two patients died more than three months after graft removal of unrelated causes (MVA and dialysis access infection.) BMI ranged from 35.1-49.4 with a median of 39 and a mean of 40.2. Most common etiologies of ESRD were type II diabetes (45%), hypertension (15%) and polycystic kidney disease (9%). Age ranged from 15-78 years with a mean of 50 and a median of 51 years. There was no perioperative death and no thromboembolic disease despite lack of routine anticoagulation in the peri-transplant period. Graft function was delayed in 32%. Length of stay ranged from 5-76 days with a mean of 10 and a median of 7 days. 15 patients (32%) experienced a total of 20 hospitalizations in the posttransplant period for 9 wound infections (19%), urinary tract repairs, rejection or other transplant-related complications. There were 13 rejections in 11 patients giving an overall acute rejection rate of 31%. The only post-transplant complication more prevalent than a concomitant group of patients with BMI<35 was deep wound infections.
Immunosuppression was standard with Basiliximab induction followed by MMF, prednisone and tacrolimus maintenance. For regrafted patients Thymoglobulin® was used for induction. Rejections were treated with steroids and Thymoglobulin®.
Conclusion: Once attempts at pre-transplant weight loss prove futile, renal transplantation should be offered to the morbidly obese since transplantation carries a survival advantage over dialysis and can be done safely.

Abstract# 1224 Poster Board #-Session: P154-III

CHANGES IN WEIGHT DURING THE FIRST YEAR POST RENAL TRANSPLANT. Ann K. Cashion,[1] Patricia A. Cowan,[1] Donna K. Hathaway,[1] Agnes Lo,[2] A. Osama Gaber.[3] *[1]College of Nursing, University of TN Health Science Ctr, Memphis, TN; [2]College of Pharmacy, University of TN Health Science Ctr, Memphis, TN; [3]Department of Surgery, University of TN Health Science Ctr, Memphis, TN.*

Purpose: Increases in weight occur following renal transplant and is a concern because of the increased risk for cardiovascular complications. This study categorizes subjects by Body Mass Index (BMI; wt [kg]/ht [m2] to determine associations with gender, race and clinical factors.
Methods: A retrospective analysis was completed on subjects transplanted between Jan 1998 and Jan 2002 who had baseline and 1-year follow-up data. All subjects had functioning grafts. BMI was defined according to clinical guidelines developed by NIH/NHLBI; normal 18.5- 24.9, overweight 25-29.9, obese ≥ 30. Clinical factors included fasting blood sugar (FBS), triglycerides (TG), cholesterol (Chol), albumin, systolic and diastolic blood pressure (BP), prednisone dose (Pred), serum creatinine (Cr), and creatinine clearance (CrCl).
Results: The sample consisted of 171 renal transplant recipients (44 ± 12.2 yrs, 33% women, 58% AA, 39% C). At 1-year PostTxp, the total group showed a significant increase in weight (6.2 ± 10.7 kg) and BMI (2.1 ± 3.8), with AA vs. C (2.6 vs. 1.3; p=0.03) and women vs. men (3.1 vs. 1.5; p=0.01) showing greater increases in BMI from baseline. When examined at baseline by BMI group, 31% were normal, 35% overweight, and 34% obese; however, by 1 yr PostTxp there were significantly more obese (49%) than normal (21%) subjects (p≤0.05). The proportion of obese individuals increased regardless of gender or race, with AA increasing more than Caucasians and women more than men (see table). At baseline, obese vs. normal BMI group was older (47 vs. 41 yrs; p≤0.05), with a lower CrCl (29.3 vs. 44.6; p≤0.003) and a higher FBS (118 vs. 95; p≤0.05). At 12 mo PostTxp differences in age, CrCl, and FBS disappeared; however the obese group had higher TG (235 vs. 165 p=0.01). At baseline and 12 mo PostTxp there was no difference in BP, Pred, Cr, Chol, or albumin levels among BMI groups.
Conclusions: We documented significant weight gain, particularly in women and African-Americans. However, demographic and clinical factors associated with weight gain do not explain post transplant obesity. We therefore speculate additional variables such as genetic factors are influencing weight gain and warrant study.

Changes in Percent of Patients per BMI category: Baseline to 12 mo PostTxp by Gender and Race

	Men (n=114)	Women (n=57)	AA (n=100)	Caucasian (n=66)
Normal	34% decrease	26% decrease	47% decrease	6% decrease
Overwt	0%	41% decrease	6% decrease	22% decrease
Obese	34% increase	65% increase	51% increase	30% increase

Abstract# 1225 Poster Board #-Session: P155-III

VALACYCLOVIR FOR CYTOMEGALOVIRUS PROPHYLAXIS REDUCES THE RISK OF ACUTE RENAL ALLOGRAFT REJECTION: A RANDOMIZED COMPARISON WITH ORAL GANCICLOVIR AND DEFERRED THERAPY. Tomas Reischig,[1] Pavel Jindra,[2] Jan Mares,[1] Karel Opatrny, Jr.,[1] Vladislav Treska,[3] Miloslav Cechura,[3] Miroslava Svecova.[4] *[1]Medicine I, Charles University Hospital, Pilsen, Czech Republic; [2]Hematooncology, Charles University Hospital, Pilsen, Czech Republic; [3]Surgery, Charles University Hospital, Pilsen, Czech Republic; [4]Virology, Charles University Hospital, Pilsen, Czech Republic.*

Purpose: Both oral ganciclovir and valacyclovir decrease the incidence of cytomegalovirus (CMV) disease after renal transplantation (RTx). Moreover, valacyclovir has been shown to reduce the risk of acute rejection in D+R- patients (pts). The aim our study was to compare the efficacy and safety of oral ganciclovir and valacyclovir in the prophylaxis of CMV disease after RTx. **Methods:** A total of 83 patients (pts) was prospectively randomized to 3-month treatment with either oral ganciclovir (1 g t.i.d., n=36, GAN group) or oral valacyclovir (2 g q.i.d., n=35, VAL group), with the dose adjusted by renal function. A third group (C, n=12) received no prophylaxis and was managed by deferred therapy. CMV seronegative recipients of seronegative grafts were not enrolled. The pts were followed up to 24 months post-RTx or until death or graft loss (mean 22.6 ± 3.3 months). The pts were monitored using CMV nested PCR in whole blood. **Results:** No differences were found among the groups in their demographic characteristics, immunosuppressive protocols, or D/R CMV serology. The 12-month incidence of CMV disease was 67% in the C group compared with 6% in the GAN group and 3% in the VAL group (P<0.001 GAN vs C; P<0.001 VAL vs C; P=1.000 GAN vs VAL). CMV viremia (positive PCR) occurred within the first 6 months in 20%, 29% and 92% pts of the GAN, VAL and C groups, respectively (P<0.001 GAN vs C; P<0.001 VAL vs C; P=0.413 GAN vs VAL). The rate of biopsy-confirmed acute rejection at 12 months was 12% in the VAL group compared with 34% in the GAN group (P=0.044) and 58% in the C group (P=0.003) (P=0.182 GAN vs C). Treatment failure (death, graft loss, CMV disease or withdrawal from study) occurred, over the follow up period, in 19%, 11% and 67% in the GAN, VAL and C groups, respectively (P=0.004 GAN vs C; P<0.001 VAL vs C; P=0.514 GAN vs VAL). The average CMV-associated costs per pt were, over the 12 months, $3,539±2,311; $3,348±2,803 and $5,651±6,550 in the GAN, VAL and C groups, respectively. **Conclusions:** Valacyclovir and oral ganciclovir are equally effective in the prevention of CMV disease after RTx. Both regimens are cost effective. Valacyclovir but not ganciclovir prophylaxis is associated with significantly reduced risk of acute rejection within first year post-RTx.

Abstract# 1226 Poster Board #-Session: P156-III

SAFETY AND EFFICACY OF A LOW-DOSE VALGANCICLOVIR REGIMEN FOR CMV PROPHYLAXIS IN ADULT RENAL TRANSPLANT RECIPIENTS RECEIVING INDUCTION THERAPY.

Patricia K. Aggers,[1] Lonnie D. Smith,[1] Troy Somerville,[1] Jacke Corbett,[1] John Holman,[1] Fuad S. Shihab.[1] *[1]Renal Transplant, University of Utah Hospitals and Clinics, Salt Lake City, UT.*

Purpose: Despite advances in anti-viral therapy for CMV prophylaxis, CMV infection is still a significant source of morbidity in renal transplant recipients. Limited data exist on valganciclovir (VGC) prophylaxis in kidney transplant recipients and the optimal prophylactic dosing regimen is yet to be established. Our institution utilizes a low-dose VGC regimen for prophylaxis in moderate and high risk renal transplant recipients. The purpose of this study is to evaluate the safety and efficacy of oral VGC at a dose of 450 mg QD for prevention of CMV infection. **Methods:** Between 11/01 and 8/03 all moderate risk (D+R+, n=55; D-R+, n=15) and high risk (D+R-, n=22) adult renal transplant recipients received VGC for CMV prophylaxis. Moderate risk recipients received VGC 450 mg QD for 1 month while high risk recipients received 450 mg QD for 3 months. The patient distribution was 48 Male/44 Female with 75% Caucasian, 10% Hispanic and 15% others. There were 36 live donor and 56 deceased donor transplants. All recipients received induction therapy with either thymoglobulin (1.5mg/kg day 0,1,4, n=86) or basiliximab (20 mg day 0,4, n=6). Maintenance immunosuppression consisted of tacrolimus (n=74) or CsA (n=18) and MMF ± pred. **Results:** At a mean follow-up of 12 months, patient and graft survival were 99% and 98%, respectively. No major side effects of VGC were noted. The most common side effects were anemia (56%), leukopenia (40%) and diarrhea (21%). Ten (11%) patients were diagnosed with CMV viremia (high risk 4/22, 18% and moderate risk 6/70 9%). Diagnosis was made by quantitative PCR (60%), a rise in acute CMV IgM titers (20%) or both (20%). All ten patients were treated with an outpatient course of VGC. There was one recurrence of CMV viremia after completing treatment, no patients developed invasive CMV disease and none required admission for CMV infection. CMV infection did not precede any acute rejection episodes (6/92). One patient developed CMV viremia 1 month after a rejection. The mean time from transplant to CMV viremia was 4.8 months (2-11). The mean time to diagnosis following VGC prophylaxis was 3.1 months (1-9). **Conclusion:** Low-Dose VGC (450 mg QD) appears to be a safe and effective regimen for CMV prophylaxis in moderate and high risk adult renal transplant recipients receiving induction immunosuppression. Low-dose VGC resulted in a low incidence of CMV infection with no need for IV therapy and no hospitalizations for CMV.

Abstract# 1227 Poster Board #-Session: P157-III

PREEMPTIVE MANAGEMENT OF CMV IS ASSOCIATED WITH LOWER RATES OF LEUKOPENIA AND SIMILAR INCIDENCE OF CMV INFECTION AS COMPARED TO ROUTINE PROPHYLAXIS IN KIDNEY TRANSPLANT RECIPIENTS RECEIVING CAMPATH INDUCTION.

Paolo Salvalaggio, Dixon Kaufman, Lorenzo Gallon, Lisa Scholz, Patrice Al-Saden, Frank Stuart, Jr., Michael Abecassis, W. James Chon, Alan Koffron, Jonathan Fryer, Talia Baker, Joseph Leventhal. *Surgery and Internal Medicine/Div. of Transplantation, Northwestern University, Chicago, IL.*

Background. CMV is a major cause of morbidity in Kidney Transplantation (KTx). Valganciclovir (Valcyte) is a recently approved anti-viral against CMV. We have observed an increased incidence of leukopenia in KTx pts receiving Valcyte vs. Ganciclovir prophylaxis and MMF-based maintenance immunosuppression (IP). Few studies have analyzed the efficacy and safety of preemptive Rx for CMV infection with the use of Valcyte. ***Aim*:** To investigate whether the preemptive management of CMV is safe, effective and associated with fewer side-effects compared to routine CMV prophylaxis in KTx pts. ***Methods*:** This is a single-center retrospective observational study that includes 322 KTx. Pts received a 30mg intraoperative dose of Campath-1H and 3-days of iv steroids for induction. Mainteinance IP consisted of MMF and Tacrolimus without steroids**.** Pts were divided into 2-eras: In **era 1** (10/01-9/02; n=164), all pts received Valcyte (450mg qd) for 3 months. In **era 2** (10/02-8/03; n=158), D^-R^- pts (n=37) were not monitored or treated; D^+R^- pts (n=13) received Valcyte (450mg qd) for 3 months and all R^+pts (D^-,n=36 and D^+,n=72) were monitored by quantitative DNA CMV PCR q2 wks for 3 months. Presence of ≥200 copies of CMV in peripheral blood initiated Valcyte (450mg qd) for 1 month or until the blood was cleared of the virus. CMV infection/disease and side effects of protocols were compared. ***Results*:** Pt demographics (age, sex, cause of ESRD, cadaver vs. living donor KTx) were comparable between eras. Mean follow-up was 12.1 months (16.5 months for era 1 and 7 months for era 2). Overall CMV infection rate was 4.6% (3.6% in era 1 and 5.7% in era 2, p=0.43). CMV tissue-invasive disease rate was 2.2% (3% in era 1 and 1.3% in era 2, p=0.45). The WBC count was 2.5±1.1 in era 1 and 3.1±1.5 in era 2 (p=0.01). The incidence of leukopenia (WBC<3) was 75% in era 1 and 47% in era 2 (p<0.0001). Admission for leukopenia dropped in era 2 (3.2%) when compared with era 1 (4.8%; p=0.58). 1-year pt and graft survival were respectively 96.6% and 95.3% in era 1 and 96.6% and 96.6% in era 2. ***Conclusions*:** Compared to a routine prophylaxis, the use of a preemptive magement of CMV in KTx pts is associated with a lower rates of leukopenia and similar incidence of CMV infection/disease and.

Abstract# 1228 Poster Board #-Session: P158-III

PROPHYLAXIS FOR CYTOMEGALOVIRUS DISEASE IN A RANDOMIZED TRIAL OF THREE DIFFERENT IMMUNOSUPPRESSIVE REGIMENS IN RENAL TRANSPLANTATION.

Gaetano Ciancio,[1] George W. Burke,[1] Adela D. Mattiazzi,[1] Jeff J. Gaynor,[1] Ramir Roohipour,[1] Lorraine Dowdy,[2] David Roth,[2] Warren Kupin,[2] Anne Rosen,[1] Delvis Jorge,[1] Nancy Johnson,[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.*

Purpose: Concerns have been raised about the possibility of increased of cytomegalovirus (CMV) disease as result of the use of potent immunosuppressive drugs. We are assessing prospectively the efficacy and safety of oral ganciclovir and valganciclovir as prophylaxis for CMV disease in patients with highly potent immunosuppression. This prospective study assessed the efficacy of 3 months CMV prophylaxis in a randomized trial of three different immunosuppressive protocols in kidney transplantation.

Method: Between May 2000 and December 2001, 150 patients were randomized in a single center prospective trial, equally divided into 3-arms, of cadaver or non-HLA identical living donor first renal transplant recipients (n=50 per group). Group A received Tacrolimus and sirolimus. Group B received tacrolimus and mycophenolate mofetil. Group C received Neoral® and sirolimus. Each group received daclizumab induction and methylprednisolone maintenance. Oral ganciclovir 1000 mg tid was used in 92 patients. The remaining 58 patients received a single dose of 900 mg/day of Valganciclovir, the valine ester of ganciclovir, which provides oral bioavailability superior to that of oral ganciclovir. Blood was sampled before transplantation from the donors and recipients for CMV serology and nested polymerase chain reaction (PCR) for CMV DNA, and after transplantation from the recipients only at monthly intervals until 6 months. Patients were observed for the development of any CMV-like illness during follow-up.

Results: The CMV serologic status was D+/R+ in 70, D+/R- in 21, D-/R- in 21 and D-/R+ in 38 pairs. Of 150 patients followed for 12 months, only 2 developed cytomegalovirus infection, both patients at 120 days posttransplantation. One of the patients received ganciclovir and the other valganciclovir. There were 11 incidents of acute rejection among the three groups. Only 10 patients discontinued treatment early, 2 due to leukopenia.

Conclusions: Oral ganciclovir has been used with success for CMV prophylaxis, but it must be administered in large, divided doses (1000 mg TID) to be effective. These results suggest that prophylaxis with a single dose of 900 mg/day of valganciclovir for at least 3 months safely and effectively prevents cytomegalovirus infection in kidney transplant recipients on highly potent immunosuppression.

Abstract# 1229 Poster Board #-Session: P159-III

RATIONALE USE OF VALGANCICLOVIR FOR THE PREVENTION OF CYTOMEGALOVIRUS DISEASE IN RENAL TRANSPLANT RECIPIENTS.

Steven Gabardi,[1,2] Colm C. Magee,[3,4] Steven A. Baroletti,[1] John Powelson,[4,5] Anil K. Chandraker.[4,6] *[1]Department of Pharmacy Services, Brigham and Women's Hospital, Boston, MA; [2]Department of Pharmacy Practice, Northeastern University, Boston, MA; [3]Division of Nephrology, Brigham and Women's Hospital, Boston, MA; [4]Harvard Medical School, Boston, MA; [5]Division of Transplant Surgery, Brigham and Women's Hospital, Boston, MA; [6]Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital Boston, Boston, MA.*

Purpose: Valganciclovir is a ganciclovir prodrug, with improved bioavailability. The appropriate dose and duration of valganciclovir for cytomegalovirus (CMV) disease prophylaxis has not been established. Many centers use 450 – 900 mg/day for three months posttransplant. We evaluated the efficacy and safety of valganciclovir in renal transplant recipients (RTR) who had received 450 mg daily for six months for CMV prophylaxis.

Methods: A retrospective analysis reviewed all RTR at risk of CMV disease receiving valganciclovir between August 2001 and November 2002. Valganciclovir was started post-operatively and dose-adjusted to renal function. Desired treatment duration was six months. CMV disease rate was the primary endpoint. Secondary endpoints included: leukopenia, thrombocytopenia, acute rejection, allograft loss, early discontinuation of therapy and patient death.

Results: 58 RTR were identified: 51.7% were female, 15.5% were black, 43.1% received cadaveric kidneys and 10.4% had a prior history of kidney transplantation. Mean age was 50 years. Donor (D) /recipient (R) CMV serostatus was 37.9% D+/R+, 29.3% D-/R+ and 17.3% D+/R-. Anti-thymocyte globulin (ATG) induction therapy was administered to 62.1% of patients. The majority of the transplant recipients were maintained on triple immunosuppression. Median follow-up was 16 months. CMV disease incidence was 0% within six months posttransplant and 5.2% at any point posttransplant (n = 3; 5.2% non-invasive, 0% tissue-invasive). All patients who developed CMV disease were D+/R- and had received ATG. The incidence of CMV disease among the D+/R- patients was 30%. A CMV disease rate of 8.1% was noted amid those patients who received ATG. Leukopenia (28%) and thrombocytopenia (24%)

were the most common valganciclovir adverse events. One patient developed acute rejection. No graft losses or deaths occurred. Early discontinuation of valganciclovir occurred in 20% of patients secondary to severe, persistent leukopenia, thrombocytopenia and/or diarrhea. None of these patients developed CMV disease.
Conclusions: A high rate of CMV disease was noted among the D+/R- population. The administration of ATG as an induction agent also increased the incidence of CMV disease. Despite the low dose of valganciclovir, hematologic adverse events were common. However, valganciclovir, at 450 mg daily for six months, is effective, safe and well tolerated for prophylaxis of CMV disease in RTR.

Abstract# 1230 Poster Board #-Session: P160-III

6 MONTHS VALGANCICLOVIR PROPHYLAXIS SIGNIFICANTLY DECREASES CYTOMEGALOVIRUS INFECTION INCIDENCE IN THYMOGLOBULIN TREATED TRANSPLANT PATIENTS. Enver Akalin,[1,2] Jonathan S. Bromberg,[2] Vinita Sehgal,[1,2] Scott Ames,[2] Lisa Daly,[2] Barbara Murphy.[1,2] *[1]Renal Division; [2]Recanati/Miller Transplantation Institute, Mount Sinai Medical School, New York, NY.*

Despite the introduction of newer anti-viral medications for prophylaxis, cytomegalovirus (CMV) infection is still the most common opportunistic infection following solid organ transplantation. We recently reported that the overall incidence of CMV disease at 1-year posttransplant was 14% (4% tissue-invasive, 10% non-invasive) in 129 kidney or pancreas transplant recipients with 3-months Ganciclovir or Valganciclovir prophylaxis. (Am J Transplant 2003; 3:731). However, of the18 patients who developed CMV disease, 83% (n=15) had received Thymoglobulin induction treatment, and 25% of all Thymoglobulin treated patients developed CMV disease. 4 of 15 (27%) patients had tissue-invasive CMV disease. In the light these findings we extended CMV prophylaxis with Valganciclovir to 6-months in Thymoglobulin treated patients and prospectively investigated the incidence, time-course, and clinical patterns of CMV disease. 50 Thymoglobulin treated transplant patients (43 kidney, 3 kidney/pancreas, 4 pancreas after kidney) received 6-months CMV prophylaxis with Valganciclovir 450 mg po qd. There were 28 male, 22 female, 39 Caucasian or Hispanic, 11 African-American, age 29-67, and median follow-up of 15 months (range 9-21 months). In terms of CMV antibody status, 25 were Donor (D) + / Recipient (R) +, 11 were D -/R+, 8 were D+/R-, and 6 were D-/R-. Only 4 patients (8%) had diagnosis of non-invasive CMV disease by blood CMV-PCR (range, 5,000-100,000) and one of them had also tissue-invasive CMV disease (CMV esophagitis). Patients developed CMV disease 4-8 weeks after completion of 6-months CMV prophylaxis, and all responded to Valganciclovir treatment.
These results indicate that 6-months Valganciclovir prophylaxis significantly decreases CMV incidence (from 25% to 8%) in Thymoglobulin treated patients.

Abstract# 1231 Poster Board #-Session: P161-III

CYTOMEAGLOVIRUS REPLICATION AS RISK FACTOR OF CARDIOVASCULAR EVENTS IN THE FIRST YEAR AFTER RENAL TRANSPLANT. E. Gómez,[1] A. S. Laurés,[1] S. Melón,[2] J. Baltar,[1] M. Oña,[2] J. Alvarez.[1] *[1]Nephology, Hospital Central de Asturias, Oviedo, Spain; [2]Microbiology, Hospital Central de Asturias, Oviedo, Spain.*

Cardiovascular (CMV) disease is now the most common cause of renal transplants failure. CMV infection has been postulated as a factor that increases the risk of arteriosclerosis in some solid organ transplants. CMV generally occurs during the first 4 months, but some patients show CMV replication during a prolonged period of time. The aim of this study was verify if the patients with CMV replication have more incidence of cardiovascular events (CVE) during the first transplant year.
106 consecutive kidney transplant recipients with a follow-up of one year was prospectively studied (70 males, 36 females, age 50±14). CMV determinations consisted in antigenemia and PCR weekly during the 4 first months after the transplant, and monthly until 1 post-transplant year. Moreover, the following parameters were analyzed: gender, age, smoking, duration of dialysis, pre-existent diabetes, pre-existent CVE, ACE and AT1R polymorphisms; and 1-year post-transplant date: CVE, body mass index, arterial hypertension, number of anti-hypertensive drugs, use of ACE and/or ARA-II inhibitors, presence of diabetes, of anemia, and immunosuppression with CyA or Tac. Homocysteine, serum creatinine, cholesterol total, HDL-cholesterol, LDL-cholesterol, PTH-i, and proteinuria were also determined. CVE were defined as presence of coronary disease, heart failure, atrial fibrillation, stroke, and/or peripheral vascular disease.
72 (67.9%) patients had CMV replication and 34 (32,1%) not. The CMV replication group had 11 CVE during the 1 post-transplant year, and the not-CMV replication group 0, p=0.01. The mean time from transplant to CMV detection in the patients with CVE was 93±28 days days vs. 112±23 in patients without CVE, p=0.3. Eleven of the 106 patients (10.3%) developed CVE during the first year of the transplant. The factors that show statistical significance between the patients with or without 1-year post-transplant CVE were: pre-existent CVE (72.5% vs. 16.8%, p=.001), age (61±11 vs. 50±14, p=.01), triglycerides (190±89 vs. 134±73, p=.02), and presence of anemia (27.7% vs. 6.6%, p=.03). However, a multiple logistic regression analysis that included gender, age, and the statistical significant variables in the univariate analysis, show that only pre-existent CVE influenced in the developed of 1-year pot-transplant CVE (OR 14.5, 95%CI: 2.4-86, p=.001).
In conclusion, CVE 1 year after the transplant is more frequent in the patients with CMV replication, but the principal risk factor is the presence of pre-existent CVE.

Abstract# 1232 Poster Board #-Session: P162-III

A NATIONWIDE SURVEY OF CYTOMEGALOVIRUS PROPHYLAXIS AND TREATMENT IN THE TRANSPLANT COMMUNITY. Rajendra S. Baliga,[1] Pradeep V. Kadambi,[1] Basit Javaid,[1] Robert Harland,[2] James W. Williams,[2] J. R. Thistlethwaite,[2] Michelle A. Josephson.[1] *[1]Section of Nephrology, Department of Medicine, University of Chicago, Chicago, IL; [2]Department of Surgery, University of Chicago, Chicago, IL.*

Introduction: Despite the voluminous literature available for cytomegalovirus (CMV) prophylaxis and therapy after renal transplantation, there are no practice guidelines or standardized recommendations.
Methods: A questionnaire regarding CMV prophylaxis and therapy practices was sent to 280 UNOS certified renal transplant centers across the country. Participation was voluntary.
Results: Of the 77 responses received, 40% were filled out by surgeons (31/77), 52% by nephrologists (40/77), 3% each by transplant physicians and nurse coordinators (2/77) and one form by an administrator. We plan to continue adding responses as they come in.
Prophylaxis Practices: The results of drugs used for prophylaxis are summarized below:
CMV prophylaxis according to serostatus

	Valganciclovir Number (%)	Ganciclovir Number (%)	Acyclovir Number (%)	No prophylaxis Number (%)
D+/R-	48 (62%)	15(19%)	2(3%)	0
D+/R+	41(53%)	9(11%)	11(14%)	5(6%)
D-/R+	37(48%)	7(9%)	10(13%)	15(19%)
D-/R-	9(11%)	5(6%)	17(22%)	41(53%)

D=donor, R=recipient, +=positive and -=negative CMV serostatus

Of the centers which use CMV prophylaxis, most used it for at least 3 months (87- 97%, across various groups), though none reported use for longer than 6 months. 29% of centers restart CMV prophylaxis after pulse steroid therapy for acute rejection, but most (>90%) restarted it after thymoglobulin or OKT3 therapy.
Diagnosis: 56% of centers use a single test for the diagnosis of CMV infection, while the rest use up to 3 tests. CMV PCR is the most commonly used single test (35%).
Treatment Practices: 44% use IV ganciclovir, 31% use valganciclovir, 10% use IV ganciclovir followed by valganciclovir, while the rest use other products, including combination therapy, for CMV disease. Duration of treatment of CMV disease varies from 2 weeks (20%) to 6 months (20%). 32% of centers do not restart prophylaxis after treatment of CMV infection, the rest restituted prophylaxis for upto 6 months.
Ganciclovir Resistance: 11 of 77 centers (14%) of centers have seen patients with ganciclovir resistance, although these centers have reported a total of 14 cases.
Conclusions: 1. Valganciclovir is most commonly used for CMV prophylaxis, usually for 3- 6 months. **2.** Although all D+/R- recipients receive CMV prophylaxis, over 50% of D-/R- recipients do not receive any anti- viral prophylaxis. **3.** IV ganciclovir remains the drug of choice for treatment of CMV disease. **4.** Ganciclovir resistance is not seen/ appreciated at most centers

Abstract# 1233 Poster Board #-Session: P163-III

PROLONGED COURSE OF PROPHYLACTIC GANCICLOVIR RESULTS IN AN LOWER RISK OF DEVELOPING CMV DISEASE IN HIGH RISK PATIENTS. Alden M. Doyle,[1] Emily Blumberg,[2] Simin Goral,[1] Roy D. Bloom.[1] *[1]Renal Electrolyte Hypertension, University of Pennsylvania, Philadelphia, PA; [2]Infectious Diseas, University of Pennsylvania, Philadelphia, PA.*

Background: Despite antiretroviral prophylaxis, CMV disease continues to be a significant problem in high risk renal transplant recipients.

Aim: To compare the tolerability and efficacy of oral ganciclovir for 6 months with the standard 3 month course.

Methods: A retrospective analysis of 108 high risk CRT and LRT patients (CMV positive donor/CMV negative recipient) followed a minimum of 9 months after transplantation. A cohort of patients transplanted between 1/01 and 4/03 (n=40) were compared with a cohort of patients treated for 3 months (n=68) transplanted during the previous 18 months. A logistic regression analysis was performed to determine risk factors for CMV disease. Variables that were statistically significant in the univariate analysis were included in the multivariable model.

Results: There were no significant differences in cadaver kidney use, recipient race, gender, age, transplant history, renal diagnosis, opportunistic infections, hepatitis C seropositivity, graft loss, or preexisting diabetes between groups. All patients were able to complete the course of ganciclovir therapy. Each patient who developed CMV disease was successfully treated. The use of any cytolytic therapy was slightly higher in the 6 months group (97.5% vs. 73.5% p=.001), however, the use of tacrolimus, MMF, and Rapamycin were not significantly different between the two groups. The multivariate model included the use of 6 months of ganciclovir, type of induction therapy, use of cytolytic therapy, and tacrolimus levels at 1 month. In this multivariable model, 6 months of ganciclovir was associated with a significantly lower risk of developing CMV disease (odds ratio 0.25, 95% confidence interval 0.07-0.95, P=.042).

Conclusion: In high risk renal transplant recipients, six months of oral ganciclovir prophylaxis is associated with a lower rate of CMV disease than three months.

Abstract# 1234 Poster Board #-Session: P164-III

A PROSPECTIVE, CROSS-OVER TRIAL COMPARING CYCLOSPORIN A + ATORVASTATIN TO TACROLIMUS ALONE AND TACROLIMUS + ATORVASTATIN IN HYPERCHOLESTEROLEMIC RENAL TRANSPLANT PATIENTS. K. Martin Wissing,[1] Isabelle Dupont,[2] Philippe Unger,[3] Nilufer Broeders,[1] Yvon Carpentier,[2] Daniel Abramowicz.[1] *[1]Nephrology, Hopital ULB-Erasme, Brussels, Belgium; [2]NUTRISUB, Hopital ULB-Erasme, Brussels, Belgium; [3]Cardiology, Hopital ULB-Erasme, Brussels, Belgium.*

Background: Hypercholesterolemia is a frequent complication in renal transplant patients who receive cyclosporine A (CsA). Whether it is preferable to treat these patients with statins or to switch them from CsA to tacrolimus (TRL) has not been investigated.

Methods: Twelve kidney transplant recipients with total cholesterol >240 mg/dl or LDL cholesterol >130 mg/dl and stable graft function under CsA were enrolled. Patients had study visits every three months and treatment was changed 1 week after each study visit. Patients were successively crossed over from CsA alone (baseline) to: 1) CsA plus atorvastatin 10 mg/d (CsA-statin); 2) CsA alone (washout); 3) conversion to TRL (TRL) and 4) TRL plus atorvastatin 10 mg/d (TRL-statin). Total cholesterol (C), LDL-C, HDL-C, LDL and HDL alpha-tocopherol content, lag-time of LDL oxidation and percentage of dense LDL were assayed at each study visit.

Results: Plasma total cholesterol levels were 284±51 mg/dl (baseline) ; 200±32 mg/dl (CsA-statin), P<0.0001 vs baseline ; 267±39 mg/dl (washout) ; 234±31 mg/dl (TRL), P<0.001 vs baseline, P=0.01 vs CsA-statin ; 175±36 mg/dl (TRL-statin), P<0.001 vs TRL, P=0.001 vs CsA-statin. LDL-C levels were 160±41 mg/dl (baseline) ; 89±22 mg/dl (CsA-statin), P<0.0001 vs baseline ; 150±26 mg/dl (washout) ; 129±22 mg/dl (TRL), P=0.01 vs baseline, P=0.001 vs CsA-statin ; 80±21 mg/dl (TRL-statin), P<0.0001 vs TRL, P=NS vs CsA-statin. HDL-C levels were unchanged by statin therapy but had a tendency to decrease after conversion to TRL. Atorvastatin reduced the LDL/HDL ratio from 2.7±0.9 at baseline to 1.6±0.6 (CsA-statin, P<0.001), whereas conversion to TRL alone had no significant effect (2.6±0.8, P=NS). The LDL/HDL ratio under TRL-statin was slightly lower than under CsA-statin (1.4±0.5, P<0.05). Neither atorvastatin therapy nor conversion to TRL significantly changed the proportion of dense LDL, lipoprotein alpha-tocopherol contents or the lag-time of LDL oxidation.

Conclusions: Administration of 10mg/d atorvastatin to CsA-treated renal transplant patients is more efficient in reducing total and LDL cholesterol levels than conversion to TRL. Conversion to TRL + atorvastatin provided a moderate additional benefit as compared to CsA + atorvastatin treatment.

Abstract# 1235 Poster Board #-Session: P165-III

IMPACT OF GRAFT MASS ON THE CLINICAL OUTCOME OF KIDNEY TRANSPLANTS. Magali Giral,[1] Yohann Foucher,[2] Georges Karam,[1] Jean Michel Nguyen,[2] Michèle Kessler,[3] Bruno Hurault de Ligny,[4] Mattias Buchler,[5] François Bayle,[6] Carole Meyer,[7] Marie Laure Martin,[2] Pascal Daguin,[1] Jean Paul Soulillou.[1] *[1]Nephrology and Clinical Immunology, Nantes Hospital, France; [2]Biostatistic, Nantes Hospital, France; [3]Nephrology, Nancy Hospital, France; [4]Nephrology, Caen Hospital, France; [5]Nephrology, Tours Hospital, France; [6]Nephrology, Grenoble Hospital, France; [7]Transplant Surgery, Strasbourg Hospital, France.*

Background. Nephronic reduction of kidney transplants has been considered benign. However, pre peri and post transplantation injuries in cadaver transplantation may have led to an underestimation of its magnitude and possible implications. Our aim was to analyse the consequences of kidney mass on transplantation outcome.

Methods. The weights of 1142 kidney grafts were prospectively collected immediately before grafting. Donors and recipients <15 years of age, simultaneous kidney/pancreas grafts and technical failures before day 7 were excluded from the analysis. The analysis was performed on Cockroft calculated creatinine clearance and proteinuria in 964 patients for whom all the necessary information was available.

Results. Using a mixed linear model to analyse the effect of the Donor Kidney Weight/Recipient Body Weight ratios (DKW/RBW) on graft function adjusted according to others studied covariates, we observed that the smallest kidneys transplanted into the largest recipients (DKW/RBW < 2 $g.Kg^{-1}$, n=88) increased dramatically their creatinine clearance by 2.38 mL/min every month for six months (p<0.0001) and by 0.27 mL/min thereafter (p<0.0001). Conversely, creatinine clearance did not change for the largest kidneys transplanted into the smallest recipients (DKW/RBW ratios ≥ 4 $g.Kg^{-1}$). Moreover, because proteinuria could be the result of an hyperfiltration and is considered to be the principal marker of kidney damage, we then investigated whether the high filtration rate observed with low DKW/RBW ratio have resulted in the occurrence of a persistent proteinuria. Using a Cox model analysis, we showed that the risk of having a proteinuria above 0.5 $g.day^{-1}$ was significantly increased for the low DKW/RBW ratios < 2 $g.Kg^{-1}$ with 50% of patients having a proteinuria in this group, compared with DKW/RBW ratios ≥ 4 $g.Kg^{-1}$ (p<0.001).

Conclusion. In cadaver transplant recipients, graft mass has a rapid impact on graft filtration rate and proteinuria. Avoiding major kidney/recipient inadequacy should have a significant impact on long term transplant function.

Liver Transplantation: Complications and Disease Recurrence

Abstract# 1236 Poster Board #-Session: P166-III

DURATION OF ABSTINENCE FROM ALCOHOL BEFORE TRANSPLANTATION FAVORABLY AFFECTS POST TRANSPLANT ALCOHOL RELAPSE IN PATIENTS WITH ALCOHOLIC LIVER DISEASE. Jennifer T. Wells,[1] Adnan Said,[1] Alexandru Musat,[1] Munci Kalayoglu,[2] Stuart J. Knechtle,[2] Thomas Chin,[2] Anthony M. D'Alessandro,[2] John D. Pirsch,[2] Michael R. Lucey.[1] *[1]Gastroenterology and Hepatology, University of Wisconsin, Madison, WI; [2]Transplant, University of Wisconsin, Madison, WI.*

Background: The optimal duration of abstinence which predicts freedom from alcoholic relapse has not been well established in patients with Alcoholic Liver Disease (ALD) being evaluated for liver transplantation. In the absence of conclusive data, six months abstinence has become a widely used requirement.

Methods: We performed a retrospective cohort study on all patients with ALD alone, who underwent a primary liver transplant at the University of Wisconsin between 1984 and 2003. Information on pre and post transplant drinking behavior, and addiction counseling and therapy were abstracted from a prospectively constructed database and medical records. Relapse was defined as any recorded alcohol use.

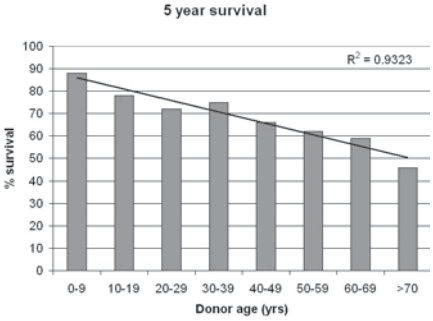
Results: Of 1100 liver transplant recipients from 1984 to 2003, 170 primary liver recipients who had ALD alone were included. All subjects had prolonged alcohol use (>50 g/day). Sixty nine percent (118/170) were male and the mean age was 52 years. Fifteen percent (25/165) had a drinking relapse with a median interval from transplantation to relapse of 37 months. In 17 of 25 (68%) the first relapse episode was 2 years or more after transplantation. Pre-transplantation duration of abstinence was categorized as < 6, 6 to12, 13 to24, and >24 months and was associated with rates of relapse of 21%, 23%, 7% and 13% respectively. Those abstinent for less than 12 months pre-transplantation had a significantly higher risk of relapse (Odds ratio 2.40, 95% CI 1.01-5.82, P=0.048) as compared to those abstinent for more than 12 months. In multivariable analysis, duration of abstinence pre-transplantation was the only significant factor predicting relapse (Odds ratio 2.98, 95% CI 1.09-8.19, P=0.03), whereas age, gender, race, pre-transplant counseling and addiction therapy did not predict relapse. Patient (log rank P=0.67) and graft survival (P= 0.82) post-transplantation did not correlate with length of abstinence from alcohol before transplantation.

Conclusion: Patients with ALD who have pre-transplant periods of abstinence of less than 12 months are at increased risk for alcohol relapse post transplantation. Patient and graft survival are not significantly impacted by alcohol relapse. Since many patients do not survive 12 months of pre-transplant observation, criteria other than duration of abstinence need to be considered in patients with shorter periods of abstinence.

Abstract# 1237 Poster Board #-Session: P167-III

DONOR AGE AND PRIMARY TRANSPLANT FOR HEPATITIS C: HOW OLD IS TOO OLD? Sasan Roayaie,[1] Gabriel E. Gondolesi,[1] Nancy R. Krieger,[1] Sukru H. Emre,[1] Myron E. Schwartz.[1] *[1]Recanati-Miller Transplantation Institute, Mount Sinai Medical Center, New York, NY.*

Background: The impact of donor age on survival after primary transplant for hepatitis C (HCV) is becoming well established. However, an acceptable upper limit has never been defined. **Methods:** Patients undergoing their first liver transplant between 6/89 and 5/00 were analyzed. Recipients with hepatocellular carcinoma (HCC) exceeding Milan criteria were excluded. Recipient and donor demographics and preoperative labs as well as era of transplant, postoperative immunesuppression, episodes of rejection, use of OKT3 and presence of hepatocellular carcinoma in the explant were analyzed. The impact of donor age on survival was also examined in a simultaneous cohort of non-HCV patients. **Results:** Of the 1324 patients transplanted during this period, 480 were HCV positive only. Of these, 31 had HCC exceeding the Milan criteria, leaving 449 patients in the study group. Median follow-up of survivors was 79.6±34 mo. Donor age, PT, BUN and creatinine were the only variables that significantly correlated with patient survival on univariate analysis for HCV patients. Donor age and recipient PT were the only variables that independently predicted survival on multivariate analysis. There was a significant inverse linear relationship between donor age and 5-year patient survival (fig 1) for HCV patients There was no correlation between donor age and survival for non-HCV patients. Donor age was an independent predictor of graft survival as well. **Conclusions:** Donor age is an independent predictor of patient and graft survival after primary transplant for HCV but not other indications. Survival at 5 year can vary from 88% to 46% based on donor age in HCV recipients. Unfortunately, there is no abrupt drop in survival to help establish an upper limit for donor age. Nevertheless, preferential use of older donors in non-HCV patients, in whom donor age does not affect survival, would result in improved survival for the entire population of liver recipients.



Abstract# 1238 Poster Board #-Session: P168-III

ANALYSIS OF OUTCOME FOLLOWING LIVER RETRANSPLANTATION FOR HCV. Ergun H. Velidedeoglu,[1] Peter L. Abt,[1] Adam M. Frank,[1] Niraj M. Desai,[1] Robert Stephenson,[1] Mary Kaminski,[2] Rajander R. Reddy,[2] James F. Markmann.[1] *[1]Surgery, Hospital of the University of Pennsylvania, Philadelphia, PA; [2]Hepatology, Hospital of the University of Pennsylvania, Philadelphia, PA.*

Background: Survival following liver transplantation for HCV is significantly poorer than for transplants performed for other causes of chronic liver disease. Retransplantation for HCV may be associated with a poor outcome, leading some to suggest that retransplantation in HCV is not justified.

Methods: A UNOS star file (5/91-12/01) was reduced to 18079 cases by excluding recipients <18yo, deaths and graft loss within 30d, recipients, multi-organ transplants, and implausible data. Kaplan-Meier graft and patient survival was calculated for primary and retransplants in the HCV- and HCV+ cohorts. Cox analysis was conducted to identify variables differentially associated with outcome in primary and secondary grafts. Variables considered included: donor and recipient age, gender, race, history of malignancy, combinations of donor-recipient gender and race, warm and cold ischemia, retransplant number, recipient labs (alb., creat., PT, T.bili), diabetes and pre-transplant life support.

Results: There were 10562 HCV- and 7517 HCV+ recipients. Mean recipient age was 49.8 ± 11.7 in HCV- versus 49.4 ± 8.4 in HCV+ recipients (p=0.03). Males comprised 55.3% of HCV- and 70.4% of HCV+ patients. The percentages of white and black recipients were 87.0% and. 7.4% in the HCV- cohort, vs. 88.1% and 7.1% in the HCV+ cohort. Retransplantation rates were 6.2% in HCV- vs. 7.0% in the HCV+ (p= .04). Reasons for retransplant in HCV- vs. HCV+ were HCV(5.2 vs. 58.3 %), HBV(8.2 vs. 1.7%), tumor(1.4 vs. 3.0%), alcohol(12.4 vs. 5.1%). Five-year graft survival of first and second grafts was 77.6 % and 59.5% (p< .000) in HCV- and 66.3% and 52.1% (p< .000) in HCV+. Retransplant, recipient and donor age, warm ischemia, and creatinine independently predicted graft survival in both cohorts, while recipient malignancy, donor race, female to male transplants, cold ischemia, albumin, and life support were independently significant in HCV- cohort. White to black race, and recipient diabetes were independently significant in the HCV+ cohort. The adjusted RR for graft failure in retransplanted patients was 1.80 in the HCV- cohort while it was 1.89 in the HCV+ cohort.

Conclusion: Multivariate analysis demonstrated that the adjusted relative risk of graft failure incurred by retransplantation is similar in HCV- and HCV+ recipients (RR 1.8 vs. 1.89). These results suggest that further careful study is required to determine cases in which retransplantation for HCV+ patients is not justified.

Abstract# 1239 Poster Board #-Session: P169-III

FACTORS DIFFERENTIALLY CORRELATED WITH THE OUTCOME OF LIVER TRANSPLANTATION IN HCV+ AND HCV - RECIPIENTS. Ergun H. Velidedeoglu,[1] Kevin C. Mange,[2] Peter L. Abt,[1] Adam M. Frank,[1] Niraj M. Desai,[1] Mary Kaminski,[3] Rajander R. Reddy,[3] James F. Markmann.[1] *[1]Surgery, Hospital of the University of Pennsylvania, Philadelphia, PA; [2]Nephrology, Hospital of the University of Pennsylvania, Philadelphia, PA; [3]Hepatology, Hospital of the University of Pennsylvania, Philadelphia, PA.*

Background: Survival following liver transplantation for hepatitis C is significantly poorer than for liver transplants performed for other causes of chronic liver disease. This is presumed to be at least in part a result of deleterious consequences of recurrent disease. However, the factors responsible for the inferior outcome in HCV+ recipients and whether they differ from factors associated with survival in HCV- recipients are unknown.

Methods: A UNOS Starfile (5/91-12/01) was analyzed to identify factors associated with outcome in HCV+ and HCV- recipients. Deaths or graft loss <30 days, recipients <18 yo, multiple organ transplants, implausible data, donors and recipient malignancy, retransplants, split and LDLT's were excluded. Kaplan-Meier graft and patient survival and Cox analysis was conducted on 13,026 transplants to identify variables differentially associated with survival in HCV- and HCV+.

Results: 7386 (56.7%) recipients were HCV- and 5640 were HCV+. Five-year patient survival was 83.5% vs. 74.6% (p< 0.00001) and five-year graft survival 80.6% vs. 69.9% (p< 0.00001) in HCV- and HCV+ populations, respectively. Multivariate analysis found donor age and recipient creatinine to be significant covariates in both groups while donor race, cold ischemia time (CIT), female to male transplants, and recipient albumin only predicted survival in HCV- recipients. In the HCV+ cohort only, recipient race, warm ischemia time (WIT), and diabetes also predicted graft survival. In addition, the negative effect of advanced donor age was greater in HCV+ recipients.

Conclusion: A number of parameters are differentially correlated with outcome in HCV- and HCV+ OLT recipients. Recipient race, warm ischemia time and recipient diabetes were uniquely associated with graft survival in HCV+ recipeints. These findings may not only have practical implications in the selection and management of liver transplant patients but also, may shed new insight into the biology of HCV infection post transplant.

Abstract# 1240 Poster Board #-Session: P170-III

ANTIVIRAL MAINTENANCE TREATMENT WITH INTERFERON AND RIBAVIRIN LIMITS FIBROTIC GRAFT DAMAGE IN LIVER TRANSPLANT PATIENTS WITH RECURRENT HEPATITIS C. Arno Kornberg,[1] Andrea Tannapfel,[2] Bernadett Küpper,[1] Johannes Scheele.[1] *[1]General and Visceral Surgery, Friedrich-Schiller-University, Jena, Germany; [2]Institute of Pathology, University Leipzig, Leipzig, Germany.*

Background:
Post-liver transplant (OLT) patients remain on continuous risk of progressive hepatitis C (HCV) after initial successful antiviral treatment, due to long-term immunosuppression. Patient and graft prognosis is determined by development of severe graft fibrosis or even cirrhosis. We demonstrate for the first time beneficial effects of the concept of an antiviral maintenance therapy after 12 months basic treatment course on graft morphology in liver transplant patients with recurrent HCV.

Patients and methods:
Between 1995 and 2003, a total of 23 patients with bioptically confirmed recurrent HCV after OLT were included in this study. Antiviral treatment started with a 12 months basic course using interferon-a 2b (IFN, IntronA™, 3x3Mio IE/week/Peg-Intron™, 50µg/week, Essex-Pharma, Germany) and ribavirin (Rb, Rebetol®, 3x200mg/day) Essex Pharma, Germany). Irrespective of virological response, a maintenance combination therapy was intended, with individual dose reduction only if necessary. Graft function (AST, ALT) and virological response (quantitative HCV-RNA) were determined continuously. Impact on fibrotic graft damage was classified according to Ishak and Knodell.

Results:
Follow-up after initiation of treatment ranges between 1 and 82 months. Treatment period exceeds 12 months in actually 20 patients. Initial biochemical response was 90%. At completion of basic treatment course 14 patients (70%) achieved serological clearance of viremia. Continuation of full dose antiviral treatment (n=14) resulted in sustained clearance of viremia up to 90 months post-OLT. Median stage of fibrosis remained low (actual 1,3± 0,8). In 3 out of 6 initial virological non-respopnders, therapy had to be stopped due to side effects. All of them developed severe biochemical and morphological disease progression (actual stage of fibrosis: 3,0). In 3 initial virological non-responders a median maintenance treatment of 16 months (range: 9-48 months) did not lead to clearance of viremia but to a limitation of graft fibrosis (actual stage of fibrosis: 2,0).

Conclusion:
We recommend antiviral maintenance therapy after a 12 months basic course, if tolerable for the patient. In virological responders, it prevents virological relapse and progression to severe graft fibrosis. In virological non-responders, it seems to be effective in limiting severe graft fibrosis despite persistent viremia.

Abstract# 1241 Poster Board #-Session: P171-III

HEPATITIS C IS A RISK FACTOR FOR DEATH AFTER LIVER RETRANSPLANTATION. Shawn J. Pelletier,[1] Douglas E. Schaubel,[1] Jeffrey D. Punch,[1] Robert A. Wolfe,[1] Friedrich K. Port,[2] Robert M. Merion.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI.*

Background: Retransplantation for liver failure associated with hepatitis C virus (HCV) has been increasing due to nearly universal posttransplant HCV recurrence and is associated with poor outcomes. We report on risk factors for death after retransplantation among liver recipients with HCV.

Methods: A retrospective cohort of liver transplant recipients who underwent retransplantation between 1/98 and 12/03 was identified in the Scientific Registry of Transplant Recipients (pediatric and multiorgan recipients excluded). Time-dependent Cox regression models were fitted to assess the relative effect of HCV on mortality risk after retransplantation, adjusted for recipient age, race, creatinine, pretransplant ICU, cold ischemia time, donor age, and time from 1st transplant to retransplantation.

Results: Of the 1807 liver retransplantations during the study period, 473 (26%) were associated with HCV. Recipients with HCV had a higher unadjusted post-retransplant death rate than those without HCV (26.9 vs. 21.2 deaths per 100 patient yrs, respectively). In the adjusted model, retransplant recipients with HCV had a 22% higher risk of death than those without HCV (hazard ratio [HR] 1.22; 95% CI 1.03-1.44; p=0.02). Other variables associated with significant relative risk of death after retransplantation included older recipient age, presence in an ICU, creatinine, and donor age >60. HCV recipients retransplanted 3-6 months and 6-12 months after the original graft had a significantly higher risk of death than non-HCV recipients (HR 1.57; CI 1.01-2.44; p=0.05 and HR 1.84; CI 1.12-3.01; p=0.02, respectively); those retransplanted earlier or later did not (0-3 mos.: HR 1.00; CI 0.80-1.26; p=0.98; 12-24 mos.: HR 1.57; CI 0.91-2.72; p=0.12; >24 mos.: HR 1.40; CI 0.84-2.32; p=0.20). Unlike retransplantation in HCV recipients aged 40+, retransplant recipients aged 18-29 and 30-39 demonstrated significantly higher mortality risk than non-HCV recipients (HR 4.55; CI 2.15-9.62; p<0.001 and HR 2.48; CI 1.42-4.33; p<0.001, respectively).

Conclusions: HCV is associated with increased risk of death, but other characteristics of HCV patients, including time from 1st transplant to retransplantation and recipient age, may identify those at particularly higher risk. For example, younger HCV patients had higher mortality than other young retransplant recipients. This may reflect willingness to retransplant younger patients with increased severity of illness or a more virulent HCV infection in this population.

Abstract# 1242 Poster Board #-Session: P172-III

EFFECT OF TIMING OF INTERFERON THERAPY ON FIBROSIS PROGRESSION IN RECURRENT HEPATITIS C INFECTION AFTER LIVER TRANSPLANTION. Marwan S. Ghabril,[1] Murli Krishna,[2] Victor I. Machicao,[1] Hugo Bonatti,[3] Ronald F. Borlaza,[1] Barry Rosser,[1] Raj Satyanarayana,[1] Jaime Aranda-Michel,[1] Bashar Aqel,[1] Andrew Keaveny,[1] Denise Harnois,[1] Winston Hewitt,[3] Hani Grewal,[3] Justin Nguyen,[3] Christopher Hughes,[3] Jeffery Steers,[3] Rolland C. Dickson.[1] *[1]Gastroenterology and Hepatology, 2 Pathology, 3 Transplantation Surgery, Mayo Clinic, Jacksonville, FL.*

The optimal timing of treatment for recurrent hepatitis C (HCV) after liver transplantation (LT) is undefined. **Aim**: To evaluate the effect of early interferon (IFN) therapy on long term fibrosis progression in recurrent hepatitis C. **Method**: Charts of all patients (pts) undergoing LT for HCV at our center from 03/14/1998 to 11/07/2000 were retrospectively reviewed. Pts with recurrent HCV and protocol biopsies at 4 month, 1 year and 3 year were considered for our study. Pts with concomitant HBV or active alcohol use after LT were excluded. Biopsies were graded (0-18) and staged (0-6) in a blinded fashion by a LT pathologist using the Ishak score. **Results:** 31 pts met criteria and were included in the study, 11 had mild recurrence and received no treatment, while 20 received IFN based treatment for significant recurrence (4 viral responders, 2 relapsed). IFN was started ≤6 months post LT (mean of 3.1) in 12 pts, and > 6 months post LT (mean 12.9) in 8. Biopsies performed prior to initiation of treatment (mean 2.4 vs mean 11 months) had similar mean grade (3.75 vs 3.63) and stage (1.0 vs 0.63) in each group respectively. Both the ≤6 month and >6 month groups received prolonged IFN treatment, mean 22 months (range 5-36) vs 18.6 months (range 3-34). Pts treated ≤6 months post LT had a slower rate of fibrosis progression and less inflammation at 3 years, compared with IFN > 6 months, despite poor viral responses, with 1 sustained responder per treated group. In addition, the stage at 3 years was similar in the early treatment group to the untreated pts who had been considered to have mild recurrence. (Table). **Conclusion:** This analysis of 3 year data on post LT recurrent HCV suggests that early and prolonged IFN therapy may be associated with a reduced rate of fibrosis progression and inflammation despite lack of viral eradication. The data is preliminary but warrants further investigation.

Histopathology results

Score (mean)	Grade			Stage			Fibrosis progression, point/yr
Time post LT	4 mth	1 yr	3 yr	4 mth	1 yr	3 yr	4 mth - 3 yr
IFN ≤ 6 mth, n=12	3.58	3.08	2.75	0.67	1.17	1.42	0.27*
IFN > 6 mth, n=8	2.65	3.88	4.63	0.25	1.25	2.38	0.77*
No IFN, n=11	2.73	2.50	2.82	0.18	0.80	1.45	0.48

* p<.05 for comparative differences in stage change

Abstract# 1243 Poster Board #-Session: P173-III

ACHIEVING EVR IN POST-LIVER TRANSPLANT PATIENTS WITH CHRONIC HEPATITIS C: IS IT A FAIR GOAL OF THERAPY? Christy Kennedy,[1] Mary McClure,[2] Timothy Brackett,[2] Bethanie Fernandes,[1] Tracy Steinberg,[2] Marcelo Kugelmas.[1] *[1]Medicine, University of Colorado, Denver, CO; [2]Surgery, University of Colorado, Denver, CO.*

Background: Early virologic response (EVR), defined by >2 log decrease in HCV RNA after 12 weeks of peginterferon + ribavirin (Peg+RBV), identifies patients with chronic hepatitis C (HCV) likely to sustain viral clearance after cessation of therapy. Aim: To measure tolerance to Peg+RBV therapy and its impact on achieving target doses (TD) and EVR in a cohort of liver transplant (LTx) recipients. Methods: We retrospectively reviewed records of all patients treated with Peg (α2a or α2b) and RBV who have completed at least 12 weeks of therapy at our institution since 2001. Therapy was initiated at half-dose Peg and either 400 or 600 mg/d of RBV, and increased at two week intervals aiming for the TD or maximum tolerated dose (MTD). Patients: Thirty-four patients (35 courses) received at least 12 weeks of Peg+RBV. Median age was 52 years (44-60), median weight was 80kg (52-136), 71% were men, and 89% were infected with HCV-1. Calculated creatinine clearance was 87.2 ml/min, 4 patients had Cr.Cl < 60 ml/min and sixteen had Cr.Cl. in between 60 and 90 ml/min. Therapy was started at a median of 19.6 months (3-102) after LTx. Patients were maintained on a variety of immunosuppressive regimens, 90% were off of steroids. Results: Fifteen patients (43%) achieved an EVR. Characteristics of these 15 patients were compared to 19 without EVR. Patients with EVR were more likely to achieve >80% of RBV TD (33% vs. 17%) and >80% of Peg TD (87% vs. 37%). The mean RBV-TD for the group was 1065 mg/d. The overall median RBV-MTD dose was 600 mg/d (0-1200) and the mean was 569 +/- 300 mg (53.4% of TD). Fourteen patients (40%) used G-CSF, 7 achieved EVR and 7 did not. Eleven (31%) patients used erythropoetin, 5 achieved EVR and 6 did not. Treatment was held in 17 patients: anemia (7), neutropenia (3), infection (3), rejection (1), depression (1), abdominal pain (1), and chest pain (1). Two patients died; one of a perforated duodenal ulcer and HCV-induced allograft failure and one from sepsis. Summary: Lower EVR in LTx recipients is related to greater HCV-1 prevalence, older age and inability to achieve TD of Peg+RBV due to renal impairment, poor bone marrow response and coexistent immunosuppression. Conclusion: Early stopping rules do not apply to treatment of HCV after LTx because of difficulty in achieving antivirals target doses. The adequate length and goals of treatment of HCV after LTx have not yet been adequately defined.

Abstract# 1244 **Poster Board #-Session: P174-III**

DETECTABLE SERUM CRYOPRECIPITATE POST TRANSPLANTATION IS ASSOCIATED WITH WORSE OUTCOME AFTER LIVER TRANSPLANTATION FOR HEPATITIS C. Stephen C. Rayhill,[1] Patricia Kirby, Michael Voigt, Douglas LaBrecque, Daniel Katz, Charles Lutz, Alan Stolpen, Rachael Miller, Roberto Kalil, Dennis Heisey, Wu You Min, Warren Schmidt. *[1]University of Iowa.*

The rapid recurrence of severe hepatitis C viral (HCV) hepatitis after liver transplantation is a problem. Positive cryoprecipitate (CP) levels are associated with poor outcome in the non-transplant population. We have demonstrated an association between CP and poor outcome after transplant. For our previous analyses, we used pre and post-transplant CP levels to define a patient's CP status (any positive equals CP positive). The effect of transplantation in CP is not known. Therefore, we analyzed the effect of post-transplant CP status. Methods: Using our longitudinal database, survival for all recipients of liver transplants for cirrhosis due to HCV was analyzed (1991 onward) based on the presence or absence of CP. Any of the 97 patients (105 grafts) with a cryocrit > trace was defined as CP positive. 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, and survival until the onset of severe activity and fibrosis were determined using Kaplan Meier estimates. Survival analyses were performed for the entire population (overall survival) and after censoring patients who died or lost their grafts for reasons other than recurrent HCV (HCV specific survival). The log rank test was used to compare survival and Cox multivariate analysis was used to determine risk ratios (RR). Results: Ninety seven patients had either a pre or post-transplant cryocrit, and 88 (91%) had a post-transplant CP level. Seventeen (19%) of the post-transplant patients were CP positive.

	Graft Survival		Survival Until Severe Activity		Survival Until Severe Fibrosis	
	RR	p	RR	p	RR	p
Overall Survival - Either Pre or Post CP Positive	1.6	0.2	2.3	0.009	2.1	0.04
Overall Survival - Post CP Positive	1.6	0.4	2.9	0.004	2.6	0.02
HCV Specific Survival - Either Pre or Post CP Positive	12.2	0.02	2.9	0.004	3.6	0.003
HCV Specific Survival - Post CP Positive	8.4	0.02	3.5	0.001	3.5	0.005

Conclusions: Liver transplant recipients with cryoglobulinemia are at greater risk for graft loss, severe early activity, and severe early fibrosis. Post-transplant CP determinations appear to be predictive.

Abstract# 1245

Abstract has been withdrawn.

Abstract# 1246 **Poster Board #-Session: P176-III**

RISK FACTORS FOR DEATH IN RECIPIENTS WITH RECURRENT HEPATITIS C CIRRHOSIS. Sammy Saab,[1,2] Henry Niho,[1] Scott Comulada,[3] R. Mark Ghobrial,[2] Jonathan Hiatt,[2] Fransico Durazo,[1,2] Steven Han,[1,2] Douglas G. Farmer,[2] Curtis Holt,[2] Hasan Yersiz,[2] Leonard I. Goldstein,[2] Ronald W. Busuttil.[2] *[1]Department of Medicine, Division of Digestive Diseases, University of California Los Angeles, Los Angeles, CA; [2]Dumont-UCLA Liver Transplant Center, Department of Surgery, University of California Los Angeles, Los Angeles, CA; [3]Department of Biostatistics, University of California Los Angeles, Los Angeles, CA.*

Background

Hepatitis C (HCV) is the leading indication for orthotopic liver transplantation in the United States, and recipients of orthotopic liver transplant for HCV invariably develop recurrent disease. As recurrent HCV is associated with an accelerated rate of cirrhosis, decompensation, and death, transplant centers will be faced with an increasing number of patients requiring retransplantation. However, the risk factors for death and retransplantation following the developing of cirrhosis from recurrent HCV is unclear.

Methods:

We reviewed records of patients who underwent liver transplantation for cirrhosis due to HCV between January 1990 and December 2001. Patients who developed recurrent cirrhosis from HCV were identified and divided into two groups, those who did and did not undergo retransplantation. We then performed a multivariate analysis of the effects of various prognostic factors on survival.

Results:

During the study period, 511 patients underwent transplantation for HCV cirrhosis. Of these, 81 patients (16%) developed recurrent cirrhosis from HCV; 51 were relisted for transplantation, and 28 underwent retransplantation. Using Kaplan-Meier survival curves, 1, 5, and 10 year patient survival was 96%, 64%, and 33%, respectively, for patients receiving a second liver transplant and 94%, 60%, and 32%, respectively, for patients who did not undergo a second transplant (p = 0.80). A multivariate Cox proportional hazards model showed patients with shorter times to clinical or pathological cirrhosis (Hazard Ratio [HR] = 0.60, Confidence Interval [CI] = 0.45 to 0.79, p < 0 .01), higher current INR levels (HR = 3.56, 95% C.I. = 2.01 to 6.31, p < 0.01) and not having diabetes mellitus (HR = 2.22, 95% C. = 1.11 to 4.55, p = 0.02) to have a higher risk of death; risk of death was not significantly different between those receiving and not receiving a second transplant (HR = 1.47, 95% CI = 0.70 to 3.08, p = 0.31).

Conclusions:

Shorter times to cirrhosis, higher levels of INR, and not having diabetes mellitus are associated with an increased risk of death following the development of cirrhosis from recurrent HCV.

Abstract# 1247 **Poster Board #-Session: P177-III**

HEPATITIS C VIRAL QUASISPECIES SELECTIVITY OF ALLOGRAFT INFECTION AFTER LIVER TRANSPLANTATION. Michael G. Hughes,[1] Tae W. Chong,[1] Robert L. Smith,[1] Heather L. Evans,[1] Robert G. Sawyer,[1] Christine K. Rudy,[1] Timothy L. Pruett.[1] *[1]Surgery, University of Virginia, Charlottesville, VA.*

Introduction: The purpose of the current study is to determine whether all hepatitis c viral quasispecies infect allografts after liver transplantation. If only a portion associate with and subsequently infect allografts, interventions targeting specific viral-allograft interactions could be developed to alter disease recurrence after liver transplantation. **Methods:** In 7 patients undergoing liver transplantation for HCV-induced end stage liver disease, blood samples were obtained prior to removal of the diseased liver, and liver biopsies were obtained from explanted liver, uninfected allograft 2 hrs after reperfusion, and infected liver 1 week to 2 months after transplantation. Cellular blood compartments (red blood cells, neutrophils, monocytes and lymphocytes) were isolated from blood samples in 2 patients by Ficoll gradient centrifugation. Serum compartments (immunoglobulin (Ig) bound virus, low density lipoprotein (LDL) bound virus and virus not bound to Ig or LDL) were isolated in 3 patients by immunoprecipitation with protein G beads and anti-LDL antibody. RNA was extracted from blood cell fractions, serum, serum fractions and liver biopsies, reverse transcribed and amplified by nested PCR with primers flanking hypervariable regions 1 and 2 (HVR1 and 2). The PCR product was cloned and directly sequenced to identify quasispecies variants within each sample. **Results:** HVR1: Genetic complexity and diversity decreased with reperfusion (complexity (Shannon entropy): serum 0.49±0.26 vs. postperfusion liver 0.29±0.14, p=0.04; diversity (maximal amino acid divergence): serum 10±7% vs. postperfusion liver 5±5%; p=0.03) but was not significantly different between postperfusion liver and infected liver quasispecies. HVR1 quasispecies not identified in the serum inoculum were recovered from postperfusion liver. These previously unidentified quasispecies were isolated from serum compartments but not cellular blood compartments. The predominant infecting quasispecies were serum rather than cell derived but were not derived or excluded from any single serum compartment. HVR2: HVR2 quasispecies were much less variable then HVR1 and did not demonstrate selective association or infection. **Conclusions:** Serum, not blood cell, derived HVR1 quasispecies selectively associate with and infect allografts after liver transplantation. This selectivity occurs primarily during reperfusion and is not due to infection by or exclusion of a single serum compartment.

Abstract# 1248 Poster Board #-Session: P178-III

PREOPERATIVE ALPHA-FETOPROTEIN SLOPE IS PREDICTIVE OF HEPATOCELLULAR CARCINOMA RECURRENCE AFTER LIVER TRANSPLANTATION. Kathy Han,[1] George Tzimas,[1] Peter Metrakos,[1] Nir Hilzenrat,[1] Philip Wong,[1] Marc Deschenes.[1] *[1]Department of Medicine, Liver Transplant Program, McGill University Health Centre, Montreal, QC, Canada.*

Liver transplantation (LT) offers a possibility of cure for patients with hepatocellular carcinoma (HCC) and cirrhosis, for it simultaneously removes the tumor and underlying liver dysfunction. However, tumor progression while on the waiting list and tumor recurrence after LT are not uncommon. We investigated the prognostic significance of various pre- and postoperative variables in terms of tumor recurrence, with emphasis on the slope of preoperative serum alpha-fetoprotein (AFP) levels. Data from 63 consecutive patients who had HCC and underwent LT between 1991 and 2003 at the McGill University Health Center were analyzed. The following was recorded age; gender; ethnic background; etiology of liver disease; Child Pugh score; pre-transplantation treatment with transarterial chemoembolization, percutaneous ethanol injection or intra-arterial chemotherapy; preoperative AFP values; result from the last preoperative imaging study; the explant histology; and, the Cancer of the Liver Italian Program (CLIP) score. Mortality data were assessed as of July 3, 2003. Univariate analysis revealed a positive correlation between the preoperative AFP slope and Cancer of the Liver Italian Program score ($p = 0.003$), vascular invasion ($p = 0.045$), tumor volume at explant ($p = 0.007$), and recurrence-free survival ($p = 0.003$). Of the pre- and postoperative variables examined, only preoperative AFP slope > 0, vascular invasion, and tumour volume at explant were identified as independent predictors of tumour recurrence by multivariate analysis. Patients with a preoperative AFP slope > 0 had a much worse 5-year recurrence-free survival rate than those with a preoperative AFP slope ≤ 0 (54 % vs 91 %, $p = 0.004$). In conclusion, as the incidence of HCC increases, the demand and waiting time for LTx continue to increase, it is important to identify prognostic factors for postoperative HCC recurrence in order to allocate organs efficiently. Despite technological advancement, the accuracy of preoperative imaging studies is limited. The present study shows that the preoperative AFP slope, which is easily obtained clinically, can be a useful preoperative predictor of tumor recurrence following LTx. This predictor variable should be examined in future studies of outcome following LTx for HCC.

Abstract# 1249 Poster Board #-Session: P179-III

OUTCOMES OF LIVER TRANSPLANT RECIPIENTS WITH HEPATITIS C RECEIVING INTERFERON / RIBAVIRIN TREATMENT. Gregory A. Smallwood,[1] Renee Devine,[1] Jennifer Lehneman,[1] Kathlene Connor,[2] Andrei C. Stieber,[2] Thomas G. Heffron.[2] *[1]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [2]Department of Surgery, Emory University School of Medicine, Atlanta, GA.*

Background: Recurrent hepatitis C (HCV) following liver transplant (OLT) currently contributes to both mortality and morbidity following transplantation and treatment includes ∝-interferon with ribavirin.

Objective: The aim of this study is to evaluate patients receiving treatment for HCV recurrence following OLT.

Methods: From February 1998 through November 2003, all liver transplant recipients with histological evidence of recurrent hepatitis C underwent treatment with ∝-interferon and ribavirin while non-responders were treated with peginterferons/ribavirin. Patients were compared on the basis of sex, age, race, immunosuppressive protocol used, days post transplant to histological recurrence, response to alpha-interferon and ribavirin, and patient and graft survival.

Results: Of 67 patients, there was a complete viral clearance in only 14.9 % (10/67). Fifty-three (79.1%) patients had a biochemical response by month 3. At 3 months there was a decrease in ALT [161 (± 91) u/L vs. 71.2 (± 130) u/L; $p = 0.003$], total bilirubin [1.6 (± 0.9) ng/dl vs. 1.2 (± 0.5) mg; $p = 0.05$], viral load (2.5×10^6 vs. 0.9×10^6, $p = 0.02$) and AST [146 (± 500) u/L vs 68 (± 103); $p = 0.002$). Of the remaining 57 patients, 25(43.8 %) were taken off of treatment due to adverse events. The remaining patients were begun on a non-responder protocol of peginterferon /ribavirin (n = 32). Six (18.7 %) of the non-responders had viral clearance at 3 months into peginterferon treatment. Sustained viral response has been documented in 2/10 (20%) of patients currently completing one year of peginterferon treatment. With the use of interferon products, mean biopsy scores indicated little progression of the disease under treatment [4.2 (± 0.4) vs. 4.3 (± 0.5); p = NS]. Race seems to play a role in overall survival with African-Americans (n = 16) having lower survival than non-African-Americans [66.7% (± 0.13) vs. 92.7% (±0.05); $p = 0.009$] with other parameters similar. Finally steroid dosing at 3 months appeared to make a difference in survival following recurrence of hepatitis C with patient expiring having a higher dose [16.4 (± 4.7) vs. 12.3 (±4.6); $p = 0.039$].

Conclusions: Following OLT, treatment of recurrent HCV is less effective than reported response rates with a high number of treatment failures, drop outs, and low survival for African-Americans or patients with higher prednisone doses. Virologic response can still be obtained with the use of peginterferons in the non-responder population.

Abstract# 1250 Poster Board #-Session: P180-III

LIVER TRANSPLANT OUTCOMES USING EXTENDED CRITERIA DONOR ORGANS: A SINGLE CENTER EXPERIENCE. M. Gupta, L. Trumbull, J. Martin,[2] J. F. Buell, T. D. Merchen, M. J. Hanaway, E. S. Woodle, J. G. Drewett,[2] S. M. Rudich. *[1]Transplant Surgery; [2]College of Pharmacy, University of Cincinnati, OH.*

Extended criteria donor (ECD) liver utilization has expanded the limited donor pool for patients awaiting orthotopic liver transplant (OLT). Many centers approach ECD livers with caution as the outcomes following their use remains unclear. **PURPOSE**: To analyze outcomes of OLT using ECD livers compared to a control group of standard criteria donor (SCD) liver allografts. **METHODS**: All OLT recipients at our center between Jan 2002-Oct 2003 were categorized as having received an ECD or SCD liver. ECD allografts 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), use of 2 or more pressors within 12 hrs of procurement, or liver function studies > 5 x upper limit of normal. Parameters that were reviewed included: recipient demographics (age, sex, MELD, mean follow-up, length of stay), graft ischemic times (total and warm), laboratory data, and clinical outcomes (death, biliary complications, rejections). **RESULTS**: 90 OLTs (42 ECD and 48 SCD) were performed during the study period. No statistical differences were observed in laboratory data including: serum creatinine, transaminases (AST/ALT), total bilirubin, and albumin at discharge, day 30, 90, and 180 for both groups. The two groups were similar with respect to rejection episodes per patient ECD (1.1) vs. SCD (1.3) [p=ns]. Five instances of vascular complications were identified (3 ECD vs. 2 SCD), although only one of those was a hepatic artery thrombosis.

	ECD (n=42)	SCD (n=48)	p value
Age (mean yrs±SD)	52.1 ± 9.2	51.6 ± 7.6	ns
Gender (M/F)	26/16	33/15	ns
Match MELD (mean±SD)	15.7 ± 7.4	18.3 ± 7.7	0.10
Lab MELD (mean±SD)	14.0 ± 6.8	14.3 ± 7.1	ns
Total Graft Ischemic time (mean mins±SD)	584 ± 92	612 ± 187	ns
Graft Warm Ischemic time (mins)	39.4 ± 6.3	39.9 ± 8.9	ns
Total OR Time (mean mins±SD)	481 ± 164	491 ± 135	ns
Length of Stay (mean days±SD)	9.7 ± 5.7	9.0 ± 6.0	ns
Follow-up (mean days±SD)	197 ± 115	279 ± 177	0.01
Deaths (n)	3 (7.1%)	3 (6.3%)	ns
Biliary Complications (n)	5 (11.9%)	5 (10.4%)	ns
Pts with Rejection (n)	12 (28.6%)	13 (27.1%)	ns

CONCLUSIONS: Clinical outcomes following OLT using ECD livers are identical to those using SCD livers. No differences were noted insofar as early or mid laboratory values, LOS, biliary or vascular complications. With intelligent recipient selection, the aggressive use of ECD liver allografts can become an important component of any transplant program.

Abstract# 1251 Poster Board #-Session: P181-III

BILIARY COMPLICATIONS IN PEDIATRIC LIVER TRANSPLANTATION: SINGLE CENTER EXPERIENCE. Manuel I. Rodriguez-Davalos,[1] Rafael J. Maurette,[1] Benjamin Shneider,[1] Gabriel E. Gondolesi,[1] Nancy Krieger,[1] Sukru Emre.[1] *[1]Pediatric Liver Transplantation, Recanati/Miller Transplantation Institute. Mount Sinai School of Medicine, New York, NY.*

Introduction

Biliary complications continue to be leading cause of morbidity and mortality after liver transplantation. Incidence of such complications is higher when cut down liver allografts such as reduced (R), split (SLT) and living donor(LDLT) are used. In this study, we reviewed our experience with biliary complications and compared the incidence and outcome of biliary complications in various graft types.

Material and Methods

Between 1/1998 and 12/2003, 158 pediatric liver transplants were performed in our institution. The following variables were analyzed: Patient demographics, type of liver allografts (R, LDLT, SLT, and Whole), graft anatomy and type of biliary reconstruction, cold and warm ischemia times and complications (biliary leak and stricture, hepatic artery thrombosis (HAT). We also analyzed the therapeutic approach to biliary complications.

Results

The mean recipient age was 6.8 years (r,18 days-20 years). 57 (36.1%) whole grafts, 32 (20.3%) split grafts, 7 (4.4%) reduced size grafts and, 62 (39.2%) living donor grafts were transplanted. Bilio-enteric anastomosis was performed in 114 (72.2%), and duct to duct anastomosis was done in 44 (27.8%). Eleven patients (6.96 %) had biliary complications. Hepatic artery thrombosis was diagnosed in 6 (3.79%); (see Table)

1 patient died on POD# 1, 2 underwent re-OLTx. Grafts were salvaged in other 3 of which, only 1 developed biliary complication. Seven of 11 biliary complications (63.6%)

required surgical intervention including drainage, revision, or conversion from duct-to-duct to hepatico-jejunostomy. Last 5 (36.3%) managed by non surgical approach including percutaneous drainage and balloon dilatation. In only one case non surgical approach was failed necessitating surgical revision.

Conclusions: Although overall incidence of biliary complications is low in our series, most of the biliary complications occurred in cut down livers. Non surgical approach should be the first choice of treatment.

Biliary Complications

	Stricture Roux-en-Y	Stricture Duct-duct	Bile Leak Roux-en-Y	Bile Leak Duct-duct	Leak from liver cut surface/or accessory duct	Biloma	HAT
Whole	-	-	-	1	1	-	-
SLT	-	-	-	-	1	-	3
LDLT	3	2	1	-	1	1	3

Abstract# 1252 Poster Board #-Session: P182-III

REJECTION IS A RISK FACTOR IN THE DEVELOPMENT OF PEDIATRIC POST-TRANSPLANT DE-NOVO AUTOIMMUNE HEPATITIS. Robert S. Venick,[1] Sue V. McDiarmid,[2] Jeff Gornbein,[3] Steven Wu,[1] Susan J. Pacini-Edelstein,[1] Mini Mehra,[1] Martin G. Martin,[1] Jorge H. Vargas,[1] Ron W. Busutill,[4] Marvin E. Ament.[1] *[1]Pediatrics, The David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Pediatrics and Surgery, The David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Biomathematics, The David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]Surgery, Dumont-UCLA Transplant Center, Los Angeles, CA.*

BACKGROUND: Post-transplant de-novo autoimmune hepatitis (d-AIH) is increasingly described as a long-term complication after pediatric liver transplantation (LT). D-AIH is characterized by graft dysfunction, the development of autoimmune antibodies (AA), and histologic evidence of hepatitis in liver transplant patients without previous history of autoimmune liver disease. This study is a matched case-control, univariate analysis which examines risk factors for developing d-AIH. **METHODS:** From 1984 to 2003, 810 pediatric LTs were performed at a single center. 50 patients developed positive AA and graft dysfunction. 9 patients were excluded because biopsy findings were not consistent with autoimmune hepatitis. The remaining 41 patients were matched with a control group for sex, initial diagnosis, and age at the time of transplant. **RESULTS:** The indications for LT were: biliary atresia n=19, fulminant hepatic failure n=14, α-1-antitrypsin deficiency n=2, OTC deficiency n=2, Alagille's syndrome n=1, Crigler-Najjar n=1, Budd-Chiari syndrome n=1, and PFIC Type 2 n=1. The overall incidence of d-AIH was 5.1 %, with 80.4% of these patients developing d-AIH after 1999. The female to male ratio was 1.7:1. The mean age at LT was 3.6 years, and the mean time from transplant to the development of autoimmune hepatitis was 7.0 years. 22 of the patients in the d-AIH group were on cyclosporine, azathioprine and prednisone as their primary immunosuppressant, while 19 were on tacrolimus and prednisone. The d-AIH group had a mean of 2.8 total episodes of rejection compared to 1.0 episodes in the control ($p<0.05$). More specifically, the d-AIH group had a mean of 2.0 vs 0.7 epsiodes of acute rejection ($p<0.05$) and 0.2 vs 0.03 episodes of steroid resistant rejection ($p<0.05$) in comparison to the control group. The following variables were analyzed and found not to be significant in the development of d-AIH: age, sex, initial diagnosis, and primary immunosuppression. **CONCLUSIONS:** The number of episodes of rejection is a significant risk factor in developing d-AIH. In this center's experience the incidence of d-AIH is 5.1%, with the majority of these cases occuring after 1999. We are in the process of investigating this trend, which may be related to changes in immunosuppression protocols. A multivariate analysis is underway.

Abstract# 1253 Poster Board #-Session: P183-III

THE EFFECT OF DONOR AND RECIPIENT HLA COMPATIBILTY ON LIVER TRANSPLANTATION OUTCOMES: AN ANALYSIS OF THE UNOS DATABASE. Charles P. Katopes,[1] Beth W. Colombe,[2] C. Victor Spain,[3] Steven K. Herrine,[1] Victor J. Navarro.[1] *[1]Gastroenterology & Hepatology, Thomas Jefferson University, Philadelphia, PA; [2]Department of Medicine, Thomas Jefferson University, Philadelphia, PA; [3]Jefferson College of Graduate Studies, Philadelphia, PA.*

Background and Purpose: The correlation between donor and recipient HLA-A, B, DR antigen matches/mismatches and liver transplant outcome is unclear. Markus et al. (1988) observed that HLA compatibility caused a trend toward lower graft survival. Neumann et al. (2003) found a trend toward reduced graft survival with certain HLA compatibilities in autoimmune diseases. To more definitively address this issue, we analyzed the United Network for Organ Sharing (UNOS) liver transplant database to determine the effect of donor and recipient HLA match/mismatch (among A, B, and DR loci) on 5-year graft survival.

Methods: We conducted a retrospective cohort study on the UNOS liver transplant database. In the analysis, we included all first liver transplants that occurred between 1987 and 2002 where complete HLA data for both the donor and recipient were available. We used Cox proportional-hazard models to evaluate associations between total number of HLA matches and mismatches (on a scale from 0 to 6) and 5-year graft survival, where graft failure was defined as retransplantation or death from transplant-related cause. Multi-variable models were used to evaluate statistical interactions and adjust for the effect of potential confounding variables.

Results: Of 61,148 liver transplants in 55,193 recipients, 29,131 first time-transplants met the selection criteria for this analysis. Among recipient-donor pairs analyzed, the median number of HLA matches was 1 (range 0 to 6), median number of mismatches was 5 (range 0 to 6). We found no significant interactions between HLA match/mismatch and ethnicity, gender, transplantation during childhood, or recipient diagnoses of viral hepatitis or autoimmune disease. After adjusting for potential confounding due to age, diagnosis, ethnicity, gender, hepatitis C status, live donor, simultaneous multi-organ transplant, recipient medical condition before transplant, and transplant year, we found no association between the degree of HLA match or mismatch and 5-year graft survival (all $p > 0.1$).

Conclusions: After controlling for potential confounding variables, the degree of donor and recipient HLA match or mismatch was found to have no effect on 5 year liver graft survival. Importantly, we found no increase in risk of graft loss with increasing HLA match level irrespective of recipient etiology of liver disease.

Abstract# 1254 Poster Board #-Session: P184-III

CARDIOVASCULAR DISEASE AFTER LIVER TRANSPLANTATION. Arie P. van den Berg,[1] Maarten Borg,[1] Stephan Bakker,[2] Wim J. Sluiter,[2] Maarten J. H. Slooff,[3] Els B. Haagsma.[1] *[1]Gastroenterology and Hepatology, University Hospital, Groningen, Netherlands; [2]Internal Medicine, University Hospital, Groningen, Netherlands; [3]Hepatobiliary Surgery and Liver Transplantation, University Hospital, Groningen, Netherlands.*

Backgrounds: Cardiovascular disease (CVD) is the main cause of death after renal transplantation. The impact of CVD after liver transplantation is less well established.

Methods: We analyzed the long-term cardiovascular morbidity and mortality in a cohort of 311 consecutive adult recipients of a first liver transplant. Patients were transplanted between 01-01-1979 and 31-12-2000, and had survived for at least 1 year after OLT. No patient was known with CVD at the time of transplantation. We determined whether cardiovascular events could be predicted at one year after transplantation by established cardiovascular risk factors or by transplant-related characteristics.

Results: The mean duration of follow-up was 6.8 years. Five- and 10- year patient survival rates were 88 and 84%, respectively. A total of 32 cardiovascular events occurred in 23 patients. Systolic hypertension, smoking, hypercholesteremia, hypertriglyeridemia, diabetes and proteinuria all were associated with CVD. Multivariate analysis showed that systolic blood pressure (P=0,01) and smoking (P=0,006) were significant and independent risk factors for cardiovascular disease. Hypertensive patients in whom treatment had controlled the systolic blood pressure to values <140 mmHg had a strongly reduced risk of CVD compared with patients with a systolic blood pressure ≥140 mmHg (with or without antihypertensive treatment). During follow-up 52 patients died, 11 of them (21%) due to a cardiovascular event. CVD was the principal cause of death after the 10th year post-transplant, causing 6 out of 11 deaths. The cumulative incidence of cardiovascular death 20 years after transplantation was 38%. The mean age at cardiovascular death was 60 years, which is low compared with a mean age of 78 years for cardiovascular death in the general Dutch population.

Conclusions: CVD is a major source of morbidity and mortality after liver transplantation, and probably occurs prematurely. CVD can be predicted by classic risk factors such as systolic hypertension and smoking. Aggressive reduction of these risk factors may effectively reduce the prevent these complications.

Abstract# 1255 Poster Board #-Session: P185-III

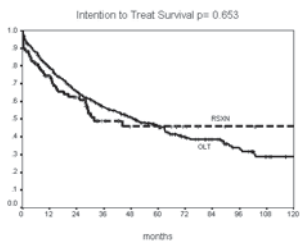
AN EVALUATION OF THE LONG-TERM CLINICAL OUTCOME AND SURVIVAL OF PATIENTS UNDERGOING LIVER TRANSPLANTATION AT AGE 65 YEARS OR OLDER. Michael A. Heneghan,[1] Paolo Muiesan,[1] Raffaeli Girlanda,[1] Mohammed Rela,[1] Matthew Bowles,[1] John G. O'Grady,[1] Nigel D. Heaton.[1] *[1]Institute of Liver Studies, King's College Hospital, London, United Kingdom.*

BACKGROUND: Significant advances in perioperative care and immunosuppression have resulted in a broadening of the indications for organ transplantation. Specifically, advanced age is no longer considered a contraindication to transplantation in most centers. Although short-term studies of elderly liver transplant recipients have demonstrated that the incidence of complications and overall patient survival are similar to those of younger adults, it is apparent that minimal center-specific, long-term data are available. Moreover, few data are available pertaining to patient survival in liver transplant recipients aged over 65 years at the time of transplant. METHODS: From January of 1998 to December of 2002, 84 patients 65 years of age or older received primary liver transplants at our center. This group of patients was compared with two groups of younger adult recipients; group 2 aged 60-64 years (n = 183) and group 3 aged between 18 years and 59 years (n = 1245) who received primary liver transplants during the same period. The most common indications for transplantation in all groups were alcohol related cirrhosis, hepatocellular carcinoma, hepatitis B and C virus infection, primary biliary cirrhosis, primary sclerosing cholangitis, and cryptogenic cirrhosis. There was no difference in the preoperative severity of illness between the groups. Results. Overall, length of hospitalization was similar for both groups, and there were no significant differences in rejection or infection rates (surgical or opportunistic), repeat operation, or need for repeat transplantation between the groups. The only significant difference identified between the groups was long-term survival. Mean survival was 102.7 months in the older group, 149 months in the 60-64 year old group and 158 months in the younger group. Overall, there was no statistically significant difference in survival between the three groups. Consistent with previous observations, the commonest cause of late mortality in elderly liver recipients was malignancy, whereas most of the young adult deaths were related to infection. CONCLUSION: Although older recipients > 65 years at this center did as well as younger recipients in the early years after liver transplantation, long-term survival is less convincing. Therefore, judicious choice of elderly candidates must be undertaken to optimize survival in this patient cohort.

Abstract# 1256 Poster Board #-Session: P186-III

TRANSPLANT VS RESECTION FOR HEPATOCELLULAR CARCINOMA IN THE CONTEXT OF HEPATITIS C:AN INTENTION TO TREAT ANALYSIS. Sasan Roayaie,[1] Gabriel E. Gondolesi,[1] Nancy R. Krieger,[1] Sukru H. Emre,[1] Myron E. Schwartz.[1] *[1]Recanati-Miller Transplantation Institute, Mount Sinai Medical Center, New York, NY.*

Purpose: To determine whether resection (RSXN) or transplant (OLT) offers better overall and disease free survival for patients with hepatocellular carcinoma (HCC) and hepatitis C (HCV) cirrhosis. **Methods:** We reviewed data on 440 consecutive patients with HCC and HCV who were candidates for RSXN or OLT. Patients were considered first for RSXN if they had Child's A cirrhosis, no encephalopathy or ascites, and platelets >100,000. Tumors had to be resectable without sacrificing a significant amount of parenchyma. Patients ineligible for RSXN were considered for OLT. **Results:** Between 1/92 and 10/03, 86 patients underwent RSXN and 354 were referred for OLT of whom 221 were transplanted, 92 died waiting for OLT, 37 are waiting, and 4 are alive but removed from the list. RSXN patients had significantly larger tumors (6.3 vs. 3.3 cm) that were more poorly differentiated and had a higher incidence of vascular invasion (78% vs. 46%). RSXN patients had a significantly higher chance of recurrence at 5 yrs (75% vs. 30%). Survival based on intention to treat was similar for RSXN and OLT (Fig 1) as was post surgical survival. Disease free survival at 5 years was significantly better for patients undergoing OLT (47% vs. 26%). Multivariate Cox regression analysis including type of surgical treatment along with other variables significantly correlating with disease free survival on univariate analysis (tumor size, grade, vascular invasion, AFP) revealed tumor size and vascular invasion as the only independent predictors of disease free survival. **Conclusions:** Survival is the same for RSXN and OLT despite more aggressive tumors in the RSXN group. Disease free survival is better for those undergoing OLT though their tumors were less advanced. Only tumor size and vascular invasion were independent predictors of disease free survival. Patients with HCV and HCC should first be considered for RSXN.



Abstract# 1257 Poster Board #-Session: P187-III

EFFICACY AND SAFETY OF LOW-DOSE VALGANCICLOVIR FOR THE PREVENTION OF CYTOMEGALOVIRUS DISEASE IN LIVER TRANSPLANT RECIPIENTS. Jeong M. Park,[1] Juan D. Arenas,[2] Robert J. Fontana,[3] Kathleen D. Lake.[2,3] *[1]Pharmacy Services, University of Michigan, Ann Arbor, MI; [2]Surgery, University of Michigan, Ann Arbor, MI; [3]Internal Medicine, University of Michigan, Ann Arbor, MI.*

Background: Valganciclovir (VGCV) is indicated for the prevention of cytomegalovirus disease (CMVD) in kidney, heart, and kidney-pancreas transplant recipients at high risk for CMVD. The efficacy and safety of VGCV in liver transplant (LT) recipients has not been established. A preliminary sub-group analysis of LT recipients in the Phase III trial (PV16000) reported a higher, but statistically non-significant, incidence of overall CMVD with oral VGCV 900 mg QD, compared to oral ganciclovir (GCV) 1 g TID. Because of its improved pharmacokinetics, 450 mg of oral VGCV offers convenient once-daily dosing while delivering systemic GCV exposure equivalent to that of oral GCV 1 g TID. The purpose of this study was to evaluate the efficacy and safety of low-dose VGCV vs. GCV for CMVD prophylaxis in LT recipients. **Methods:** In 2002, CMVD prophylaxis regimen at our transplant center was changed from GCV to VGCV. LT recipients at high risk (D+/R-) or moderate risk (D±/R+) for CMVD received either oral GCV 1g TID or VGCV 450 mg QD (both adjusted for renal function) for 90 days following transplant. Adult LT recipients between 01/01/01-02/28/03 were retrospectively reviewed for their CMVD prophylaxis and immunosuppression regimens, occurrence of CMVD (CMV viremia with fever or tissue invasive CMVD), and hematological toxicity. **Results:** Forty seven GCV recipients (9 D+/R- and 38 D±/R+) and 42 VGCV recipients (7 D+/R- and 35 D±/R+) were identified. Mean follow-up was 27 ± 5 months for the GCV cohort and 15 ± 3 months for the VGCV cohort. All but 3 VGCV recipients (no steroids) initially received tacrolimus/MMF/steroids for immunosuppression and a majority of them remained on the same regimen. Fifteen GCV recipients (13 with steroids and 2 with antibody) and 8 VGCV recipients (7 with steroids and 1 with antibody) were treated for rejection. Two patients in each cohort developed CMVD (4% in the GCV cohort vs. 5% in the VGCV cohort, p=1.0), including 1 CMV hepatitis in the GCV cohort and 1 CMV gastritis in the VGCV cohort. All CMVD cases occurred in the D+/R- group and after completion of CMVD prophylaxis (113-261 days post-transplant). None of these 4 patients were treated for rejection before the onset of CMVD. No patients exhibited ANC < 500 /mm^3 during CMVD prophylaxis. **Conclusions:** Compared to oral GCV, low-dose VGCV appears equally efficacious and safe in the prevention of CMVD following LT. Our findings support the high-risk of D+/R- recipients and the concern for delayed-onset CMVD after completion of CMVD prophylaxis.

Abstract# 1258 Poster Board #-Session: P188-III

EFFICACY OF CMV ANTIGEEMIA DIRECTED PREEMPTIVE THERAPY WITH GANCICLOVIR AT ONE YEAR IN LIVER TRANSPLANT RECIPIENTS PRETREATED WITH THYMOGLOBULIN OR ALEMTUZUMAB. Eun J. Kwak,[1] Shahid Husain,[1] David L. Paterson,[1] Paulo Fontes,[2] Thomas Cacciarelli,[2] Amadeo Marcos,[2] John Fung,[2] Bijan Eghtesad.[2] *[1]Div. of Infect Dis, Univ.of Pitt Med Ctr, Pittsburgh, PA; [2]Thomas E . Starzl Trans Inst, Pittsburgh, PA.*

OBJECTIVE. To compare the efficacy of preemptive therapy (guided by CMV antigenemia) with ganciclovir among liver transplant recipients pretreated with thymoglobulin or campath to that of standard immunosuppression.**METHODS.** We studied consecutive liver transplant recipients at our center from Jan 2001 to April 2003. From Jan - June 2001 patients received only postoperative maintenance immunosuppression with tacrolimus and prednisone (Group 1).From July 2001- Sept 2002 patients were pretreated with thymoglobulin 5mg/kg body weight (Group2) and as of Sept 2002 received pretreatment with 30 mg of alemtuzumab (Group 3). Both groups (Group 2 and 3) also received maintenance immunosuppression with tacrolimus. Significant CMV antigenemia was defined as presence of 1 positive cell/2x10^5 leukocytes in high risk (Donor +/ Recipient -) or ≥ 10 CMV positive cells/2x10^5 leukocytes in low risk group (Recipient + or Recipient/Donor-). All transplanted patients with significant antigenemia received preemptive therapy with 5mg/kg /day of IV ganciclovir titrated to renal function, continued until two antigenemia tests were negative.CMV disease was diagnosed histologically. **RESULTS.** Of 201 patients 65 belonged to Group 1, 64 to Group 2, and 72 to Group 3. The three groups did not differ in respect to sex, age, indication of transplantation, MELD score and donor or recipient CMV status. CMV antigenemia developed in 55 %(36/65) in Group 1, 47 % (30/64) in Group 2 and 54 % (39/72) in Group 3 respectively (p=.5). Significant CMV antigenemia was noted in 22 % (14/65) in Group 1, 28 % (18/64) in Group 2 and 26% (19/72) in Group 3 respectively (p=.2). Recurrence of CMV antigenemia was found in one case each of Group 1 and Group 2. Positive CMV antigenemia occurred predominantly within a month of transplantation (Median 30 days; 25th-75th = 19-50days). Median duration of positive CMV antigenemia was 7 days in each group (25th-75th = 3-21 days). Histologically proven CMV disease was noted in 1 patient in Group 1and 2 patients in Group 2. None of the patients in Group 3 developed histologically proven CMV disease.**CONCLUSION.**CMV antigenemia directed preemptive therapy with ganciclovir is comparable in preventing CMV disease at one year in liver transplant recipients pretreated with thymoglobulin or alemtuzumab

Abstract# 1259 Poster Board #-Session: P189-III

LOW DROP-OUT RATE FROM LIVER TRANSPLANT WAITING LIST IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC) TREATED WITH INTRA-ARTERIAL CHEMO/ EMBOLIZATION (TACE/TAE). Giovanni B. Vizzini,[1] Angelo Luca,[2] Adele D'Antoni,[1] Roberto Miraglia,[2] Ugo Palazzo,[1] Giada Pietrosi,[1] Lucio Mandalà,[3] Salvatore Gruttadauria,[3] Tommaso Piazza,[4] Bruno Gridelli.[3] *[1]Hepatology and Gastroenterology, ISMETT-UPMC, Palermo, Italy; [2]Radiology, ISMETT-UPMC, Palermo, Italy; [3]Transplant Surgery, ISMETT-UPMC, Palermo, Italy; [4]I.T., ISMETT-UPMC, Palermo, Italy.*

Liver transplantation is considered the only curative treatment for HCC, because it simultaneously cures the malignant disease and the cirrhosis. However, due to the organ shortage and the increasing waiting time, intention to treat analyses showed a reduced efficacy of OLT, due to tumor progression and drop-out from the list (up to 20% in published series). TACE/TAE has been used as neo-adjuvant therapeutic option in patients with HCC in the waiting list for OLT, with conflicting results. In this study, we analyzed the outcome of a group of patients with HCC, treated with TACE/TAE during the waiting time for OLT.

Patients: from July 1999 to July 2003 we treated with TACE/TAE 302 patients at our Institution, equal to 698 treatment sessions in total. Fifty-nine of them fitted our criteria for liver transplantation ("Milano" criteria) and were treated with 74 sessions of TACE/ TAE (1.2 treatments/pt) during the waiting time: 38 patients had single lesion < 5 cm, 12 patients had 2 or 3 lesions, max 3 cm. Nine patients fitted the criteria after reduction of the tumor size (HCC down-staging) with TACE/TAE.

Results: Twenty-one patients underwent liver transplantation after a mean waiting time of 289 days (range 25-740); 3 patients died during the waiting time, due to liver failure, and 27 patients are still alive in the waiting list without tumor progression. There were 6 (10.2%) drop-outs because of tumor progression, with a cumulative probability of drop-out at 6, 12, 18 and 24 months of 3.9%, 7.1%, 12.5% and 17.1%, respectively. Out of the 9 down-staged patients, one had tumor progression and was excluded from the list, one died during the waiting time due to liver failure, 3 patients underwent liver transplantation, and 4 are still alive in the waiting list without tumor progression. Out of 21 transplanted patients (mean follow-up 11.4 months, +/-10.3), one single HCC recurrence occurred, 20 months after OLT.

Conclusion: TACE/TAE seems to be an effective means to control tumor growth in patients with HCC awaiting OLT, and it allowed to achieve a low drop-out rate from the waiting list. It can be particularly helpful in centers where the pre-OLT waiting time is estimated to be long.

Abstract# 1260 Poster Board #-Session: P190-III

LEVETIRACETAM (KEPRA) HAS SUPERIOR EFFICACY AND IS ASSOCIATED WITH REDUCED DOSES OF ANTI-REJECTION MEDICATIONS AFTER LIVER TRANSPLANTATION. Abigail Mithoffer,[1] Graham Glass,[2] James Stankiewicz,[2] Peter Bergethon,[2] Jeffery Cooper,[1] Richard J. Rohrer,[1] Richard B. Freeman.[1] *[1]Division of Transplant Surgery, Department of Surgery, Tufts-New England Medical Center, Boston, MA; [2]Neurology, Tufts-New England Medical Center, Boston, MA.*

The incidence of seizures (SZ) after liver transplant (LT) is reported to be 10-40% and are a major cause of morbidity. Treatment of seizures after LT is complicated by the fact that most anti-epileptic drugs (AEDs) induce hepatic cytochrome P450,34A enzymes and interfere with immunosuppressant drug metabolism. The best treatment regimen for SZ after LT has yet to be defined. We retrospectively reviewed 532 LT cases performed between 1993 and 2003 for the incidence of and efficacy of treatment for, SZ after LT. Fifty one (10.0%) patients had SZ post LT of which 32 were treated with an AED and 19 were treated with benzodiazepines and/or a change in their immunosupressant regimen. The annual incidence rates varied between 4.5% and 14.7% with no discernable increasing or decreasing trend over time. Of the 32 who were treated with an AED, 11 remained on phenytoin at 30 days and 6 remained on levetiracetam at 30 days with the remaining15 having their AED discontinued within 30 days. In the phenytoin treated group, only 6 of 11 (55 %) remained seizure–free at 30 days while 6 of 6 (100%) in the levetiracetam group were seizure free at 30 days post-transplant (P<0.001). Patients who were treated with levetiracetam required significantly lower doses of cyclosporin (P=0.02) and FK-506 (one third to one half the comparable dose) to maintain therapeutic immunosupressant levels compared to patients who were treated with phenytoin. The frequency of rejection was similar (9% vs. 0%) in the phenytoin and levetiracetam groups. In our experience, SZ occur in approximately 10% of LT recipients. Treatment with levetiracetam, which does not induce the hepatic metabolism of various immunosuppressant drugs, is more efficacious and is associated with significantly lower doses of immunosuppressant medications and no increase in rejection episodes. Levetiracetam appears to be both efficacious and economically valuable as a treatment regime in LT patients.

Abstract# 1261 Poster Board #-Session: P191-III

OUTCOME OF LIVER TRANSPLANTATION FOLLOWING KIDNEY-ONLY TRANSPLANTATION. John R. Lake,[1] Sarah H. Rush,[2] Robert M. Marion.[3] *[1]Medicine, University of Minnesota, Minneapolis, MN; [2]SRTR/University Renal Research and Education Association, Ann Arbor, MI; [3]SRTR/University of Michigan, Ann Arbor, MI.*

Introduction

Liver disease, particularly from viral hepatitis, is a common co-morbidity in renal transplant recipients and has traditionally has been an important late cause of death.We examined the use and outcome of liver transplantation following kidney transplant in a national cohort.

Methods

Using SRTR/OPTN data, liver and liver/kidney transplant recipients from 1995 to 2001, who were previous recipients of a kidney alone, were identified. Unadjusted Kaplan-Meier patient and graft survivals were determined and compared by etiology of liver disease. Deaths and retransplantations were included as graft failures. In order to study those patients who only underwent liver transplantation for hepatic disease, patients with primary hyperoxaluria were excluded resulting in 143 patients who were included in the analysis.

Results

The annual number of liver and liver/kidney transplants in patients with previous kidney transplant remained relatively constant over the duration of the study, ranging from 10 to 21. Hepatitis C (HCV) was the underlying diagnosis in 35% of the recipients, hepatitis B in 9%, and either congenital hepatic fibrosis or polycystic liver disease in 26 %. Acute liver failure accounted for 11% of the cases.

Unadjusted Patient Survival by Time since Liver Transplant

	1 month	3 months	1 year	deaths(n)
HCV diagnosis	84.3%	63.2%	55.3%	19
Non-HCV diagnosis	85.5%	78.3%	72.3%	22
All	85.1%	72.9%	66.3%	41

p<.01 at 1 year (HCV vs. non-HCV)

Unadjusted Graft Survival by Time since Liver Transplant

	1 month	3 months	1 year	failures(n)
HCV diagnosis	80.0%	60.0%	52.5%	25
Non-HCV diagnosis	84.3%	75.7%	68.3%	21
All	82.7%	70.0%	62.5%	46

p<.01 at 1 year (HCV vs. non-HCV)

The primary cause of death was infection in 41% of patients, 5% died of renal failure. None were reported to have died of recurrent liver disease.

Conclusion

The outcome of liver and liver/kidney transplantation after prior kidney transplantation is relatively poor when compared to primary liver recipients. The outcome is significantly worse in patients transplanted with HCV as compared to non-HCV disease. Future studies should focus on understanding the mechanisms accounting for these poor outcomes.

Abstract# 1262 Poster Board #-Session: P192-III

STEROID-FREE INDUCTION FOR LIVER TRANSPLANRT RECIPIENTS WITH HEPATITIS C - A PROSPECTIVE RANDOMIZED STUDY. Tomoaki Kato,[1] Hideo Yoshida,[1] Olivia Hung,[1] Marzia Montalvano,[1] Guy Neff,[2] Kamron Sadfar,[2] Seigo Nishida,[1] Jang Moon,[1] Juan Madariaga,[1] Genarro Selvaggi,[1] Juan Madariaga,[1] Phillip Ruiz,[3] Andreas Tzakis.[1] *[1]Liver and GI Transplant, University of Miami, Miami, FL; [2]Hepatology, University of Miami, Miami, FL; [3]Immunopathology, University of Miami, Miami, FL.*

Background: Recurrence of Hepatitis C (HepC) has been a most difficult dilemma in liver transplantation (OLT). Effect of immunosuppresion on recurrence of HepC including the use of steroid, mycofenolate mofetil(MMF), and IL-2 antibody has been reported with large discrepancies. Since 1999, we have been performing a prospective randomized study comparing immunusuppression protocols that includes steroid-free regimen in patients with HepC. The first 50 patients were randomized to Daclizumab+tacrolimus (FK) or Steroids +FK without preemptive antiviral strategy (Phase I) and we have had a mixed results (unpublished). We are currently conducting the second phase with a modified protocol including use of MMF and preemptive antiviral therapy. Here are our early results from the second phase of the study.

Patients and Methods: OLT recipients with HepC were randomized to 2 treatment arms: Daclizumab+FK+MMF (study arm) and Steroids+FK+MMF (control arm). Study group received no steroids except for the treatment of biopsy proven rejection. Both arms received pre-emptive anti-viral therapy with Pegasys ® and ribavirin.

Results: Twenty-six patients have been enrolled (10 in study arm and 16 in control). All patients are alive. One patient in the study arm had suspected profound neurological toxicity from tacrolimus and was withdrawn from the study. Incidences of rejection in the first 3 months were 0% in study arm and 40% in control arm. A patient in the study arm developed severe rejection after 6 months requiring OKT 3. This patient was crossed over to the steroid (control) arm. Eight have received protocol 1-year biopsy (6 in control 2 in study arm, Table). Two patients in the control arm (33%) showed advanced fibrosis but none in the study arm. Comparing the common side effects of steroids (study vs control): hypertension (33% vs 63%), PTDM (11% vs 44%), and wound infection (22% vs 31%) (all of them, p=NS).

Conclusions: OLT recipients with HepC tolerated steroid free protocol. There is a trend in lowering steroids related side effects in steroid-fee patients. However, its effect on HepC recurrence has yet to be determined until the completion of the study.

1-year protocol biopsy results

Fibrosis Sage	Study Am	Control Arm
0	1 (50%)	1 (17%)
1	0	2 (33%)
2	1 (50%)	1 (17%)
3	0	2 (33%)
4	0	0

Abstract# 1263 Poster Board #-Session: P193-III

ARTERY STENTING FOR HEPATIC ARTERY STENOSIS IN LIVER TRANSPLANTATION. Takehisa Ueno,[1] Greg Jones,[2] Adrian Martin,[1] Edmund Sanchez,[1] Srinath Chinnakotla,[1] Sherfield Dawson,[1] Henry Randall,[1] Nicolas Onaca,[1] Robert Goldstein,[1] Marlon Levy,[1] Goran Klintmalm.[1] *[1]Baylor Regional Transplant Institute, Baylor University Medical Center, Dallas, TX; [2]Vascular and Interventional Radiology, Baylor University Medical Center, Dallas, TX.*

Purpose

Hepatic artery (HA) stenosis is a common complication of post liver transplantation. Surgical reconstruction has been the first choice for treatment. Emerging interventional radiologic technique makes it possible to repair without surgery. The aim of this presentation is to evaluate current indication of HA stenting and outcome.

Methods

23 patients underwent HA stent for HA stenosis between 1998 and 2003 in our institution. Origins of ESLD were Hepatitis C (n=15), Hepatitis B (n=2), Cryptogenic (n=2), Laennec's cirrhosis (n=1), α-1 anti-trypsin deficiency (n=1), Allagera syndrome (n=1) and polycystic liver (n=1). 14 patients were female and 9 were male. Age ranged from 21 to 61 year old (Mean 49). 8 patients (35%) had history of previous HA revision. 15 patients newly developed HA stenosis. Stenosis locations were anastomosis (n=13), distal (n=7) and proximal side (n=3). Patients were followed by DUS and Angiography. Charts were reviewed retrograde.

Results

20 patients survived after the HA stenting procedure more than 2 months. 3 patients died from liver failure (n=2) and HA aneurysm. 20 patients were followed for mean 26±13 month (3-62 month). One of 20 patients died from renal failure 2 year after HA stenting. Post stenting anticoagulation was both of Aspirin and Plavix (n=9), Aspirin only (n=8), Plavix only (n=4) and none (n=2). The indication for angiography which found stenosis were abnormal DUS (n=11), follow up angiography post surgical HA revision (n=5), liver enzyme elevation (n=2), Routine follow up DUS post OLTx (n=2). Mean hospital days were 2.7 days (n=18). 10 patient's hepatic arteries were normal after stenting. However, 10 patients (43%) developed hepatic arterial complications again. 8 patients developed restenosis, total HA thrombosis (n=1) and left HA thrombosis (n=1). 8 patients of the 10 patients who developed recurrent problem were successfully treated interventionaly: re-stent (n=7) and dilation (n=1). The remaining 2 patients required surgical repair. Mean time to HA complication post stent was 166±113days.

Conclusions

Stenting HA for hepatic stenosis is an excellent treatment for HA stenosis. Stenting is well tolerated requiring short admissions with excellent short-term successful rate. However, half of patients developed restenosis in mean 6 months with one in five patients required surgical repair. Patient long-term outcome of stenting needs further follow up.

Liver, Heart, Lung and Pancreas/Islets: Donation, Preservation, and Outcomes

Abstract# 1264 Poster Board #-Session: P194-III

IN VIVO VIABILITY ASSESSMENT OF ISLETS ISOLATED FROM THE PANCREAS PRESERVED BY THE TWO-LAYER METHOD. Keitaro Kakinoki,[1] Yasuhiro Fujino, Yasuyuki Suzuki, Takuro Yoshikawa, Tomohiro Tanaka, Shiri Li, Naohiro Goto, Yasuki Tanioka, Tetsuya Sakai, Yoshikazu Kuroda. *[1]Gastroenterological Surgery, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan.*

(Background) Although outcomes of islet transplantation have improved due to the new regimen of immunosuppression by the Edmonton protocol, there remains a major obstacle to isolate viable islets from long-term preserved pancreas. It is considered that preservation technique play a very important role in subsequent success in islet isolation. Previously we have reported that the two-layer cold storage method (TLM) improved yield and function of isolated islet from long-term preserved pancreas. Recently, it has been reported that TLM allowed successful transplantation from one donor to one recipient in clinical setting.

(Objective) In this study, we performed in vivo more accurate functional analyses of islets isolated from the pancreas preserved by TLM in rat to nude mouse islet transplantation model.

(Method) We used Lewis rats and Balb-c nude mouse for donors and recipients, respectively. Rat islets were isolated immediately after harvesting (Fresh group), and after 24 hour pancreas preservation by TLM (TL group) or conventional cold storage in UW solution (UW group), 200 isolated islets in each group were transplanted under the renal capsule of Streptozotocin induced diabetic nude mice. To investigate the function of transplanted islets, we monitored recipient blood sugar levels, and performed intra peritoneal glucose tolerance testing (IPGTT) at day 21 posttransplant.

(Results) Graft survival rates were 100%, 80%, and 0% in Fresh, TL, and UW groups, respectively. IPGTT at day 21 posttransplant showed the blood sugar curve of TL group was comparable to that of Fresh group, while in UW group the graft function decreased compared to Fresh and TL group.

(Conclusion) The function of the islets isolated from the pancreas preserved by TLM for 24 hours is comparable to freshly isolated islets, better than that preserved by conventional UW solution. The TLM is an excellent preservation technique to isolate islets with great viability.

Abstract# 1265 Poster Board #-Session: P195-III

DOES DURATION OF DONOR BRAIN INJURY AFFECT OUTCOME AFTER ORTHOTOPIC PEDIATRIC HEART TRANSPLANTATION? Jonah Odim, Hillel Laks, Chris Vincent, Charles Murphy, Ana Banerji, Kaushik Mukherjee, David Gjertson, and the UCLA Heart Transplant Team. *Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Objective: There are scarce data relating donor brain injury to outcomes after heart transplantation. We tested the hypothesis that the duration of donor brain injury had an adverse effect on recipient rejection and mortality in pediatric heart transplantation.

Methods: Ninety-three pediatric patients from 1997 to 2003 underwent orthotopic heart transplantation at our center. The donor and recipient medical records were reviewed. The primary outcomes were the number of rejection episodes and the time to first rejection. Secondary outcome was mortality.

Results: Of the 93 recipients of cardiac allografts, 8 (9%) and 2 (2%) received second and third allografts respectively. Overall mortality for the group was 7% (6/93). Median time duration of donor brain injury to declaration of death, death to organ removal, and graft ischemia time were 38, 24, and 3.3 hours respectively. Cox regression analysis (adjusting for UNOS status, ventilator dependence, ECMO/VAD status, diagnosis of congenital heart disease, gender and CMV mismatches, and type of immunosuppression) demonstrated that recipients of donor hearts with relatively long periods from brain injury to death declaration (96 hours) or from death to organ removal (34 hours) had significantly lower rejection rates [HRs = 0.3 (p=0.01) and 0.5 (p=0.05) for injury and death times, respectively]. In this series, prolonged donor heart ischemia (4.6 hours) did not impact rejection rates.

Conclusions: The longer duration of neurohormonal discharge and stress related to donor brain injury and death, may attenuate factors in the recipient associated with rejection. Further study of these phenomena are warranted.

Abstract# 1266 **Poster Board #-Session: P196-III**

DOES TYPE OR CONSTITUENT OF PRESERVATION SOLUTIONS INFLUENCE EARLY GRAFT FAILURE AFTER CARDIAC TRANSPLANTATION? A MULTI-INSTITUTIONAL STUDY. N. Moazami, R. C. Bourge, R. N. Brown, L. E. Wagoner, J. P. Boehmer, G. Ewald, E. K. Kasper, J. O'Donnell, B. K. Rayburn, B. Czerska, P. Solon. *and The Cardiac Transplant Research Database, University of Alabama at Birmingham, Birmingham, AL.*

Background: In the current era of heart transplantation, there are large institutional variations in choice of solutions for heart preservation during organ procurement. This multi-institutional study was undertaken to examine trends in preservation solutions over the past decade and their potential impact on early graft failure (EGF). **Methods:** Analysis of 7323 heart transplants from 34 institutions between 1990 and 2002 identified the preservation solution in 92% of transplants. By multivariate analysis, the interaction between ischemic time and preservation solutions on the likelihood of EGF was examined. **Results:** There was a temporal trend toward decreasing use of Stanford solution, Plegisol, and Eurocollins solution over time. During the same period, the use of UW and Celsior solutions increased, accounting for 42% and 23% respectively of all donor procurements in 2002. Freedom from EGF for donor ischemic time < 4 hours was similar for all solutions used, regardless of composition. However, for donor ischemic time > 4 hours, freedom from EGF was significantly better for intracellular type solutions (high K^+) compared to extra cellular (high Na^+) or Stanford-type solutions (p=0.01). **Conclusion:** 1. Institutions using standardized solutions have increasingly switched to UW or Celsior for donor procurement. 2. Early graft failure is not affected by the type or constituents as long as the donor ischemic time is less than 4 hours. 3. For ischemic time > 4 hours in clinical heart transplantation, intracellular based solutions appear to be the most reliable.

Abstract# 1267 **Poster Board #-Session: P197-III**

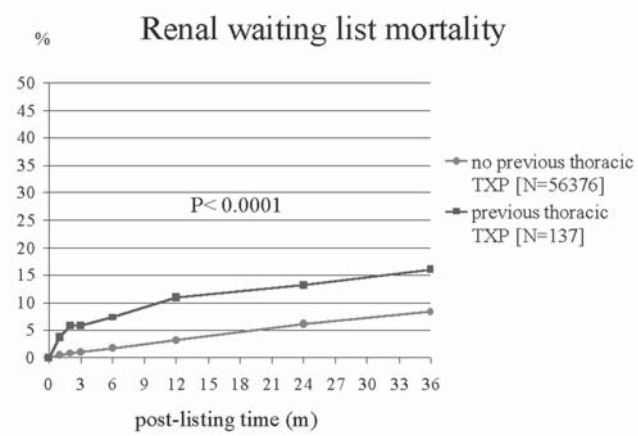
OUTCOMES IN ELDERLY PATIENTS RECEIVING ORTHOTOPIC HEART TRANSPLANTS. G. V. Gonzalez-Stawinski,[1] P. M. McCarthy,[1,3] K. Hoercher,[1,3] J. Feng,[3] E. A. Beyer,[1] E. McGee,[1] P. E. Parrino,[1] D. O. Taylor,[2,3] R. C. Starling,[2,3] J. B. Young. *[1]Thoracic and Cardiovascular Surgery, The Cleveland Clinic Foundation, Cleveland, OH; [2]Cardiovascular Medicine, The Cleveland Clinic Foundation, Cleveland, OH; [3]Transplantation Center, The Cleveland Clinic Foundation, Cleveland, OH.*

Purpose: Traditionally advanced age has been considered a contraindication for heart transplantation (Tx). However, this concept has been challenged, with patient selection relying on physiologicage rather than chronologic age. The following study was conducted to determine the impact of advanced recipient age on postoperative outcomes following Tx. **Methods:** Between January 1 1990 and August 27 2003, 928 patients underwent first time cardiac transplantation. For the purpose of this analysis, patients were stratified according to age (< 65 years vs. 65 years) and early and late outcomes were compared. **Results:** Among 928 transplant recipients, 101 patients were 65 years of age or older while 827 were younger than 65 years. Mean follow-up was 4.63 years (range 6 months to 11 years). Survival rate for older recipients was 95%, 91%, 89%, 84%, 80%, and 74% at 1 -, 6 months, 1-, 3-, 5-, and 7 years. Survival for younger recipients was 99%, 91%, 89%, 83%, 77%, and 51% for the same time periods (early phase: p=0.94, late phase: p=0.53). Early on older patients had a greater number of readmissions when compared to the younger cohort (p<0.0001). However, readmission due to infection was similar between the two study groups (Early phase: p=0.77, Constant phase: p=0.57). **Conclusion:** Orthotopic heart transplantation in older patients is feasible with long term survivals similar to younger cohorts. Given these data selected patients 65 years or older should not be denied orthotopic heart transplantation.

Abstract# 1268 **Poster Board #-Session: P198-III**

SHOULD THORACIC TRANSPLANT PATIENTS BE PRIORITIZED IN THE RENAL ALLOCATION SCHEME? Jacqueline M. Smits,[1] Erwin de Vries,[1] Mike Smith,[1] Guido G. Persijn,[1] Gunther Laufer,[2] Ulrich Frei.[3] *[1]Eurotransplant Int'l Foundation, Leiden, Netherlands; [2]Thoracic Surgery, University Hospital Innsbruck, Innsbruck, Austria; [3]Nephrology, Charite, Berlin, Germany.*

Introduction. The goal of the Eurotransplant renal allocation scheme is to maintain the highest possible level of objectivity, transparency and equity for all patients on the list. In the past rescue allocation algorithms were designed for patients for whom this condition of equity no longer could be sustained. Patients with a low chance of receiving a transplant (highly sensitized patients) or patients with a high waiting list mortality (old end-stage renal disease (ESRD) patients) were served with an adapted scheme. The post-thoracic transplant candidates with an ESRD caused by calcineurin inhibitor toxicity is another group of patients that deserves further attention. *Aim*: Investigate if prioritization on the renal transplant waiting list is needed for thoracic transplant patients. *Patients and Methods:* Between January 1, 1990 and December 31, 2000, 56637 patients were registered for a renal transplant in Eurotransplant. At the end of the study period (December 2003), 37734 patients were transplanted, 1570 were removed from the list, 7854 had died while waiting for their allograft and 9479 were still waiting. Of all these patients listed for a renal transplant, 137 patients had previously been transplanted with a thoracic allograft. Major endpoint was death on the renal waiting list. *Results*: Renal waiting list mortality for patients with a thoracic transplant was significantly higher compared to patients with no previous thoracic transplant: at 1, 2 and 3 years after listing the rates were 11%, 13% and 16% and 3%, 6% and 8% for previous thoracic transplant patients and no previous transplant patients, respectively (p<0.0001). *Conclusion*: If the view is held that all patients listed for a renal transplant should have the same chance of surviving until an organ becomes available, thoracic transplant patients should be prioritized in the renal allocation schemes.



Abstract# 1269 Poster Board #-Session: P199-III

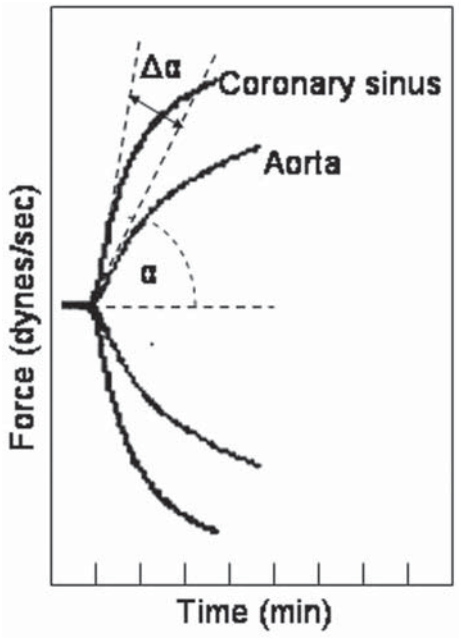
PLATELET FUNCTION AND CORONARY FLOW RESERVE PROVIDE A POINT OF CARE ASSESSMENT OF CORONARY ACTIVATION AFTER BRAIN DEATH. Robert S. Poston,[1] Junyan Gu,[1] Deyanira Prastein,[1] James Gammie,[1] James Brown,[1] Richard N. Pierson III,[1] Bartley P. Griffith.[1] *[1]Division of Cardiac Surgery, University of Maryland School of Medicine, Baltimore, MD.*

INTRODUCTION: Brain death (BD) induces an inflammatory and procoagulant phenotype which can lead to microvasculature dysfunction and activation of platelets perfusing the donor heart. Point of care assays for objectively assessing organ viability were established in a dog model and the clinical relevance confirmed in human hearts.

METHODS: BD was induced in dogs by inflation of an intracranial foley balloon (n=6). After 1 hour, BD hearts were assessed by immunohistochemistry (IHC) for ICAM-1, e-selectin, thrombomodulin and tissue factor (scored 0-3) and compared to normal hearts (control, n=10). Endothelial function was assessed using hyperemic flow response in the LAD after a 10 second of occlusion (transit time flowmeter) and % transgraft change (aorta-coronary sinus) in platelet function, measured using TEG-α (see fig) and whole blood aggregometry (Ω). Ten consecutive human heart donors underwent a similar analysis to assess correlation with primary graft dysfunction after transplantation, defined as the postoperative need for mechanical support.

Results: IHC showed an increase in tissue factor (2.5±0.6 vs. 1.6±0.9) and decrease in thrombomodulin (1.2±1.1 vs. 2.0±0.4) in BD vs. control hearts. Point of care tests showed an increase %transgraft change in platelet activation by TEG-α (8.4±1.9 vs. 4.3±3.5%) and aggregometry (8.1±6.2 vs. 2.4±3.1%) and decreased coronary flow reserve (1.6±0.8 vs. 2.7±0.7, p<0.01) with BD. Three of 10 human hearts developed primary graft dysfunction after transplantation. These hearts showed an increased baseline %transgraft change in TEG-α (10.3±3.0 vs. 6.3±2.8%, p=0.08) and Ω (9.2±3.1 vs. 5.9±1.9%, p=0.06).

Conclusion: Point of care assays can effectively monitor microvascular function and a procoagulant graft phenotype which may lead to a more objective assessment of the viability of hearts from marginal donors.



Abstract# 1270 Poster Board #-Session: P200-III

LIVER TRANSPLANTATION FROM CONTROLLED NON-HEART BEATING DONORS: AN IMPORTANT NEW SOURCE OF GRAFTS. Paolo Muiesan,[1] Raffaele Girlanda,[1] Heather Tollerton,[2] Wayel Jassem,[1] Michael Heneghan,[3] Mohamed Rela,[1] Nigel David Heaton.[1] *[1]Department of Liver Transplantation, King's College Hospital, London, United Kingdom; [2]South Thames Transplant Coordination Service, King's College Hospital, Dulwich, London, United Kingdom; [3]Institute of Liver Studies, King's College Hospital, London, United Kingdom.*

Background. To increase the number of livers available for transplantation a non-heart beating donor (NHBD) liver transplant program was started after obtaining hospital ethical committee approval. Methods. Controlled donors with a warm ischaemia of less than 30 minutes were considered, preferably less than 65 years of age. A 5-minute 'stand off ' period was observed from asystole to skin incision. A super-rapid technique with aortic and superior mesenteric vein perfusion was utilised for the retrieval. Methods used to assess the suitability of these livers for transplantation included serum liver function tests, morphological and histological assessment and hepatocyte viability testing. Results. In the first 2-½ years of the programme 38 livers were retrieved from NHBDs. Of these 21 were considered suitable for transplantation. A further 17 were not used due liver appearance in 12 (steatosis in 9, abnormal consistency in 3), prolonged hypoxia and hypotension post withdrawal of therapy in 4 and poor perfusion in one. Mean donor age was 40.5 years (range 0.75-67), 21 were males. Cause of death was head trauma in 6 donors, intracranial bleed in 16 and anoxic / ischaemic brain injury in 16. Mean warm ischaemia time (systolic BP < 50 mmHg to aortic perfusion) was 14.1 minutes (7-31). Twenty-two patients were transplanted (one liver split for 2 grafts) and the indications included viral hepatitis in 10, familial amyloid polyneuropathy in 2, primary sclerosing cholangitis in 2, acute liver failure in 2, autoimmune hepatitis in 2 and, alcoholic liver disease, cryptogenic cirrhosis, factor VII deficiency and hepatoblastoma in one each. The average age of the recipients was 37.6 years (0.7 – 72 years) and 13 were males. All grafts had good early function except one right lobe split graft. There were three deaths due to ischaemic brain injury, chronic rejection and multi-organ failure following re-transplantation for primary non function respectively. One graft was lost to hepatic artery thrombosis and the patient was retransplanted. Overall patient and graft survival is 86% and 82% respectively at a mean follow up of 12 months.Conclusion. Grafts from controlled NHBDs should be considered for organ donation. The procedure is demanding on staff, time and resources. Early results suggest that this is a significant new source of grafts but careful donor selection and short cold ischaemia are mandatory.

Abstract# 1271 Poster Board #-Session: P201-III

CAN LIVER DONORS AGE SEVENTY AND OLDER BE USED ROUTINELY FOR LIVER TRANSPLANTATION? Glenn Halff,[1] Ken Washburn,[1] Robert Esterl,[1] Greg Abrahamian,[1] Alejandro Mejia,[1] Kermit V. Speeg,[1] Francisco Cigarroa.[1] *[1]Surgery, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Purpose: The purpose of this study was to analyze the results with transplantation of older cadaveric donor livers to determine if there is a role for their routine use to help alleviate the shortage of donor livers.

Methods: The results of 23 liver transplants using cadaveric donor livers between the ages of 70-84 during the period 2001-2003 were retrospectively reviewed. These represented 95% of donor livers this age in our organ procurement organization (OPO) that were used for transplant during this time period. The other older liver was exported to another OPO. The mean recipient age was 59 years (range 42 - 73 years). The median and mean true Mayo Endstage Liver Disease (MELD) score for the older donor recipients at the time of transplant was 20 and 19.2. True mean MELD score for all patients transplanted in our center during this period was 20.3. Ten (43%) of the recipients had hepatitis C. None of the donors had hepatitis C.

Results: Older donor one year graft and patient survival is 80%. Overall programmatic 1 year graft and patient survivals are 86% and 88%. Mean cold ischemic time was 4 hours and 8 minutes. Mean recorded warm ischemic time was 28 minutes. No patients had primary nonfunction. One patient acquired hepatitis C post transplant from an unknown source and this was the only patient where liver dysfunction contributed to the patient's death, which was from sepsis ten months post transplant. Two patients who died had pre transplant MELD scores of 34 and 23. Another died of metastatic tumor and the fourth was comatose pre transplant and did not recover mental status post transplant. The incidence of recurrent hepatitis C disease is 40% with all patients with elevated liver function tests having been biopsied and all being stage 0 except one that is stage 2. Protocol biopsies were not done on all patients.

Conclusion: Cadaveric livers over the age of 70 can be used with a good success rate when the warm and cold ischemic times are short and the recipients are not extremely ill prior to the transplant.

Abstract# 1272 Poster Board #-Session: P202-III

IS COLD ISCHEMIA TIME BECOMING LESS IMPORTANT IN LIVER TRANSPLANTATION? Erick B. Edwards,[1] Richard B. Freeman.[2] *[1]Research, United Network for Organ Sharing, Richmond, VA; [2]Transplant Surgery, Tufts University School of Medicine-New England Medical Center, Boston, MA.*

Introduction. Although organ preservation techniques have improved over the years, prolonged cold ischemia time (CIT) has been shown to be detrimental to deceased donor liver transplant outcomes. Anecdotal evidence suggests that this effect has become less significant over time. In order to test this assertion, we analyzed both the short-term and the long-term impact of CIT on graft survival in 4 separate eras.

Methods. Using Cox regression models adjusted for several donor and recipient factors, we estimated the impact of CIT on both short-term (0-90 days post-transplant) and long-term graft failure. For the long-term analysis, only grafts functioning for a minimum of 90 days were included, and the relative risk of failure within 3 years post-transplant was estimated. Multiorgan, split/reduced and heterotopic transplants were excluded. The distribution of CIT among the transplants still functioning at 90 days was similar to that of all transplants. The 4 transplant eras were chosen as follows: 1988-1991 (N=9231); 1992-1995 (N=13155); 1996-1999 (N=15670); 2000-2001 (N=8449).

Results. With the exception of the earliest era, after adjusting for donor and recipient factors, CIT had a statistically significant effect on outcome in the early post-transplant period (see table). Further, this effect appears to be increasing over time. CIT did not have an impact on long-term outcomes in any of the four eras.

Conclusions. Extended CIT continues to have a negative impact on liver transplant outcomes, even more so in recent years. Recipients of extended CIT livers should be carefully chosen in order to minimize short-term graft losses.

The Effect of CIT on Short-Term Graft Failure

Era	Relative Risk*	Lower 95% CI	Upper 95% CI
1988-1992	1.05	0.99	1.12
1992-1995	1.12	1.05	1.20
1996-1999	1.22	1.13	1.31
2000-2001	1.22	1.02	1.44

*16 hours CIT vs. 8 hours CIT

Abstract# 1273 Poster Board #-Session: P203-III

A COMPARISON OF MEDICAL OUTCOMES AND QUALITY OF LIFE (QOL) SURVEYS IN RIGHT LOBE (RL) VS. LEFT LATERAL SEGMENT (LLS) LIVER DONORS. Abhinav Humar,[1] Elizabeth Larson,[1] Brooke Glessing,[1] John R. Lake,[1] William D. Payne.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Background: Concern remains regarding the possibly higher donor risks associated with RL vs LLS donation. We studied medical outcomes and responses to QOL surveys in donors of a RL vs. LLS to determine if there were differences.

Results: Between 96-03, 42 LDLTs were performed: 26 RL and 16 LLS. Mean donor age, male/female ratio, and donor size were similar between the 2 groups. A significantly higher liver mass was resected in RL vs. LLS donors (720 vs. 310 gm, p=0.01) with greater blood loss (398 vs. 240 mls, p= 0.04) and operative time (7.2 vs. 5.7 hours, p=0.05). However this did not translate into significant differences in recovery. No donor required extraneous blood transfusion. RL donors had a higher serum ALT and bilirubin early posttx (ie, 1 day, and 1 week); but by 1 month there was no significant difference. Mean hospital stay was slightly higher in RL donors (7.8 days vs. 6.9 days, p=ns). Complications were not different between the 2 groups: 1 central line infection in each group, colitis in 1 RL donor, and 1 bile leak in a LLS donor (which resolved spontaneously). All donors were sent a QOL survey at 6 months post donation. A total of 24 surveys were returned: 16 RL and 8 LLS. RL donors noted persistent pain for a mean of 25.6 days postdonation, vs. 17.6 days for LLS donors. Time until donors noted no major restriction to daily activity was 65.6 in RL donors vs. 54.7 days in LLS donors. Mean time off work was 59.4 days for RL donors and 32.4 days for LLS donors. RL donors reported 92.4 days before they reached their predonation level of exercise; LLS donors 100 days. Half of LLS donors reported their recovery was shorter than expected; the remainder was as expected. With RL donors, 38% noted their recovery was shorter than expected, 38% as expected, and 24% longer than expected. 93.7% of RL donors and 87.5% of LLS donors reported their current health to be the same or better compared to predonation. 93.7% of RL donors described their health currently as being excellent or very good, vs. 75.0% of LLS donors. None of the donors reported problems in obtaining health or life insurance after surgery. All donors reported that they would still donate, and would suggest it to others.

Conclusions: While RL vs LLS donation is associated with greater operative stress for the donor, this need not translate into a more complicated recovery. Overall donor satisfaction and current QOL were not significantly different in the 2 groups.

Abstract# 1274 Poster Board #-Session: P204-III

TRANSIENT IMPAIRED FUNCTION OF SYNTHESIS OF ELDERLY GRAFTS IN THE POSTOPERATIVE PERIOD AFTER LIVER TRANSPLANTATION. Alfonso S. Serralta,[1] Francisco C. Orbis,[1] Fernando R. Sanjuan,[1] Eugenia I. Pareja,[1] Angel H. Moya,[1] Rafael Lopez-Andujar,[1] Juan C. Vila,[1] Manuel B. Juan,[1] Jose P. Mir.[1] *[1]Division of Liver Surgery and Transplantation, University Hospital La FE, Valencia, Spain.*

Introduction: Synthesis is one of the most relevant functions of the liver, and could be altered when the liver is suffering the stress of transplantation.

Objective: To evaluate the function of synthesis of the liver from aged donors in the postoperative period.

Material and methods: Case-control study with 65 liver transplants with a graft from donors older than 60 yrs of age, and 65 cases from younger donors. All were elective, primary, whole organ. 76 variables were analysed to evaluate comparability of groups. There were no differences between groups except for cause of death and levels of hepatic enzymes of the donor (lowers in the aged donor group). The reconstruction of main bile duct continuity was performed with end-to-end anastomoses with a T-tube inside in all these cases. Daily postoperative bile production was measured through collection in the T-tube drainage bag. Daily blood samples were collected during fist 15 days after transplantation. Initial graft function was quantitatively measured with the Hospital Clinic of Barcelona Score. Data were compared with the student's t test and chi-square with Fisher's correction when appropriate. A value of $p< 0.05$ was considered significant.

Results: There were no differences between groups for blood transfusion, Haemonetic use, plasma transfusion (5.1±0.3 vs 4.9±0.4, p=0.958) and platelet transfusion during surgery.

There were no differences between groups in the level of AST and ALT, bilirrubin, GGT, AF, or albumin. There were no differences between groups in primary non-function, graft dysfunction or graft loss during fist month after transplantation. There were no differences between groups for the bile production at the moment of implantation.

Protrombine time and bile production were lower in the group of aged donors during first week after transplantation. Differences disappeared at 15-day. Although the total number of cases with intrabdominal bleeding was not different between groups (6.2% vs 12.7%, p=0.238), there were more postoperative laparotomies because of bleeding in the group of aged donors (1 vs 8, p<0.018).

Conclusion: Although global evaluation of graft function of the grafts from aged donors showed no differences when compared with younger donors, more intensive care in the replacement of liver function synthesis products could be necessary after liver transplantation with grafts from donors over 60 years of age.

Abstract# 1275 Poster Board #-Session: P205-III

ISCHEMIC PRECONDITIONING IN CADAVER DONOR LIVER TRANSPLANTATION: A PROSPECTIVE RANDOMIZED CLINICAL TRIAL OF SAFETY AND EFFICACY. B. Koneru, A. Fisher, J. Skurnick, K. Klein, Y. He, D. Wilson, A. De la Torre, R. Arora, A. Merchant, A. Samanta. *Division of Transplant Surgery, Pathology and Biostatistics and Epidemiology, University of Medicine and Dentistry, Newark, NJ.*

Introduction : Ischemic preconditioning (IPC) protects the liver against cold and warm ischemia and improves graft function following transplantation in animals. No clinical trials of IPC have been reported. Therefore, a randomized prospective trial of safety and efficacy of five min of hepatic IPC was carried out in human cadaver donors.

Methods: Donors of 62 adult recipients at our center were randomized 1:1 either to standard (STD) recovery or to IPC. Hepatic hilum was clamped for five min soon after laparotomy and before intraperitoneal dissection. Primary end points of graft injury and function were peak aspartate (pAST) and alanine transferases (pALT) (U/L) and international normalized ratio of prothrombin (pINR) after transplant.

Results: Sixty two donors were enrolled; 28 to STD recovery and 34 to IPC. Age, gender, body mass index (BMI), and % donors with macrosteatosis were not significantly different between the two groups. All continuous data represent means +/- SD.

	Age(yrs)	Gender M/F	BMI	% with macrosteatosis
STD	46+/- 16	17/11	25+/-5	37
IPC	47+/-18	17/17	27+/-7	28

Heart rate, mean blood pressure and pressor use in operating room did not change significantly in IPC group before and after IPC vs. STD group. Recipient age, disease severity score and cold ischemia were similar in both groups. The mean warm ischemia was significantly longer in the IPC group.

	Age(yrs)	MELD	CI (min)	WI (min)	pAST	pALT	pINR
STD	53+/-8	20+/-7	415+/-87	37+/-6	1651+/-1313	1214+/-1257	2.2+/-0.5
IPC	49+/-10	19+/-8	384+/-92	41+/-6	2159+/-2504	1510+/-2090	2.3+/-0.8

All grafts functioned immediately and one patient in STD group was retransplanted within 6 mths. Histological parameters were similar between IPC and STD groups. Median length of stay in IPC recipients was 8 vs.10 days in STD. This difference was not significant. Six mth graft and patient survival was 91 in IPC vs. 79 and 82% in STD, respectively, not significantly different. Although we intended to enroll 126 patients, the study was terminated due to the apparent lack of benefit from IPC.

Conclusions: 1. Five minutes of IPC in cadaver liver donors is safe and does not cause splanchnic congestion, when performed immediately after opening the abdomen. 2. Five min of IPC did not decrease allograft injury or improve function. 3. Alternative protocols of IPC must be considered, such as longer intervals of hilar clamping.

Abstract# 1276 Poster Board #-Session: P206-III

COMPOSITE TISSUE ALLOTRANSPLANTATION: DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL. Linda C. Cendales,[1] Xiaojie Zhang,[1] He Xu,[1] John Bacher,[2] Allan D. Kirk.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]Division of Veterinary Resources, NIH, Bethesda, MD.*

Composite tissue allotransplantation (CTA) is an emerging field requiring an amalgamation of microsurgical technique and immunosuppressive management. Although the technical aspects of CTA have followed logically from replantation, the discipline remains immunologically undefined. Furthermore, of the immunosuppressants available for use in CTA, many have human specificity and cannot be evaluated in small animals. Based on an evaluation of specimens from transplanted human limbs, we have recently introduced a classification system to standardize CTA rejection. We have shown that vasculitis, dermitis, myositis and perineural involvement can be seen during rejection, but given the sporadic availability of human tissue, the progression of the immune response remains undefined. To permit systematic study of the progression of CTA rejection, we have established a non-human primate (NHP) model. NHPs provide anatomical, antigenic and immunological similarity to humans, and can be used to test most human-specific agents. Limb transplantation in NHPs could markedly impair their social development. We have therefore designed our model to incorporate all elements of a limb CTA yet leave no functional deficit even in the case of rejection or excision. Our model involves a transplanted radial forearm flap including bone, muscle, nerve, tendon, artery, vein and skin. To validate this model, 5 animals have undergone autotransplantation and 8 have received allografts with and without immunosuppression (subtherapeutic tacrolimus, mycophenolate and prednisone to allow for rejection to be temporally characterized). Donor-recipient pairs for CTA were selected based on MHC non-identity, blood type and size. All procedures were well tolerated by all animals with no adverse functional consequences. The model allows for protocol biopsies to be performed without threatening the viability of the flap. Autografts showed no evidence of rejection and served as the baseline for CTA comparison. Non-immunosuppressed allografts showed an infiltrate initially arising within 3 days in the perivenular tissue leading to graft congestion and failure without a prominent dermal infiltrate. Subtherapeutically immunosuppressed animals rejected in 2 weeks with a marked dermal lymphocytic infiltrate similar in magnitude and distribution to human cases seen to date. Thus, we have established a NHP model for CTA that is responsive to immunosuppression and allows for the systematic evaluation of CTA histology and therapeutic maneuvers to improve allograft survival.

Abstract# 1277 Poster Board #-Session: P207-III

γδ T CELLS INDUCE EPITHELIAL REGENERATION AFTER INJURY OF THE LUNG. Ruedi K. Braun,[1] Robert F. Zink,[1] Zhuzai Xiang,[1] William J. Burlingham,[1] Robert B. Love.[1] *[1]Surgery, University of Wisconsin Madison, Madison, WI.*

Chronic rejection of the graft after lung transplantation is characterized by massive fibrosis of the small airways. Stable lung allograft outcome has been correlated with the persistence of donor-derived leukocytes including T cells and alveolar macrophages in the bronchoalveolar lavage (BAL). Since γδ T cells have also been shown to be important for the homeostasis of the epithelium we investigated their possible protective role in maintaining lung epithelium using the model of bleomycin-induced fibrosis.

Epithelial injury was induced by intratracheal instillation of 0.1 U bleomycin in C57Bl/6 wild type and γδ T cell knockout mice. Cellular analysis of the BAL leukocytes was done using flow cytometry (FACS Calibur). To monitor epithelial proliferation miniosmotic pumps containing BrdU were implanted subcutaneaously. Immunohistochemistry and morphometric analysis on 30 µm section was used to asses the number of proliferating cells per volume of epithelium.

Analysis of the cellular infiltration into the airways revealed a significant reduction in total number of macrophages and T cells that migrated into the airways in the γδ-TcR knockout mice suggesting retention of these cells within the lung parenchym. Histological analysis of lung sections revealed that bleomycin-treated C57Bl/6 wild-type mice had multifocal and random areas of consolidation of the pulmonary parenchyma. In contrast C57Bl/6 γδ TcR knockout mice showed a diffuse and homogeneous consolidation of the pulmonary parenchyma throughout all lung lobes. The "volume density of lesion" revealed a significant greater volume of lesion in the γδ TcR knockout mice than in the C57Bl/6 wild-type strain at day 14. Evaluation of the epithelial repair using BrdU incorporation showed that the number of proliferating epithelial cells in the airways was significantly higher in the first week, but significantly lower in the second week in the C57Bl/6 wild-type mice compared to the C57Bl/6 γδ TcR knockout mice.

Conclusion: These results suggest that γδ T cells induce early epithelial repair following epithelial injury. Retention of donor-derived γδ T cells may therefore protect the grafted lung from ischemia-reperfusion injury and alloimmune insult, thus partly accounting for the better graft outcome in transplant recipients with persisting donor passenger leukocytes.

Abstract# 1278 Poster Board #-Session: P208-III

HEMATOPOIETIC STEM CELL TRANSPLANTATION RESTORES IMMUNE SYSTEM FUNCTION IN Atm DEFICIENT MICE. Jessamyn Bagley,[1] John Iacomini.[1] *[1]Transplantation Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA.*

PURPOSE: Ataxia-telangiectasia (A-T) is an autosomal recessive disease caused by mutations in the gene encoding ataxia-telangiectasia mutated (*ATM*), characterized by progressive cerebellar degeneration, variable immunodeficiency, and extreme radiation sensitivity. Recurrent sino-pulmonary infections due to immunodeficiency are a major cause of mortality in A-T patients. In mice, mutation of *Atm* leads to a phenotype that recapitulates many of the symptoms of A-T. In this study we investigated whether the T cell deficiencies observed in this model were due to defects in the thymic microenvironment in which T cells develop, or defects intrinsic to T cell progenitors.

METHODS: Effects of thymic environment were examined by reconstituting irradiated wild-type C3H ($H\text{-}2^k$) mice with $Atm^{-/-}$ ($H\text{-}2^b$) or wild-type ($Atm^{+/+}$) bone marrow cells. Effects of progenitor intrinsic defects on T cell development were examined using a clinically applicable conditioning regimen to engraft wild-type bone marrow in $Atm^{-/-}$ recipients. To measure functional immunity, we compared the ability of C3H/$Atm^{-/-}$ chimeras and $Atm^{+/+}$ controls to reject skin allografts.

RESULTS: Both $Atm^{-/-}$ and $Atm^{+/+}$ bone marrow cells engrafted in irradiated C3H recipients, resulting in greater than 99% donor type multi-lineage chimerism. Analysis of T cell development in the thymus of bone marrow transplant recipients revealed defects in the ability of T cell progenitors derived from $Atm^{-/-}$ HSCs to develop. Treatment of $Atm^{-/-}$ recipients with anti-T cell antibodies, and a single dose of cyclophosphamide was sufficient to result in greater than 99% donor type multi-hematopoietic lineage chimerism. Unmanipulated $Atm^{-/-}$ mice ($H\text{-}2^b$) exhibited delayed rejection of skin allografts from allogeneic B6.CH-2^{bm1} mice (Median survival time (MST) = 17 days, n =7) when compared to normal littermate $Atm^{+/+}$ controls (MST= 11 days, n=5, P=0.002). In contrast, $Atm^{-/-}$ mice reconstituted with wild-type C3H bone marrow were able to reject skin allografts with the same kinetics observed for $Atm^{+/+}$ mice receiving conditioning alone (MST=13 vs. 12 days respectively, P=0.52). These data suggest that replacement of $Atm^{-/-}$ hematopoietic compartment by bone marrow transplantation can overcome the immunodeficiency observed in $Atm^{-/-}$ mice.

CONCLUSIONS: We show that the replacement of the hematopoietic stem cell compartment in $Atm^{-/-}$ mice using a non-myeloablative host conditioning regimen can overcome the immune deficiencies observed in these mice. Therefore, hematopoietic stem cell transplantation may prove to be of therapeutic benefit in A-T patients.

Abstract# 1279 Poster Board #-Session: P209-III

THE THYMUS IS INDISPENSIBLE FOR A BONE MARROW DERIVED $CD8^+$ TCR^- LYMPHOCYTE SUBPOPULATION THAT FACILITATES ALLOGENIC STEM CELL ENGRAFTMENT IN MICE. Nupur N. Gangopadhyay, Matthew J. Schuchert, James D. Luketich, Rodney Landreneau. *Department of Surgery, University of Pittsburgh, Pittsburgh, PA.*

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality from allogenic stem cell transplantation. We have previously shown that a $CD8^+/TCR^-$ lymphoid bone marrow population (Facilitating Cell, FC) can promote engraftment of allogenic hematopoietic stem cells (SC) in mice without inducing GVHD. Phenotypically, FCs express CD8 as well as CD3 in association with TCRβ and a unique 33 kD protein (FCp33). FC has low-level expression of co-stimulatory m-RNA (CD28 and CD40L) but high abundance of Il2 and IL10. Also, FCs express various markers (CD44, c-kit, Thy1.2 and CD25) common to developing T-cells. Considering the phenotypic similarity of FC to prothymocytes, experiments were designed to elucidate 1) the role of thymus in FC mediated allogenic bone marrow transplantation and 2) possible production of other mediators (TGFβ, IFNγ and NO synthetase) known to assist in allogenic transplantation through immuno-suppression. Thirty thousand male FCs ($CD8^+/TCR^{dim/-}$) and ten thousand female SCs ($SCA1^+/c\text{-}Kit^+/Lin^-$) were inoculated into lethally irradiated (950 cGy) female allogenic and syngenic mice. Thymocytes, splenocytes and bone marrow cells were collected on day 5 post transplantation. Cells were further subjected to genomic DNA PCR for the male Y chromosome (SRY) to allow tracking of the donor FC. Due to low number of FC in the recipients, exact quantification of FC migration to the various organs failed when a real time PCR was used. Using a ^{32}P labeled primer however, allowed us to obtain SRY (FC) and GUS specific PCR product in allogenic and syngenic recipients. In allogenic recipients, the highest amount of FC specific (SRY) PCR product was detected in the spleen followed by thymus and bone marrow. In syngenic recipients, thymus had more FC specific (SRY) PCR product than spleen followed by bone marrow. The importance of the thymus in this setting is further underscored by the fact that FC function is abrogated in thymectomyzed allogenic recipients. In separate RT-PCR experiments, expression of TGFβ, IFNγ and NO synthetase was found to be higher in FC than in T-cells. These data demonstrate for the first time that the thymus plays an important role in FC function. These findings also support the notion that FCs may be specially equipped to facilitate allogeneic SC transplantation without inducing GVHD through various immuno-regulatory mediators. (Supported by the ASTS -ROCHE Surgical Scientist Scholarship, CLRA and Milheim Foundation grants)

Abstract# 1280 Poster Board #-Session: P210-III

DONOR SPECIFIC SPLEEN CELL INJECTION LEADS TO ANTIBODY UNRESPONSIVENESS TO A SINGLE TRANSGENIC MHC CLASS I (HLA-A2) DISCORDANCE. Tetsu Sado,[1] Raju Radha,[1] Stanley C. Jordan,[1] Mieko Toyoda.[1] [1]*Transplant Immunology, Cedars-Sinai Medical Center, Los Angeles, CA.*

Background: Multiple transfusions, previous transplants or pregnancies cause sensitization to MHC antigens which limits access to transplantion. However, in some cases, donor specific transfusion (DST) induces tolerance and prolongs graft survival. DSTs between HLA class I and/or II haplo-mismatch combinations induce long-term allograft acceptance in humans. Here we examined the effect of DS spleen cell injection with single MHC class I (HLA-A2) discordance between donor and recipient on humoral responses in an HLA-A2 transgenic mouse model. **Methods:** Spleen cells ($1x10^5$-$1x10^7$ cells) of HLA-A2 transgenic C57BL/6 mice were injected IV in C57BL/6 mice (n=10) at week 1, 2 and 3 to induce sensitization to HLA-A2. Blood was collected every week for 2 months after the injection. These mice were challenged with $1x10^5$ HLA-A2 transgenic spleen cells at week 20 after the initial sensitization followed by collection of blood every week for 1 month post-challenge. Anti-HLA-A2 IgG and IgM antibody levels were measured by ELISA to determine if mice were sensitized or developed unresponsiveness to HLA-A2. A human anti-HLA class I and class II ELISA (One Lambda) was modified for detection of mouse anti-HLA-A2 IgM and IgG. **Results:** Anti-HLA class I IgM and IgG, but not anti-HLA class II, were detected in all mice which received the first 3 injections, while non-treated mice were negative for both antibodies. The level of anti-class I IgM peaked at week 2 and decreased promptly (74±9.1, 77±9.6, 311±196 and 127±44 arbitrary units at week 0,1,2,3 post-sensitization, respectively). The anti-class I IgG level began to increase at week 2 and reached peak levels at 3 to 4 weeks, then decreased gradually (78±24, 216±161, 516±253, 512±232 and 417±135 at week 1,2,3,4,7 respectively). These results suggest that mice were sensitized to HLA-A2. However, no anti-class I IgM or IgG were detected in these mice after the challenge at week 20 (IgM: 81±18, 88±16, 81±12, 86±11; IgG: 374±126, 308±129, 305±116, 359±167 at week 19,20,21,22 respectively), suggesting induction of non-responsiveness against HLA-A2. Confirmation of tolerance induction in these mice is underway using skin transplantation. **Conclusions:** 1) Donor specific spleen cell injection induces antibody unresponsiveness in an HLA-A2 transgenic mouse model with a single MHC class I (HLA-A2) discordance between donor and recipient. 2) The mechanisms responsible are unclear, but initial sensitization appears to be important in development of antibody unresponsiveness.

Abstract# 1281 Poster Board #-Session: P211-III

CD28/CD154 SIGNALS RENDER CD4+ MEDIATED REJECTION RESISTANT TO GROWTH FACTOR BLOCKADE. Minh Diem Vu,[1] Gulcin Demirci,[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 and growth factor signals are of central importance in T cell activation and T cell effector function. However, it is unclear how these signals interact with each other in regulating CD4+ T cell activation and CD4+ dependent allograft rejection. A better understanding of the potential crosstalk between costimulatory pathways and growth factor signaling during CD4-mediated rejection is important in transplantation research. The aim of this study is to evaluate the role of CD28/CD154 costimulation and growth factor signals in the CD4-mediated allograft rejection.

Materials and methods: Fully mismatched DBA/2 skin allograft was transplanted onto the wt C57BL/6 mice and B6.CD8$^{-/-}$ knockout (CD8KO) mice. Graft survival was determined in the absence or presence of CTLA-4Ig/MR1 treatment (0.5mg/each, i.p., given on days 0, 1, 3) to block CD28/CD154 costimulation and/or rapamycin (RAPA) treatment (3mg/kg, i.p., given on days 0, 1, 2, then every other day for 14 days) to block growth factor signaling. Also, the DBA/2 skin allograft was transplanted onto CD28$^{-/-}$/CD154$^{-/-}$ double knock out (CD28/CD154DKO) mice and treated with RAPA, and graft survival was determined and compared with the wt controls.

Results: C57BL/6 mice vigorously rejected the DBA/2 skin allograft regardless of RAPA treatment. Similarly, CD28/CD154DKO mice promptly rejected the DBA/2 skin allograft. Depletion of CD8+ T cells in the CD28/CD154DKO mice failed to prevent rejection. However, treatment of CD28/CD154DKO mice with RAPA induced long-term skin allograft survival. Furthermore, CD8KO mice can promptly reject the DBA/2 skin allografts, and neither CD28/CD154 costimulatory blockade nor RAPA alone can prevent the allograft rejection. In contrast, treatment of CD8KO mice with RAPA along with CD28/CD154 blockade induced indefinite skin allograft survival.

Donor	Recipient	Treatment	Survival (days)	MST (days)
DBA/2	Wt C57BL/6	RAPA	11, 12, 13, 14, 17, 18, 22	16
DBA/2	CD28/CD154DKO	No treatment	10, 10, 10, 11, 11, 13	11
DBA/2	CD28/CD154DKO	RAPA	46, 71, >120 (x 3)	>120
DBA/2	CD8KO	RAPA	17, 18, 19, 19, 21, 22	19
DBA/2	CD8KO	CTLA-4Ig/MR1/RAPA	>100 (x 5)	>100

Conclusion: Our data suggest that CD28/CD154 costimulatory signals and RAPA-sensitive growth factor signals have overlapping roles in determining the effector function of CD4+ T cells, CD28/CD154 signals confer growth factor blockade resistance rejection and blocking CD28/CD154 signals or genetic mutation of CD28/CD154 molecules renders CD4-mediated rejection sensitive to RAPA.

Abstract# 1282 Poster Board #-Session: P212-III

EXOGENOUS IL-12 ENHANCES DONOR REACTIVE IMMUNITY AND PRECIPITATES REJECTION OF MINOR ANTIGEN DISPARATE MALE HEART GRAFTS IN MICE. Chunshui He,[1] Anna Valujskikh,[1] Peter S. Heeger.[1] [1]*Immunology and The Glickman Urologic Institute, The Cleveland Clinic Foundation, Cleveland, OH.*

Interactions between the innate and the adaptive immune systems are poorly understood. Notably, murine viral infections activate recipient DCs to produce IFNa and IL-12, and induce a low frequency of T cell precursors ($1/10^5$-$1/10^6$) to differentiate and markedly expand (up to 10,000 fold) into a potent high frequency effector repertoire capable of curing the infection. The frequency of minor antigen reactive, anti-male T cell precursors in female C57BL6 (B6, H-2^b) mice is similar to the frequency of anti-viral T cell precursors in these animals. Still, transplantation of male heart grafts into syngeneic female recipients induces only a modest expansion (20-100 fold) of effector T cells that do not mediate rejection. To better understand the influence of innate cytokines on the effector T cell repertoire in this model, we transplanted male B6 hearts into female B6 recipients and administered recombinant IL-12, IFNa or control PBS. 7/10 mice treated with 1 mcg IL-12 on day 3 posttransplant acutely rejected the male heart grafts (MST 15 d). None of the control male hearts were rejected (MST >60 d, n=8). Syngeneic female hearts transplanted into female recipients and treated with IL-12 were not rejected (MST >60d) suggesting that the effect of the IL-12 was to activate the anti-male T cell repertoire. All heart grafts in IL-12-treated recipients exhibited histology consistent with acute rejection. We next performed recall IFNg ELISPOT assays using recipient spleen cells isolated on d 7 and 14 posttransplant to directly assess the effects of IL-12 on the donor-reactive immune repertoire. IL-12 specifically induced a10 fold increase in the numbers of anti-donor CD4 and CD8 T cells following graft placement (1000-2000 anti-male IFNg producers per million) compared to controls (100-300 per million n=3-6 per group, $p < 0.05$). In contrast to the marked effects of IL-12 on graft outcome, exogenous IFNa did not induce clinical or histologic evidence of male heart graft rejection (MST>30d, n=3) and there was no effect on the recipients' anti-male immune repertoire. The findings demonstrate that minor antigen disparate male heart grafts can be rejected by syngeneic females if sufficient innate immune signals, particularly IL-12, are present to fully expand/activate the anti-graft T cell repertoire. Further definition of the relevant links between innate and adaptive immunity in cardiac transplantation could provide novel targets for controlling the anti-graft immune responses and ultimately prolonging graft survival.

Abstract# 1283 Poster Board #-Session: P213-III

RECIPROCAL ROLE OF CYCLINS AND CYCLIN INHIBITOR p21 IN ALLO-IMMUNE ACTIVATION, INFLAMMATION AND GRAFT SURVIVAL. Ashwani K. Khanna.[1] [1]*Medicine (Nephrology), Medical College of Wisconsin, Milwaukee, WI.*

Allo-immune activation is one of the most significant post transplant events, which results in increased expression of pro-inflammatory cytokines. Our studies have demonstrated that the immunosuppressive agents cyclosporine (CsA), tacrolimus (TAC) and sirolimus (SRL) induce expression of cyclin inhibitor p21, implicating it to be a unifying mediator of their anti-proliferative effects. Cyclin inhibitor p21 plays a unique role in cell cycle regulation. Therefore, we hypothesized that cyclins and cyclin inhibitor p21WAF1/CIP1 are key components of allo-immune activation and inflammation. In vitro and in vivo alloantigen and mitogen stimulation of lymphocytes results in increased expression of cyclins (D3, G and E), IL-2 and pro-inflammatory cytokines, which was inhibited by cyclosporine. Lymphocytes from p21WAF1/CIP1 -/- mice exhibited augmented response to allogeneic and mitogenic stimuli. The over-expression of p21WAF1/CIP1 inhibited circulating and intra-graft expression of proinflammatory cytokines and prolonged graft survival in a rat heart transplant model. Therefore, p21 can potentially inhibit lymphocyte proliferation and alloimmune activation similar to immunosuppressive drugs. In this study, we also provide evidence that recombinant p21 protein inhibits lymphocyte proliferation by entering into cells and localizes in side nucleus, interact with transcription factors nuclear factor-kB (NF-kB), and nuclear factor of activated T cells (NFATc and NFATp) and also by inhibition of pro-inflammatory cytokines. The results were compared with immunosuppressive drugs; CsA, TAC and SRL currently used to treat organ transplant recipients. Our studies provide evidence that recombinant p21 protein is a novel and viable alternate strategy to achieve immunosuppression.

Abstract# 1284 Poster Board #-Session: P214-III

IMPACT OF *IN VIVO* BLOCKADE OF SLP-76/GADS INTERACTION. Jonathan S. Maltzman,[1,2] Martha S. Jordan,[1] Amrom Obstfeld,[1] Andrew L. Singer,[1] Gary A. Koretzky.[1,3] *[1]Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA; [2]Medicine, University of Pennsylvania, Philadelphia, PA; [3]Pathology, University of Pennsylvania, Philadelphia, PA.*

Background: The adaptor protein SLP-76 is critical in linking proximal T cell receptor signaling events to downstream second messengers. Recruitment of SLP-76 to glycolipid-enriched microdomains (GEMs) through its constitutive association with the adaptor protein Gads is required for generation of a productive signal. We have developed a polypeptide fragment of SLP-76 that includes the proline-rich Gads binding site; expression of this fragment in the Jurkat T cell line acts in a dominant negative fashion through blocking the association of SLP-76 and Gads. The goal of these studies is to evaluate the effects of expressing this dominant negative peptide on T cell development and T cell function in an in vivo setting. **Methods**: A retroviral vector containing a chimeric cDNA including a 150bp region of SLP-76 fused with the fluorochrome DS-RED2 was generated (hereafter termed the Gads Binding Fragment or GBF). Bone marrow infected with the GBF retrovirus was then used in the generation of radiation bone marrow chimeras. Single cell suspensions from thymus, spleen and lymph node were evaluated for CD4 and CD8 populations. Functional studies to evaluate calcium flux and CD69 upregulation following T cell receptor (TCR) crosslinking were preformed using FACS. **Results**: In radiation chimeras expressing the GBF, total thymocyte cell number was reduced. There was impaired development of single positive thymocytes with a decrease in the CD4+CD8- to CD4-CD8+ ratio. In response to TCR stimulation, there was decreased calcium flux in both the double positive and single positive subpopulations. Analysis of peripheral lymphoid organs demonstrated a severe decrease in the number and proportion of both CD4+ and CD8+ T lymphocytes. **Conclusions**: Blockade of the interaction between SLP-76 and Gads adaptor proteins can be achieved in vivo through expression of the GBF and results in impaired thymic development and T lymphocyte function. These findings suggest that conditional expression of the GBF in mature T lymphocytes may allow for modulation of in vivo immune responses.

Abstract# 1285 Poster Board #-Session: P215-III

DIFFERENTIAL EXPRESSION AND FUNCTION OF PD-L1 AND PD-L2 ON HEPATIC AND SPLENIC DENDRITIC CELLS. Audrey H. Lau,[1] An de Creus,[1] Alison J. Logar,[1] Angus W. Thomson.[1] *[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

BACKGROUND: Manipulation of classic costimulatory pathways such as the B7-family (CD80/86)-CD28 or CD40-CD154 pathways has been shown to modulate immune responses and to promote tolerance induction. More recently, new "alternative" costimulatory molecules, such as the B7-family programmed death-1 ligands (PD-L)1/B7-H1 and PD-L2/B7-DC, have been suggested to play a role in determining the outcome of immune responses, including graft rejection. We have investigated the expression and function of these alternative costimulatory molecules on dendritic cells (DC) obtained from the liver (an inherently tolerogenic organ), spleen, or bone marrow.
METHODS: DC mobilized *in vivo* with the DC hemopoietin Flt3 ligand were isolated from the livers and spleens of C57BL/10J mice while bone marrow (BM) DC were generated *in vitro* by culture in GM-CSF or GM-CSF+IL-4. Freshly-isolated DC or DC stimulated with the Toll receptor ligands LPS or CpG were analyzed for expression of PD-L1 and PD-L2 by flow cytometric analysis, for naïve T cell (C3H/HeJ) allostimulatory activity (MLR, in the absence or presence of neutralizing anti-PD-L mAbs), and cytokine production.
RESULTS: *In vitro*-propagated "immature" BMDC constitutively and differentially expressed these ligands depending on the presence of IL-4. Freshly-isolated liver and splenic DC expressed low constitutive levels of PD-L1, with higher levels on splenic DC, while PD-L2 was not detected. Upon stimulation of hepatic and splenic DC with LPS or CpG, expression of both ligands was upregulated to similar levels. Hepatic DC were poorer allostimulators in comparison to splenic DC when freshly-isolated and upon stimulation with LPS. This differential allostimulatory capacity was lost when hepatic and splenic DC were triggered with CpG. Blockade of PD-L1 and PD-L2 with specific mAbs increased T cell proliferation induced by hepatic or splenic DC in MLR, with hepatic DC requiring a greater amount of blockade in comparison to splenic DC.
CONCLUSIONS: These data provide the first description of expression of PD-L1/2 on freshly-isolated and stimulated DC from liver and secondary lymphoid tissue. The upregulation of these inhibitory molecules upon stimulation and their ability to regulate T cell responses suggests that they may play a key role in modulation of immune reactivity, including that induced by liver transplantation.

Abstract# 1286 Poster Board #-Session: P216-III

A ROLE FOR GRAFT SITE AND LYMPHATICS IN THE EFFECTIVENESS OF COSTIMULATION BLOCKADE-BASED STRATEGIES IN MURINE MODELS OF TRANSPLANTATION. Sulaiman A. Nanji,[1] Colin C. Anderson,[1] Bin Luo,[1] Wayne W. Hancock,[2] Colleen D. Schur,[1] Rena L. Pawlick,[1] Lin Fu Zhu,[1] Norman M. Kneteman,[1] A. M.James Shapiro.[1] *[1]Department of Surgery, University of Alberta, Edmonton, AB, Canada; [2]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.*

Short-term combination therapy with immunosuppressive drugs and costimulation blockade has demonstrated considerable success in preventing graft rejection in a variety of transplant models. However, the rate of successful graft acceptance varies considerably depending on the type of tissue transplanted. In a fully MHC-mismatched mouse model, we have previously shown that costimulation blockade of the inducible costimulator molecule (ICOS) in combination with cyclosporine induces permanent abdominal cardiac allograft survival, but does not facilitate renal subcapsular islet allograft survival. We herein explored the possible factors responsible for the divergent findings in cardiac and islet allograft survival in this model.
Methods. BALB/c mice were used as donors for islet and cardiac tissue, while intact or splenectomized and renal lymph node resected B6 mice were used as recipients. Islet transplantation was performed under the renal capsule of streptozotocin-treated diabetic recipients, while heterotopic abdominal or renal subcapsular cardiac transplantation was completed in normal recipients. Transplanted mice were treated with anti-ICOS mAb (12A8, 0.1 mg/d) and cyclosporine (10 mg/kg/d). Reagents were administered i.p. for 14 days.
Results. The divergent findings between cardiac and islet allograft survival may be related to the site of transplantation and the presence of intact lymphatic drainage in recipient kidneys. This is based on the findings that a) unlike abdominal cardiac transplants, cardiac tissue transplanted under the renal capsule of mice receiving 12A8 + cyclosporine was rejected, and b) recipient mice undergoing splenectomy and renal lymph node resection at the time of islet transplantation and combination treatment demonstrated significantly prolonged graft survival compared to recipient mice maintaining normal lymphatic drainage (MST=33 vs 17 days respectively, $p<0.01$).
Conclusions. These data demonstrate that the graft site and the presence of intact lymphatic drainage influence the immunologic patterns of graft acceptance or rejection.

Abstract# 1287 Poster Board #-Session: P217-III

NK RECEPTOR, RAT NKP30, IS A POLYMORPHIC GLYCOPROTEIN INVOLVED IN THE IMMUNE RESPONSE DURING ALLOGENEIC LIVER TRANSPLANTATION. Christine L. Hsieh,[1] Yasuhiro Ogura, Hideaki Obara,[1] Unzila Ali,[1] Wei-wei Su,[1] Guadalupe Rodriguez, Ronald Nepomuceno, Olivia M. Martinez,[1] Sheri M. Krams.[1] *[1]Surgery, Stanford University, Stanford, CA.*

Transplanted solid organs are placed in stressful conditions that can illicit innate immune responses from sources such as natural killer (NK) cells. Using a rat model of orthotopic liver transplantation, we have previously observed that a significant population of recipient NK cells infiltrate allografts. As an effector cell, NK cells likely contribute to the graft cytokine environment and may mediate cytotoxicity against stressed or foreign cells. To better understand the role of these infiltrating NK cells, we sought to characterize receptors expressed by these cells and determine what functions these receptors may mediate during transplantation. We identified and cloned the rat homolog to the human NK activation receptor, NKp30, directly from rejecting liver allografts. Rat NKp30 (rNKp30) mRNA is constitutively expressed in primary rat NK cells. RNKp30 RNA expression was detected specifically in allografts, but not in syngeneic grafts nor cyclosporine-treated allografts by Northern blot analysis. These data suggest that NKp30 may affect transplantation.
In this report we characterized the expression of rNKp30 and its putative ligand(s). NK cell receptor diversity may contribute to effective immunity. To determine the variance of rNKp30, rNKp30 cDNA was cloned from multiple rat strains. Sequence analysis identified five single-nucleotide polymorphisms. To examine the cellular localization of rNKp30, we generated antibodies and determined that rNKp30 is expressed on the cell surface of transfected cells by flow cytometry and fluorescent microscopy. Immunoprecipitation and Western blot assays of either tunicamycin-treated or endoglycosidase-treated rNKp30 transfected cells reveal that this receptor varies in its glycosylation pattern of N-linked carbohydrates. Western blot analysis of labeled membrane proteins demonstrates that all glycosylated forms of rNKp30 are transported to the surface. These carbohydrate molecules may prove important for ligand recognition. Putative rNKp30 ligand expression was determined by flow cytometry on cells stained with an rNKp30-Fc fusion protein. Putative ligands are expressed by rodent cell lines, Nb2 and Yac1, the rat hepatoma line, FaO, and a subpopulation of primary rat peripheral blood mononuclear cells. Further studies characterizing rNKp30 and identification of its ligand will help elucidate the role of NK receptors in transplant immunology.

Abstract# 1288 Poster Board #-Session: P218-III

EXPRESSION OF SECRETORY LEUKOCYTE PROTEASE INHIBITOR (slpi) IN EXPERIMENTAL ACUTE CARDIAC ALLOGRAFT REJECTION. Sheri E. Kelemen,[1] Michael V. Autieri,[2] Anbin Mu,[1] Howard J. Eisen.[1] *[1]Cardiology, Temple University School of Medicine, Philadelphia, PA, American Samoa; [2]Physiology, Temple University School of Medicine, Philadelphia, PA.*

Introduction:Transcriptional profiling of gene expression by microarray analysis during the allograft response is a powerful approach in the characterization of the molecular processes responsible for transplant rejection. Microarray analysis was utilized to identify genes that changed significantly in response to allograft rejection.
Methods: Thirty-two heterotopic rat heart transplants were performed. Sixteen allografts (Wistar-Furth to Lewis) and 16 isografts (Lewis to Lewis). Four rats in each group were sacrificed on days 1, 3, 5 and 7. Messenger RNA expression in transplanted rat hearts was examined by cDNA microarray analysis using the Affymetrix RG_U34 gene chip, representing 30,000 sequences. Subsequent fold change analysis was performed comparing isografted left ventricle (LV) to allografted LV.
Results: One gene that changed significantly during allograft rejection was secretory leukocyte protease inhibitor (SLPI). SLPI is part of the inflammatory response serving to minimize local tissue damage and cell necrosis through inactivation of leukocyte serine proteases produced by infiltrating leukocytes during graft rejection. Fold change analysis showed that compared to isografted left ventricle, expression of SLPI was higher in allografted LV by factors of 12 fold, 52 fold and 10 fold on days 3, 5, and 7 post-transplant, respectively. There was no significant fold change between the two groups on day 1 post-transplant. To confirm the microarray data, immunohistochemistry was performed on corresponding left ventricles showing an increase in SLPI protein in allografted tissue compared to isografted tissue for days 3, 5, and 7 post-transplant. In addition to the expected expression of SLPI in neutrophils and macrophages, SLPI is also seen in the cardiac myocytes which is a novel finding.
Conclusion:This study shows that SLPI is upregulated in allograft rejection. SLPI expression in myocytes suggests a mechanism of protection from tissue damaging proteases. Serine protease inhibitors have been shown to reduce myocardial necrosis in a rabbit model of heart transplant and therefore may be useful therapeutic agents to enhance the body's control of tissue damage associated with transplant rejection.

Abstract# 1289 Poster Board #-Session: P219-III

EPSTEIN BARR VIRUS CAN PROTECT LATENTLY INFECTED B CELL LYMPHOMAS FROM FASL/TRAIL-INDUCED APOPTOSIS. Andrew L. Snow,[1] Sheri M. Krams,[1] Olivia M. Martinez.[1] *[1]Department of Surgery, Stanford University School of Medicine, Stanford, CA.*

B cell lymphomas associated with Epstein Barr virus (EBV) infection remain a serious complication in immunocompromised individuals, including iatrogenically immunosuppressed transplant recipients. EBV-specific cytotoxic T cells prevent the outgrowth of activated, proliferating B lymphoblasts in immunocompetent individuals through induction of apoptosis. We have previously shown that EBV+ B cell lymphomas derived from patients with post-transplant lymphoproliferative disorder (PTLD) show differential sensitivity to FasL-mediated apoptosis, and complete resistance to TRAIL-mediated apoptosis. Based on these findings, we sought to determine whether EBV itself could modulate apoptosis through dedicated death receptors in infected B cell lymphomas.
Unlike uninfected control cells, BJAB lymphoma cells latently infected with the B95.8 strain of EBV (BJAB_B95) are completely resistant to apoptosis triggered through Fas or the TRAIL receptors DR4 and DR5. However, BJAB_B95 cells remain sensitive to etoposide- and staurosporine-induced cell death, indicating mitochondrial-dependent pathways of apoptosis are intact. Flow cytometry shows moderately decreased expression of Fas at the cell surface in BJAB_B95 compared with BJAB, whereas the expression of TRAIL receptors is unchanged. Western blot analysis reveals impaired processing and activation of caspases 8 and 3 following death receptor engagement in BJAB_B95 cells. However, concomitant treatment with cycloheximide restores caspase activity and sensitizes BJAB_B95 cells to both FasL- and TRAIL-mediated apoptosis, suggesting the translation of inhibitory protein(s) driven by latent EBV infection confers resistance to death receptor apoptosis in EBV-infected BJAB cells. Furthermore, expression of LMP1 in BJAB cells does not affect FasL/TRAIL sensitivity, demonstrating the expression of this single viral gene is not sufficient for establishing apoptosis resistance. Alternatively, we have developed reagents for silencing various EBV latent genes using RNA interference to determine which EBV gene is necessary for mediating resistance to FasL- and TRAIL-driven apoptosis.
In summary, we conclude that EBV itself can protect latently infected host B cells from death receptor-dependent apoptosis. Determining how EBV infection contributes to this apoptosis resistance and which latent genes are involved may aid in the treatment of various EBV-associated neoplasms.

Abstract# 1290 Poster Board #-Session: P220-III

DOWN-REGULATION OF DONOR-SPECIFIC ANTIBODY: A T CELL AFFAIR? Andrea A. Zachary,[1] Robert A. Montgomery,[2] Dessislava Kopchaliiska,[1] Mary S. Leffell.[1] *[1]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.*

We have developed a protocol for down-regulating antibody to donor HLA (DSA), permitting successful transplantation of patients with DSA. In 49 patients treated, 63% had no DSA at the end of treatment while 73% sitll had Ab to 3rd party HLA. At a mean follow-up time of 13 mos., 89% of patients had eliminated DSA but only 19% had no 3rd party Ab. The differences were highly significant (P<0.001) at both time periods. Presence/absence of antibodies were determined using the most sensitive and specific tests available: solid phase immunoassays and flow cytometric crossmatches. Tests for anti-idiotypic Ab revealed anti-Id in 6 of 10 patients tested. Although failure to find anti-Id may have been due to lack of an appropriate specimen, these results suggest that anti-Id is not the primary means of long-lasting elimination of DSA. Ab elimination does not appear to be due to clonal deletion. In a patient who discontinued immunosuppression, Ab of the original specificity for donor recurred and, in at least one patient, we were able to detect B cells specific for donor antigen after DSA was eliminated. We examined the effect of anti-thymocyte serum (ATS). In 2 patients receiving ATS weeks after completing the desensitization protocol and in 6 patients who were sensitized only to 3rd party, ATS treatment had no effect on the patient's Abs. However, a recurrence or increase of DSA occurred in 8 of 8 patients who received ATS while undergoing desensitization. Although ATS binds to B cells, cell activation assays provided no evidence that the binding activated B cells. Rather we believe that the failure of antibody to recur, after desensitization treatment is completed, reflects a balance between regulatory T cells and donor-specifc B cells and until that balance is acheived, elimination of regulatory T cells permits continued differentiaion of these B cells to plasma cells. This process may also require adequate antigen presentation as stopping treatment without transplanting results in a huge rebound of DSA and antibodies that cannot be routinely eliminated are those specific for antigens expressed at low levels, DRw53 and DRw53.

Abstract# 1291 Poster Board #-Session: P221-III

REDUCED NUMBER OF $V\alpha24^+V\beta11^+$ NKT CELLS IN PERIPHERAL BLOOD OF LONG TERM REJECTION-FREE RECIPIENTS OF HUMAN KIDNEY ALLOGRAFTS. Nelson Z. Galante,[1] Esper G. Kallás,[2] Marcos A. Cenedeze,[1] Reinaldo Salomão,[2] Alvaro Pacheco-Silva,[1] Niels O. S. Câmara.[1] *[1]Laboratório de Imunologia Clínica e Experimental - Hospital do Rim e Hipertensão, Disciplina de Nefrologia - Universidade Federal de São Paulo, São Paulo, Brazil; [2]Laboratório de Imunologia - Disciplina de Doenças Infecciosas e Parasitárias, Universidade Federal de São Paulo, São Paulo, Brazil.*

There are still lacking data supporting a role for NKT cells in the maintenance of human tissue-specific tolerance. We are interested in studying NKT cell role in kidney transplants and its correlation with graft function. Peripheral blood TCR $V\alpha24^+V\beta11^+$ NKT cells were phenotyped according to CD4 and CD8 expression in normal controls (NC), in 10-years rejection-free cadaver kidney allografts maintained with minimal immunosuppression (long term rejection free [LTRF]), in patients with acute rejection (AR) and in patients with acute tubular necrosis (ATN). Results were expressed as percentages of $CD4^+CD8^-$ ($CD4^+$ NKT) or $CD4^-CD8^-$ (double negative - DN NKT) $V\alpha24^+V\beta11^+$ cells. The percentages of $V\alpha24^+V\beta11^+$ cells were 0.09, 0.14, 0.02 and 0.09 % on gated lymphocytes respectively in AR, ATN, LTRF and NC groups (ANOVA, p=0.263). $CD4^+$ NKT cells account for 42.86, 6.17, 11.11 and 17.86 % and NKT DN cells for 37.5, 11.04, 22.22 and 52.11 % of $V\alpha24^+V\beta11^+$ cells, respectively in the same groups. The differences between the frequencies of $CD4^+$ and DN $V\alpha24^+V\beta11^+$ cells were only significant in NC groups (p<0,05). Although total $V\alpha24^+V\beta11^+$ cells did not significant differ among the groups, the lowest frequency was observed in the LTRF group. Probably, intragraft localization of NKT cells as part of a tolerogenic status is a plausible explanation. Of note, the greater number of $CD4^+$ NKT (IL-4 producing NKT cells) observed in AR patients, compared to DN NKT, raises the question about the importance of specific subsets in precise events, such as kidney allograft rejection. Functional studies associated with intragraft cell population phenotyping are critical to establish the role of NKT in kidney allografts.

Abstract# 1292 Poster Board #-Session: P222-III

IDENTIFICATION OF HEPATITIS B VIRUS (HBV) - SPECIFIC LYMPHOCYTES IN HUMAN LIVER ALLOGRAFTS FROM HBV IMMUNE DONORS. Ying Luo,[1] Chung-Mau Lo,[1] Cindy Ka-Yee Cheung,[1] George Ka-Kit Lau,[2] Sheung-Tat Fan,[1] John Wong.[1] *[1]Department of Surgery, University of Hong Kong Medical Center, Queen Mary Hospital, Hong Kong, China; [2]Department of Medicine, University of Hong Kong Medical Center, Queen Mary Hospital, Hong Kong, China.*

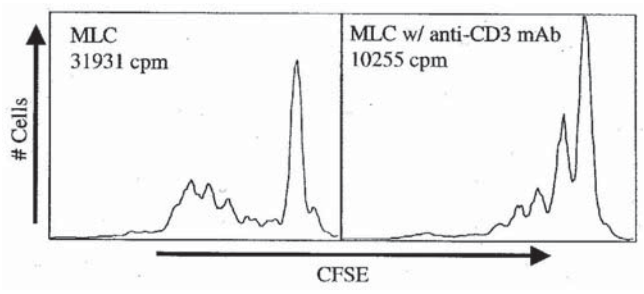
Both murine and human studies have suggested the possibility of adoptive transfer of HBV-specific immunity after liver transplantation, which may be attributed to the presence of significant number of functionally active lymphoid cells in the liver allografts from HBV immune donors. In this study, we characterized the resident lymphocytes, and identified the HBV-specific lymphocytes in liver allograft biopsies taken from 18 HBV immune donors. Liver-derived lymphocytes were prepared by mechanical dissociation and enzymatic digestion. The number, location, and phenotype of resident lymphocytes in huam liver allografts were determined by immunostaining and flow cytometry. The frequencies of HBV-specific T cells and B cells were determined by enzyme linked immunospot (ELISPOT) assay. 0.6-6(3.7 ± 1.9)$\times10^6$ lymphocytes could be recovered from 1 gram of liver tissue. These resident lymphocytes were predominantly located in the portal tracts and also scattered throughout the liver parenchyma. The majority of CD3+ T cells expressed CD8 ($67.3\pm14.1\%$), while a smaller proportion expressed CD4 ($23.3\pm9.7\%$). Cells expressing the natural killer cell marker CD56 constituted $25.4\pm9.1\%$. The frequencies of hepatitis B surface antigen (HBs-Ag) or hepatitis B core antigen (HBc-Ag)-specific IFN-γ-secreting T cells were 6.4 ± 5.7 and $11.6\pm9.9/10^6$ resident lymphocytes respectively. The antibody against HBs-Ag (anti-HBs)-secreting B cells could also be detected in 4 liver allografts ($3.2\pm4.2/10^6$ resident lymphocytes). In conclusion, considerable number of HBV-specific lymphocytes reside in liver allografts from HBV immune donors, and these may account for donor-derived immune response in liver recipients.

Abstract# 1293 Poster Board #-Session: P223-III

ANTI-CD3 MONOCLONAL ANTIBODIES INDUCE MITOGENIC PROLIFERATION BUT PREVENT EXPANSION OF ALLOREACTIVE T CELLS. Sicco H. Popma, Don Griswold, Lily Li. *Immunobiology Research, Centocor, Inc., Malvern, PA.*

OKT3 is a potent immunosuppressive agent that has been used to successfully reverse acute transplant rejection for decades. A humanized OKT3 with two alanine replacements on the CH2 domain (hOKT3γ1 Ala-Ala) was generated to retain the immunosuppressive activity of OKT3 but to reduce the side effects. This study reports the effect of OKT3 and hOKT3γ1 Ala-Ala on T-cell proliferation and phenotype in human mixed lymphocyte cultures (MLC).

Human $CD3^+$ T-cells were purified from peripheral blood mononuclear cells (PBMC) via density centrifugation and consequently labeled with CFSE. These cells were then co-cultured for 7 days with irradiated allogeneic PBMC in a MLC in the presence or absence of OKT3 or hOKT3γ1 Ala-Ala. CFSE labeling allowed us to assess the phenotype of the proliferating T cells within MLC via multiparameter flow cytometry. Phenotypic analysis of proliferating cells in MLC demonstrated an early logarithmic expansion of mainly $CD4^+$, $CD45RO^+$ memory T cells, whereas OKT3 or hOKT3γ1 Ala-Ala prevented the logarithmic expansion of T cells in MLC (see figure).



Both OKT3 and hOKT3γ1 Ala-Ala induced expansion of mainly $CD8^+$ T-cells, with both memory and naïve phenotypes. However, at day 7 the T cells in the mAb treated cultures showed diminished activation demonstrated by reduced CD25 expression and ^{3}H-thymidine incorporation. In contrast, untreated MLC showed high levels of CD25 and ^{3}H-thymidine incorporation at this time. In addition, no obvious activation induced T cell death was observed in the anti-CD3 treated MLC.

These results suggest that anti CD3 mAb (OKT3 and hOKT3γ1 Ala-Ala) induced mitogenic proliferation of T cells, which prevented clonal expansion of alloreactive T cells in MLC. The kinetic advantage of the rapidly induced T cell proliferation by anti-CD3 mAb may affect alloantigen specific T cell proliferation and play a role in therapeutic effects of these anti-CD3 antibodies.

Abstract# 1294 Poster Board #-Session: P224-III

PROTEOMIC ANALYSIS OF THE ALLOGRAFT RESPONSE. Basset El Essawy,[1] Hassan H. Otu,[2] Aviado Manuel,[2] Yon Su Kim,[1] Towia Libermann,[2] Terry Strom.[1] *[1]Department of Medicine, Harvard Medical School, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA 02215; [2]BIDMC Genomic Center, harvard Institute of Medicine, Boston, MA 02215.*

The development of noninvasive diagnostic tests that provide insights into the presence or mechanisms of allograft response,e.g. rejection or tolerance, is still a challenge. Recently the advent of Protein Chip technology has extended the application of mass spectrometry to the study of proteins from complex biological samples. We applied Protein Chip technology toward discovery of allograft response biomarkers. Three groups (4 recipients/group) of skin transplant recipients were studied. In group I, C3H recipients of DBA2 skin allograft were tolerized with MR1 (anti-CD154) plus CTL4Ig plus rapamycin therapy. The group II, C3H recipients of DBA2 grafts were untreated, while untreated C3H recipients in group III received syngeneic skin transplants. Clinically apparent rejection in group II was noted at day 10. Blood samples were drawn on day 7 before overt rejection and the serum was filtered. Protein chips were spotted robotically with day 7 serum and the protein profiles were identified by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (Ciphergen). On day 7, 161 peaks (proteins) were identified. The inter-group comparison was analyzed via a t-test like score with a measure of correlation accounting for the "signal-to-noise" ratio of choosing a mass point as being differentially expressed. The significance of results was assessed using permutation analysis. Peaks that have a 90% of confidence value as being differentially expressed are designated as different. On day 7, expression of 23 proteins were elevated in group I as compared to group II and 26 proteins were elevated in group I vs. III. The protein peaks that were decreased in group I when compared to groups II and III were 6 and 11 respectively. In contrast, no significant difference was detected between groups II and III. These findings support the potential of proteomic pattern technology as a noninvasive diagnostic test for monitoring the allograft rejection. Moreover, the patterns noted in tolerant allograft recipients and recipients of syngeneic grafts were identified at day 7. We will now focus on precise identification of proteins that predict tolerance or allograft rejection.

Abstract# 1295 Poster Board #-Session: P225-III

HLA-G MODIFIES THE LEVEL OF COSTIMULATORY MOLECULES ON RAT DENDRITIC CELLS. Maurice A. Smith,[1] Anatolij Horuzsko,[2] Stephanie L. White,[1] Oscar Grandas.[1] *[1]Department of Surgery, Medical College of Georgia, Augusta, GA; [2]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA.*

Costimulatory molecule expression on dendritic cells can be down regulated with corticosteroids and IL-10, yet this has not translated to induction of tolerance in vivo. It has been demonstrated that soluble HLA-G can bind and induce apoptosis of $CD8^+$ activated T cells and is involved in the induction of tolerance. Recent studies have suggested that tolerance of alloantigen specific T-cells is induced by semi-mature dendritic cells, defined by the expression of costimulatory molecules CD86 and CD80. The goal of our study is to evaluate the effect of HLA-G tetramer treatment of immature dendritic cells on their expression of CD86 and CD80. Bone marrow cells were cultured from BN rat femurs and sorted with anti-rat dendritic cell (OX-62) Micro Beads. Dendritic cells were treated with HLA-G tetramer for three days. Cultured cells were analyzed by flow cytometry using antibodies for MHC class II (I-A), CD80 and CD86. Treatment with HLA-G tetramer produced a significant increase in CD80 expression, decrease in CD86 while the expression of MHC II was not altered.

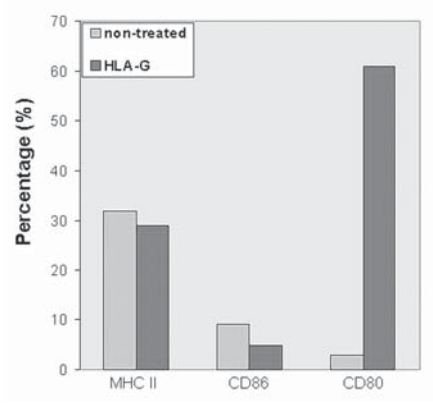


Figure 1: Results of flow cytometry of immature dendritic cells

HLA-G treated cells retained their immature stage as confirmed by low expression of MHC II, down regulated CD86 expression, and allowed the expression of CD80. It can be concluded that the expression of CD86 and CD80 on dendritic cells is modified by treatment with HLA-G. Studies are necessary to further characterize modified dendritic cells. We will analyze the function of HLA-G modified dendritic cells in vitro using antigen-presentation assays and also observe their effects in vivo via transplant models.

Abstract# 1296 Poster Board #-Session: P226-III

IMMUNE REGULATION WITHIN THE GRAFT IS MEDIATED BY FLIP AND FOXP3. Carla C. Baan,[1] Barbara J. van der Mast,[1] Esme Dijke,[1] Hester A. de Groot-Kruseman,[1] Sander S. Korevaar,[1] Lex P. Maat,[2] Aggie H. Balk,[2] Willem Weimar.[1] *[1]Internal Medicine; [2]Thorax Center, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands.*

Immune responses of allo-reactive T-cells are controlled by mechanisms like activation induced cell death (AICD) and Foxp3+ regulatory T-cells. AICD is triggered by IL-2-dependent activation of the Fas-FasL pathway and can be inhibited by FLICE inhibitory protein (FLIP). The transcription factor Foxp3 has been identified as the key gene for the development and function of CD25+ regulatory T-cells. To assess whether both mechanisms are operational during immunological quiescence and acute rejection after clinical heart transplantation, we measured mRNA expression levels of IL-2, TCR, FasL, FLIP and Foxp3 in endomyocardial biopsies (N=17) sampled before, during and after acute rejection by real time RT-PCR.

Rejection biopsies had elevated IL-2 and FasL mRNA expression levels compared to the expression levels before and after acute rejection ($p=0.03$, and $p=0.11$, ANOVA, respectively), whereas FLIP mRNA expression levels significantly decreased during rejection ($p=0.05$). Messenger RNA expression of Foxp3 was present in all analyzed biopsies. The highest Foxp3 expression levels were found during acute rejection ($p=0.01$) and correlated with IL-2 and TCR mRNA expression levels ($p=0.01$ and $p<0.0001$, respectively).

Our results demonstrate that immune regulatory mechanisms are induced within the transplanted heart during acute rejection. Both Foxp3+ T-cells and FLIP controlled AICD are involved in this process.

Abstract# 1297 Poster Board #-Session: P227-III

FEEDBACK INHIBITION OF T CELL RESPONSES BY ACTIVATED HEPATIC STELLATE CELLS, A POSSIBLE MECHANISM OF HEPATIC TOLERANCE. Cheng-Hsu Chen,[1] Ming-Chin Yu,[1] Lianfu Wang,[1] Shekhar Gandhi,[1] John J. Fung,[1] Lina Lu,[1] Shiguang Qian.[1] *[1]Thomas E. Starzl Transplantation Institute, Surgery, University of Pittsburgh, Pittsburgh, PA.*

Spontaneous liver allograft acceptance is associated with activated T cell apoptosis in the liver, suggesting unusual interactions between the hepatic milieu and immune cells. Hepatic stellate cells (HSC) located in the sinusoid and presinusoidal space represent 5-8% of liver cells. HSC 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). Quiescent HSC expressed very few surface molecules and cytokines. Activation by *in vitro* culture induced expression of MHC class I, CD40, CD80 and ICAM-1, but not MHC class II, and also induced expression of IL-1Rα, TGFβ1, MIF and IL-6 (RNase protection assay). Further stimulation by IFN-γ did not enhance cytokine expression, however, exposure to DC activated allogeneic T cells markedly upregulated expression of inflammatory cytokines including IL-1α, its receptor and IL-6, but expression of MHC class II remained negative, suggesting a lack of APC machinery. Consistently, activated HSC stimulated low thymidine incorporation and CTL activity in allogeneic C3H ($H2^k$) T cells. Interestingly, when B10 HSC were added into a DC/T allogeneic MLR culture, T cell proliferative responses were significantly inhibited in a dose dependent manner, which appeared to be related to activation of HSC, as quiescent HSC showed 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, as HSC from C3H (syngeneic to T cells) also suppressed T cell proliferative responses. Despite inhibition of T cell responses, IFN-g production was not reduced, suggesting that HSC do not interfere with T cell activation. The TUNEL staining data indicated that the low [^{3}H]TdR uptake was associated with elevated levels of T cell apoptosis. We utilized TCR transgenic mouse lines to examine the influence of HSC on antigen-specific T cell responses, and revealed a massive reduction in the antigen-specific $CD4^+$ and $CD8^+$ T cells after stimulation of antigens for 48 and 72 hours, respectively. These data strongly suggest that HSC are capable to regulate immune responses, which is initiated by becoming activation by stimulation of activated T cells, and then induce massive activated T ell apoptosis.

Abstract# 1298 Poster Board #-Session: P228-III

ONLY RAPAMYCIN DOES NOT INTERFERE WITH FOXP3 EXPRESSION OF HUMAN ACTIVATED PERIPHERAL BLOOD CELLS. Carla C. Baan,[1] Annemiek M. Peeters,[1] Dennis A. Heselink,[1] Wendy M. Mol,[1] Barbara J. van der Mast,[1] Willem Weimar.[1] *[1]Internal Medicine, Erasmus MC, Rotterdam, Netherlands.*

The transcription factor Foxp3 has been identified as the key molecule controlling the development and function of regulatory CD25+ T-cells. To understand the biology of Foxp3 in allogeneic reactions and to study the effect of immunosuppressive drugs on Foxp3 expression, we measured mRNA expression levels of unstimulated PBMC obtained from patients treated with Rapa/CsA (N=12), Tac/MMF/steroids (N=18), or Tac/MMF/anti-CD25 mAb (N=16). We also analyzed Foxp3 expression in a mixed lymphocyte reaction (MLR) of PBMC from healthy volunteers in the presence and absence of Rapa (10 ng/ml), anti-CD25 mAb (5 µg/ml), Tac (10 ng/ml) and CsA (100 ng/ml).

In unstimulated PBMC from patients we could not show an effect of the immunosuppressive treatment, as comparable mRNA expression levels were measured before and after kidney transplantation (14 days-6 months post-transplantation). However, Foxp3 mRNA expression was significantly induced after *in vitro* allo-stimulation. Kinetic examination of the MLR showed a 20-fold higher Foxp3 mRNA expression after 5 days of culture. Both calcineurin inhibitors CsA and Tac, and anti-CD25 mAb significantly inhibited the *in vitro* induced Foxp3 gene transcription (range 70-90%), whereas Rapa did not inhibit but rather delayed the induction.

In conclusion, allogeneic activation of human peripheral blood cells leads to the production of the transcription factor Foxp3. This suggests that the regulatory activity of CD25+ T-cells or the generation of these cells is induced by allo-antigen. Rapamycin, in contrast to calcineurin inhibitors or anti-CD25 mAb, does not interfere with this immunosuppressive countermechanism.

Abstract# 1299 Poster Board #-Session: P229-III

TOWARDS THE DEVELOPMENT OF A DENDRITIC CELL-BASED VACCINE FOR HEPATITIS B: INDUCTION OF TUMOR IMMUNITY AND CYTOTOXIC T LYMPHOCYTE RESPONSES USING DC TRANSFECTED BY AN ADENOVIRAL VECTOR ENCODING HEPATITIS B SURFACE ANTIGEN. Shuangjian Qiu,[1] Michael E. de Vera,[1] Shiguang Qian,[1] Lina Lu,[1] C. Andrew Bonham.[1] *[1]Surgery, Starzl Transplantation Institute, Pittsburgh, PA.*

Recurrent hepatitis B virus (HBV) infection after liver transplantation can be a significant problem. Although hepatitis B immune globulin and nucleoside reverse transcriptase inhibitors are effective in the prevention and treatment of recurrent hepatitis B, resistance to these drugs as well as cost issues require the development of other novel treatment options. To this end, we have sought ways to develop a dendritic cell (DC)-based vaccine for the treatment of HBV infection. DC are specialized antigen-presenting cells with powerful immunostimulatory properties. In this study, murine bone marrow-derived DC propagated *in vitro* were transfected with an adenoviral (Ad) vector to express the hepatitis B surface antigen (HBsAg). Ad-HBsAg-transfected DC stimulated strong and specific cytotoxic T lymphocyte responses to HBsAg-expressing tumor cells. Furthermore, vaccination of mice with Ad-HBsAg-transfected DC also protected these animals from a subsequent lethal tumor challenge, whereas no protection was conferred in mice immunized with Ad-EGFP (green fluorescence protein)-transfected DC. Immunity was antigen-specific, as wild-type tumor (HBsAg negative) grew normally. Vaccination with HBsAg protein, a clinicially utilized preparation that confers immunity to HBV infection, did not protect against tumor challenge even though it induced a strong antibody response. These studies describe for the first time the contributions of humoral and cellular immune responses to tumor immunity induced by Ad-transfected DC compared to protein vaccination. DC-based vaccination can be a potential treatment not only for HBV but for other viral infections as well after liver transplantation

Many patients with HCC demonstrate evidence of prior HBV exposure, suggesting that the presence of the virus in a quiescent state may promote tumorigenesis.

Abstract# 1300 Poster Board #-Session: P230-III

HUMAN RENAL TUBULAR EPITHELIAL CELLS MODULATE T CELL RESPONSES VIA ICOS-L AND B7-H1. Cees van Kooten,[1] Simone de Haij,[1] Andrea M. Woltman,[1] Leendert A. Trouw,[1] Astrid C. Bakker,[1] Lieping Chen,[2] Richard A. Krozcek,[3] Mohamed R. Daha.[1] *[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Immunology, Mayo Clinic, Rochester; [3]Molecular Immunology, Robert-Koch Institute, Berlin, Germany.*

Renal tubular epithelial cells (TEC) play a central role in renal inflammation. There are conflicting results whether TEC induce T cell activation or hyporesponsiveness. Recently, new costimulatory molecules (ICOS-L and B7-H1) have been described, which appear to be involved in regulation of peripheral T cell activation. In the present study we investigated expression of ICOS-L and B7-H1 on human TEC and performed T cell co-cultures to investigate the role of these novel B7 family members in regulation of T cell responses.

Primary TEC were cultured from human kidney, and compared with the human TEC cell line HK-2. Expression of different B7 molecules was studied by PCR and FACS analysis, or in situ in renal sections by immunostaining. Cocultures were performed with purified peripheral blood T cells, which were stimulated with a non-mitogenic anti-CD3 antibody. T cell activation was monitored by proliferation (thymidine incorporation) or production of cytokines (IFN-gamma and IL-10) as measured by ELISA.

We found that TEC lack expression of CD80 and CD86, but do express ICOS-L and B7-H1 *in vitro* and *in vivo*. Stimulation with IFN-gamma resulted in increased expression of B7-H1, whereas ICOS-L expression was marginally increased upon stimulation with CD40L, with no effect of IL-1, IL-17 or TNF-alpha. Furthermore, we show that TEC are able to provide a costimulatory signal to TCR-triggered T cells, resulting in strong IL-10 production. Blocking studies revealed that IL-10 production was partially mediated by ICOS-L. In contrast, B7-H1 appeared to be involved in inhibition of proliferation and cytokine synthesis. In addition, we demonstrate that TEC are able to alter the cytokine profile of fully activated T cells, which were incubated with anti-CD3 and anti-CD28 antibodies. Coculture with TEC resulted in an inhibition of IFN-gamma production by activated T cells, but a strongly increased production of IL-10 (up to 5 ng/ml).

In conclusion, interaction of tubular epithelial cells and kidney infiltrating T cells via ICOS-L and B7-H1 may change the balance of positive and negative signals to the T cells, leading to IL-10 production and limitation of the local immune response.

Abstract# 1301 Poster Board #-Session: P231-III

HYPOXIA INDUCIBLE FACTOR-1α REGULATES TCR SIGNAL TRANSDUCTION. Aaron K. Neumann,[1,2] Mangat P. Biju,[1] Randall S. Johnson,[3] Volker H. Haase,[1] Laurence A. Turka.[1,2] *[1]Department of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Immunology Graduate Group, University of Pennsylvania, Philadelphia, PA; [3]Department of Biology, University of California, San Diego, CA.*

Von Hippel-Lindau protein (pVhl) regulates the mammalian hypoxia response through continuous degradation of α-subunits of the Hypoxia Inducible Factor (Hif) family of transcription factors under conditions of normal oxygen tension. Hypoxia, or deficiency in *Vhl*, leads to the accumulation of active Hif-α with subsequent transcription of hypoxia response genes. We have observed that thymocytes from mice with T lineage-specific deletion of *Vhl* (hereafter, *Vhl-/-*) exhibited increased apoptosis *in vivo* and *in vitro*.

As thymocyte death is regulated by the strength of signals received through the TCR, we examined the responsiveness of *Vhl-/-* thymocytes to TCR crosslinking. Unlike controls, *Vhl-/-* thymocytes fail to induce capacitative Ca+2 entry (influx mediated by phospholipase C activation and release of intracellular Ca+2 stores) upon TCR crosslinking; however, thapsigargin-releasable intracellular Ca+2 stores are intact, and these stores induce control equivalent extracellular Ca+2 influx in these cells. Ca+2 mobilization following TCR crosslinking is rescued in thymocytes from mice bearing conditional deletion of both *Vhl* and *Hif-1α* demonstrating the essential role of *Hif-1α* in the Ca+2 defect of *Vhl-/-* cells. Thus, *Vhl-/-* thymocytes are capable of normal Ca+2 signaling via intracellular stores, but have a specific defect in Ca+2 flux in response to TCR stimulation, which is a result of increased *Hif-1α* activity. TCR proximal signaling events cause activation of phospholipase C-γ_1 (PLCγ_1) followed by inositol-1,4,5-trisphosphate (IP_3) mediated release of intracellular Ca+2 stores and opening of plasma membrane Ca+2 channels. Interestingly, similar patterns of total phospho-tyrosine blotting were observed in stimulated control and *Vhl-/-* thymocytes, and surprisingly, PLCγ_1 is phosphorylated in *Vhl-/-* cells upon TCR crosslinking.

Together these data suggest that activation of *Hif-1α* inhibits TCR-induced capacitative Ca+2 entry, although TCR proximal signals and activation of PLCγ_1 are intact. Since low oxygen tension alters lymphocyte activation *in vitro* and hypoxic foci are present in lymphoid organs, *Hif-1α* may regulate the responsiveness of lymphocytes stimulated under physiological conditions. Given the key role of a rise in intracellular calcium in coupling TCR signals to downstream events such as cytokine gene expression and cell cycle entry, these findings demonstrate a novel target and approach to modulating T cell responses.

Abstract# 1302 Poster Board #-Session: P232-III

PRE-OPERATIVE DELTA-MELD SCORE DOES NOT INDEPENDENTLY PREDICT MORTALITY AFTER LIVER TRANSPLANTATION. Patrick G. Northup,[1] Carl L. Berg.[1] *[1]Digestive Health Center of Excellence, University of Virginia Health System, Charlottesville, VA.*

Background: It has been recently demonstrated that a change in MELD score of 5 points or greater over 30 days (Δ-MELD) is an independent predictor for death in patients awaiting liver transplantation and that a higher absolute MELD score at the time of transplantation increases post-transplant mortality, particularly at high MELD scores. We hypothesized that patients with a progressive rise in MELD immediately prior to transplant might experience greater post-operative mortality than patients with identical but stable MELD scores.

Methods: Using the STAR dataset, the data from all adult liver transplantations (recipient age >17) performed in the U.S. between 2/1/2002 and 11/14/2003 were analyzed. MELD scores from the day of transplantation and from 25-35 days prior to transplantation were calculated and used to compute a Δ-MELD score. Post-transplant survival times and 90-day mortality rates were primary endpoints. Logistic regression and proportional hazard models were developed to analyze the influence of the dependent variables.

Results: There were 1510 liver transplants included in the analysis. The average MELD score at transplantation was 24.0 (23.6-24.4, 95% CI) and the average 30-day Δ-MELD prior to transplantation was 3.1 (2.8-3.4). 202 patients (13.4%) had improvement in MELD score in the 30 days prior to transplant, 528 (35.0%) had no change in MELD, and 780 (51.7%) had worsening of MELD score. 429 patients (28.4% of the total) had 30-day Δ-MELD > 5. Patients with a Δ-MELD > 5 had higher absolute MELD scores at transplant (31.9 vs. 20.9, p<0.0001), shorter mean post-transplant survival (218 days vs. 244, p=0.003), and higher 90-day mortality (20.7% versus 10.6%, p<0.0001). However, logistic regression modeling showed that none of the excess mortality was attributable to the high Δ-MELD score (p=0.22 for Δ-MELD > 5) and the excess risk was attributable to absolute MELD score at the time of transplantation (p=0.02).

Conclusion: Pre-operative Δ-MELD is not an independent risk factor for post-liver transplant mortality. Mortality of patients with rapidly worsening chronic liver disease who undergo transplantation depends primarily on absolute MELD score at the time of transplantation but not the rate of change immediately preceding transplant. Since it has been shown that patients with a high Δ-MELD score have increased mortality without transplantation, allocation policy should consider these patients as high risk.

Abstract# 1303 Poster Board #-Session: P233-III

SPECIFIC LABORATORY METHODOLOGIES ACHIEVE HIGHER MODEL FOR ENDSTAGE LIVER DISEASE (MELD) SCORES FOR PATIENTS LISTED FOR LIVER TRANSPLANTATION. Russ R. Arjal,[1] James F. Trotter,[1] Brad Brimhall.[2] *[1]Department of Gastroenterology/Hepatology, University of Colorado Health Sciences Center, Denver, CO; [2]Department of Laboratory Medicine, University of Colorado Health Sciences Center, Denver, CO.*

Background: Priority for liver transplantation is based on the Model for Endstage Liver Disease (MELD) score which was developed, in part, to permit only objective variables (creatinine (Cr), bilirubin and international normalized ratio (INR)) to determine status for liver transplantation. We performed a study to determine if higher MELD scores could be achieved by using selected laboratory methods which: 1) differ in terms of instrumentation or methodology from those used in our clinical laboratory and 2) were selected specifically to return the highest Cr, bilirubin and INR. **Methods:** Twenty-nine consecutive patients listed for liver transplantation who received phlebotomy for routine indications were enrolled in this IRB-approved study. MELD scores were calculated using bilirubin, Cr and INR from standard methods in our hospital laboratory (standard) and using the highest Cr, bilirubin and INR from standard or alternative methods (alternative). Changes in priority for transplantation were measured based on percentile change on the transplant list at our center. The standard determination of serum Cr and bilirubin in our laboratory is the Roche Hitachi 917 instrument and INR is determined by the Stago STA-R instrument. The Ortho Vitros 950 and Dade Dimension RXL were used to measure Cr and bilirubin and the BeckmanElectra 1400C and Instrumentation Laboratory ACL3000 instruments were used to measure INR by alternative methods. **Results:** The mean increase in the MELD score between standard (13.6) and alternative (18.4) methods was 4.8 points or 26 %, (p = 0.004). The mean difference for each laboratory variable was (standard vs. alternative): Cr (0.95 vs. 1.13 mg/dl, p = 0.06), bilirubin (3.6 vs. 3.6 mg/dl, p = ns) and INR (1.40 vs. 1.98, p < 0.0001). The mean increase in priority for transplantation was 22.3 percentiles (standard, 58th percentile vs. alternative methods, 80th percentile) and 14 % of patients moved to one of the top five positions on the list. **Conclusions:** 1) Patients listed for liver transplantation at our center achieved higher MELD scores and therefore a higher priority for liver transplantation using specific laboratory methods which yield higher

laboratory values than standard methods used in our clinical laboratory. 2) While the MELD score is determined only by "objective variables," there may be considerable and clinically relevant variation in the MELD score based on the selection of laboratory methods used for the determination of Cr, bilirubin and INR.

Abstract# 1304 Poster Board #-Session: P234-III

REDUCTION IN PRIORITY POINTS FOR LIVER TRANSPLANT CANDIDATES WITH HEPATOCELLULAR CARCINOMA (HCC) DID NOT INCREASE DROP OUT RATES FROM THE WAITING LIST. Richard B. Freeman,[1] Erick B. Edwards,[2] Ann Harper.[2] *[1]Division of Transplant Surgery, Department of Surgery, Tufts-New England Medical Center, Boston, MA; [2]Research Division, United Network for Organ Sharing, Richmond, VA.*

The original MELD/PELD liver allocation plan allowed candidates with Stage I and Stage II HCC to receive 24 and 29 MELD points respectively to approximate a 15% and 30% risk of progression beyond Stage II. Early results indicated that, at this level of priority, these candidates had an extremely high 90-day transplant rate and essentially no drop out rate. In deference to standard MELD score candidates who experienced much lower transplant rates and higher death rates compared with HCC candidates under the original policy, HCC priority was reduced to 20 and 24 points for Stage I and Stage II candidates on 2/27/03. To determine the effect of this change we used a competing risk approach to compare the cumulative probability of still waiting, and removal for transplant, death/too sick, or other reason at 30, 45 60 and 90 days after listing for candidates granted increased priority due to their HCC diagnosis before (N= 834) and after (N=390) 2/27/03. After the policy change, HCC candidates experienced a trend toward increased probability of still waiting that did not reach statistical significance at any time point. We found a significant reduction in cumulative probability of transplant at 45 (42.1% vs. 31.0%), 60 (50.4% vs. 35.9%) and 90 (58.2% vs. 44.9%) days after listing. The cumulative probability for removal for death/too sick did not change with the change in HCC policy for 30 (1.20% vs. 0.26%), 45 (1.68% vs. 0.26%), 60 (2.52% vs. 0.51%), and 90 (3.36% vs. 3.59%) day endpoints. The cumulative probability for removal due to other reasons was also unchanged with the new lower HCC priority (0.48% vs. 0.26% at 30, 0.60% vs. 0.51% at 45, 0.84% vs. 0.77% at 60 and 1.32% vs. 1.79% at 90 days). Our results support recent single center reports of very low drop out rates for early stage HCC at 90 days. The reduction in HCC priority resulted in lower probability of transplant for HCC candidates but did not increase their drop out rate as measured by probability of removal for death/too sick or other reason. HCC candidates still have a high probability of transplant within 90 days even after the reduction in HCC priority.

Abstract# 1305 Poster Board #-Session: P235-III

DECEASED DONOR LIVER TRANSPLANT SURVIVAL RATES UNDER THE MELD/PELD SYSTEM. Richard B. Freeman,[1] Ann Harper,[2] Erick Edwards.[2] *[1]Division of Transplant Surgery, Department of Surgery, Tufts-New England Medical Center, Boston, MA; [2]Department of Research, United Network for Organ Sharing, Richmond, VA.*

The MELD/PELD (M/P) system for liver allocation was implemented on February 27 2002. Reporting of 6-month post transplant survival data is 84% complete for the 4204 liver transplants performed with deceased donors from 2/27/02 through 12/31/02. For non-status 1 recipients, actual 6-month patient survival was 91.38% (CI=90.40,92.25) and was significantly better than the 79.5% (CI=75.46,83.55) survival rate for status 1 candidates. Overall 6-month graft survival for M/P recipients (87.56, CI=86.49,88.62) was also superior to the status 1 recipients (74.66%, CI=70.38,78.94). Non-status 1 children (92.98%, CI=89.79,96.21) have slightly better 6-month survival rates compared with adults (91.20%, CI=90.24,92.17) but this difference is not statistically significant and 6-month pediatric graft survival (88.16%, CI=84.17,92.14) was also similar to adults (87.51, CI=86.40,88.62). When stratified by PELD score at transplant, pediatric recipients had excellent 6-month patient survival with no difference in patient survival rates among the strata. Pediatric graft survival was also not different among the PELD strata. However when adults are stratified by MELD score at transplant, recipients with MELD>35 had acceptable but inferior patient [figure1] and graft survival compared with the other strata. Adult recipients with HCC MELD exceptions had improved 6-month patient survival (90.70%, CI=88.73,92.67) over non-HCC exceptions (86.46%, CI=82.40,90.52) and standard MELD (86.64%, CI=82.40,90.52) recipients but these differences were not statistically significant. We conclude that early post transplant patient and graft survival remains excellent under the MELD/PELD system with even the most ill adult and pediatric chronic disease recipients achieving 84.3% and 86.8% 6-month patient survival rates respectively.

Abstract# 1306 Poster Board #-Session: P236-III

IMPACT OF DIFFERENT THROMBLOPLASTINS ON THE INTERNATIONAL NORMALIZED RATIO AND MELD SCORE IN PATIENTS WITH CIRRHOSIS LISTED FOR LIVER TRANSPLANTATION. Andres E. Ruf,[1] Marta E. Martinuzzo,[2] Graciela S. Cerrato,[2] Lila L. Chavez,[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; [2]Division of Hematology, Fundacion Favaloro, Buenos Aires, Argentina.*

MELD has been found to accurately estimate death within 3 months in patients with cirrhosis awaiting liver transplantation (OLT). The formula of MELD includes prothrombin time (PT) expressed as International Normalized Ratio (INR). The INR system was introduced to standardize reports of PT using thromboplastins (TBP) of different sensitivities in patients on oral anticoagulation. However, INR has not been validated for patients with liver failure and coagulopathy. **Goal:** To assess the impact of utilizing different TBP on INR and MELD scores in patients with advanced cirrhosis. **Patients and Methods**: We studied 51 adults with cirrhosis (24 females, 27 males) listed for OLT. INR was investigated in the same blood sample utilizing 4 TBP of different origin and sensitivity, as defined by their International Sensitivity Index (ISI): TBP-1 (rabbit brain, ISI: 1,31); TBP-2 (human recombinant, ISI: 0,93); TBP-3 (human placenta, ISI: 1,03) and TBP-4 (rabbit brain, ISI: 2,22). This resulted in 4 MELD scores per patient according to the results of INR for each TBP. Statistical analysis included the Friedman test and Dunn's multiple comparisons test. **Results:** INR and MELD scores for each TBP are shown in the table. Global analysis of the values obtained with the 4 TBP resulted in statistically significant differences both for INR ($p<0.001$) and MELD ($p<0.001$). Comparison between each TBP is shown in the table. When analyzing the most divergent results obtained with the 4 TBP, changes in INR resulted in a median variation of 2 points (0-7 points) in the MELD scores for the same patient: 1 point in 14 (28%), 2 points in 20 (39%) and ≥ 3 points in 15 (29%). **Conclusions**: Use of different TBP resulted in significant variations in INR and MELD for the same patient. This suggests that in patients with the same degree of coagulopathy, MELD scores may differ according to the TBP used in each center. It has to be established whether this TBP-dependent variability in the INR and MELD scores has significant implications in the allocation of organ donors.

Median (range) of INR and MELD scores for the different thromboplastins

	TBP-1	TBP-2	TBP-3	TBP-4
INR	1.4 (0.9-5.5)≠	1.3 (1.1-3)*	1.4 (1-3.9)	1.5 (1.1-5.8)‡
MELD	16 (7-38)≠	14 (8-32)*	16 (8-35)	17 (9-39)‡

≠ $p< 0.01$ vs TBP-2; * $p< 0.001$ vs TBP-3; ‡ $p< 0.001$ vs TBP-1, TBP-2 and TBP-3

Abstract# 1307 Poster Board #-Session: P237-III

MELD OR PELD: WHICH IS THE BETTER PREDICTOR OF PRE-TRANSPLANT MORTALITY IN PEDIATRIC CANDIDATES? Erick B. Edwards,[1] Ann M. Harper,[1] Richard B. Freeman.[2] *[1]Research, United Network for Organ Sharing, Richmond, VA; [2]Transplant Surgery, Tufts University School of Medicine-New England Medical Center, Boston, MA.*

Introduction. Since 2/27/02, deceased donor livers have been allocated in the U.S. based on the MELD/PELD scoring system. Although the MELD system has been shown to accurately predict 90-day waiting list mortality in adult candidates, similar analyses of the PELD system have been hampered by the small numbers of deaths among pediatric candidates since implementation. Results from the first 18 months of PELD allocations were used to compare the predictive ability of both MELD and PELD in pediatric transplant candidates.

Methods. We analyzed the waiting list outcomes of 938 pediatric patients with both MELD and PELD data who were waiting on 2/27/02 or were added prior to 8/31/03. Outcomes at 90 days were determined from the point of first available MELD/PELD laboratory value for all patients. Candidates removed for death or too sick to transplant were counted as events. Concordance with 90-day mortality and an approximate 95% confidence interval were computed for each system using a modification of the standard method to accommodate censored data.

Results. In this cohort laboratory MELD values ranged from 6 to 53; laboratory PELD values ranged from -11 to 60. About 36% of candidates were aged < 2. A total of 51 patients died (or were too sick to transplant) within 90 days of the date of the initial MELD/PELD score entry. Of these candidates, 38 were aged < 2. Concordance with 90-day mortality was higher for PELD (c=0.88, 95% CI [0.84, 0.92]) than for MELD (c=0.86, 95% CI [0.82, 0.90]). In a subset analysis where only patients aged < 2 were considered, concordance with 90-day mortality was equivalent with PELD (c=0.79, 95% CI [0.73, 0.85] and MELD (c=0.79, 95% CI [0.73, 0.85]).

Conclusions. The PELD score for pediatric liver allocation is an excellent discriminator of mortality risk in the overall pediatric population. Both PELD and MELD were less precise in predicting pre-transplant mortality in the very young pediatric candidates. A larger cohort is needed in order to investigate if other factors, such as diagnosis, could potentially enhance this result.

Abstract# 1308 Poster Board #-Session: P238-III

IMPROVEMENTS IN THE MODEL FOR END-STAGE LIVER DISEASE (MELD) SEVERITY SCORE. Dawn M. Dykstra,[1] Russell H. Wiesner,[2] Friedrich K. Port,[1] Robert A. Wolfe,[3] Robert M. Merion.[3] *[1]URREA, Ann Arbor, MI; [2]Mayo Clinic, Rochester, MN; [3]University of Michigan, Ann Arbor, MI.*

Background: Since its inception in February 2002 as the severity-based liver allocation policy for adults, ongoing efforts have assessed the impact of MELD on waitlist outcomes. We sought to determine whether the value of MELD for predicting waitlist death could be improved by further evaluating the component (creatinine, bilirubin and INR) effects and including additional factors in the score.

Methods: Adults with chronic liver disease placed on the liver transplant waitlist (excluding exception cases) between 2/27/02 and 2/27/03 were followed through 7/31/03 (n=7,995 patients with 32,673 lab MELD scores). The effects of creatinine, bilirubin, INR (log and linear effects for each), ΔMELD (change in MELD score divided by days between current and previous score), albumin, diagnosis, ascites, encephalopathy, and variceal bleed on mortality risk were assessed using a time-dependent Cox model censoring at waitlist removal.

Results: Using both log and linear terms to model the effects of creatinine, bilirubin, and INR significantly improved the predictive value of these components ($p<0.05$). The importance of ascites (χ^2=21.4 and 40.3 for slight and moderate, respectively, $p<.0001$), encephalopathy grade 3-4 (χ^2=26.8, $p<.0001$) and ΔMELD (χ^2=17.1, $p<.0001$), while not as great as the original components, was still noteworthy. The log (ln) of albumin, while significant (χ^2=9.4, p=0.002), was less predictive than the factors above. Variceal bleeding also had a significant association with waitlist mortality (χ^2=6.9, p=0.01), whereas primary diagnosis did not.

Conclusion: Although the original MELD components remain the most statistically important predictors of waitlist mortality, these results suggest that incorporation of additional factors into a modified MELD score should be considered.

Risk Factor	Relative Risk	Chi-Square	P-value
Ln Creatinine	34.51	83.2	<.0001
Creatinine (linear)	0.39	29.4	<.0001
Ln Bilirubin	2.45	135.3	<.0001
Bilirubin (linear)	0.99	5.9	0.0154
Ln INR	5.30	136.3	<.0001
INR (linear)	0.90	11.2	0.0008
ΔMELD	1.02	17.1	<.0001
Ascites - slight	1.83	21.4	<.0001
Ascites - moderate	2.36	40.3	<.0001
Encephalopathy grade 1-2	1.04	0.2	0.6857
Encephalopathy grade 3-4	1.95	26.8	<.0001
Ln Albumin	0.65	9.4	0.0022
Variceal Bleed	1.39	6.9	0.0086
Diagnosis (7 categories)	-	7.7	n.s.

Abstract# 1309 Poster Board #-Session: P239-III

MODEL FOR END-STAGE LIVER DISEASE SCORES DO NOT PREDICT LIVER TRANSPLANTATION WAITING LIST MORBIDITY. Terianne Cowling,[1] Linda W. Jennings, Takehisa Ueno, Patrick Martin, Edmund Q. Sanchez, Srinath Chinnakotla, Henry Randall, Sherfield Dawson, Robert M. Goldstein, Goran B. Klintmalm, Marlon F. Levy.[2] *[1]Transplant Services, Baylor University Medical Center, Dallas, TX; [2]Liver/Kidney Transplantation, Baylor All Saints Medical Center, Fort Worth, TX.*

The model for end-stage liver disease (MELD) was implemented in-part to reduce waiting list (WL) mortality among patients awaiting liver transplantation (LTX). It is unknown if MELD scores can predict patient morbidity while on the WL. This study was conducted to determine if MELD is predictive of LTX WL morbidity. **Methods**: Between 5-98 and 12-98, all noncomatose LTX candidates consecutively listed for LTX at our institution were asked to participate in this study. Subjects were telephoned bi-weekly to assess the frequency of events experienced while awaiting LTX. Events included: number of doctor/clinic visits, outpatient labs, outpatient and inpatient diagnostic tests and procedures, and hospital and intensive care unit (ICU) days. Follow-up ceased at patient death, removal from active status on the WL, study termination (10-00), or LTX. Frequency of events were also examined in relation to MELD scores obtained both at listing and at LTX.

Results: Sixty-three patients were enrolled. Mean age at listing was 50 years. Patients were followed a total of 25,859 days, averaging 410.5 days per patient (range 10-904). Listing MELD scores ranged from 6-25, median = 13. LTX MELD scores ranged from 8-28, median = 15. Listing MELD scores correlated with transplant MELD scores (P=0.0002). Findings between MELD scores and frequency of events are summarized below:

Parameter	Correlation with listing MELD scores ≤ 60 days of listing	Correlation with LTX MELD scores ≤ 60 days from LTX
Number of Outpatient events	P values	P values
Doctors/clinic visits	0.682	0.258
Labs	0.239	0.446
Diagnostic tests	0.124	0.332
Procedures	0.124	0.274
Number of Inpatient events		
Hospital days	0.936	0.046*
ICU days	0.152	0.969
Diagnostic tests	0.328	0.332
Procedures	0.476	0.362

* $P<0.05$ denotes significance

In the vast majority of measured events, MELD scores were not significantly associated with the frequency of these events. Significant correlations between MELD scores and frequency of events were noted only among hospitalized days in the 60 days immediately preceding LTX.

Conclusions: The MELD scoring system does not appear to accurately predict morbidity among patients awaiting LTX. If waiting list morbidity is to be reduced, a more accurate and comprehensive tool is needed to both measure morbidity and to reduce it.

Abstract# 1310 Poster Board #-Session: P240-III

WHICH PELD FACTORS HAVE THE STRONGEST ASSOCIATION WITH PRE- AND POST-TRANSPLANT OUTCOMES? Erick B. Edwards,[1] Ann M. Harper,[1] Richard B. Freeman.[2] *[1]Research, United Network for Organ Sharing, Richmond, VA; [2]Transplant Surgery, Tufts University School of Medicine-New England Medical Center, Boston, MA.*

Introduction. Analyses of the impact of PELD allocation factors on outcomes have been hampered by both small numbers and short duration of follow-up. Using data collected since the inception of PELD on 2/27/02, we analyzed the effect of bilirubin, INR, growth failure, age at listing, and serum albumin on pre-transplant mortality and post-transplant graft failure. Additionally, the effect of serum creatinine (a MELD factor) on these outcomes was also examined.

Methods. Using Cox regression, we analyzed the outcomes of 1020 patients who were waiting on 2/27/02 or were added to the waiting list prior to 8/31/03. Only the time spent on the list after 2/27/02 was used for patients actively waiting on that date. With the exception of age < 1 at listing, all model factors were treated as time-dependent variables. All observations were censored after 365 days. Removals for death and too sick to transplant were counted as events in the analysis of pre-transplant mortality. A separate Cox regression model was used to analyze post-transplant outcomes for patients in this cohort who received deceased donor liver transplants between 2/27/02 and 6/30/03. Follow-up was censored after 180 days post-transplant.

Results. A total of 61 deaths were observed within 365 days of listing and 375 patients received deceased donor liver transplants. With the exception of albumin, all factors had a statistically significant association with pre-transplant mortality (see Table 1). With the exception of albumin and growth failure, all factors had a statistically significant association with post-transplant graft failure (see Table 2).

Conclusions. These preliminary results suggest that the effect of serum albumin in the PELD model should be re-evaluated. Serum creatinine appears to be a good candidate for inclusion in the PELD model.

Table 1. PELD Components and Pre-Transplant Mortality.

Component	Relative Risk	p-value
log(INR)	5.21	< 0.001
log(Bilirubin, mg/dl)	2.37*	< 0.001
log(Creatinine, mg/dl)	2.02*	< 0.001
log(Albumin, mg/dl)	0.611*	0.357
Growth Failure	1.91	0.025
Age < 1 at List	2.91	< 0.001

*per unit increase

Table 2. PELD Components and Post-Transplant Graft Failure.

Component	Relative Risk	p-value
log(INR)	2.06	0.032
log(Bilirubin, mg/dl)	1.31*	0.026
log(Creatinine, mg/dl)	1.53*	0.038
log(Albumin, mg/dl)	1.09*	0.883
Growth Failure	1.05	0.857
Age < 1 at List	2.52	0.004

*per unit increase

Abstract# 1311 Poster Board #-Session: P241-III

SHORT- AND LONG-TERM OUTCOME OF ORTHOTOPIC LIVER TRANSPLANTATION USING HEPATITIS C VIRUS-INFECTED ALLOGRAFTS. Fabio Vasta,[1] John J. Fung,[2] Bijan Eghtesad,[2] DeAnna Blisard,[2] A. Jake Demetris,[2] Thomas V. Cacciarelli.[2] *[1]IsMeTT, University of Pittsburgh, Palermo, Sicily, Italy; [2]Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Hepatitis C Virus (HCV) infection remains the main indication for orthotopic liver transplantation(OLTx).The use of HCV + donors as an alternative donor pool for these patients is unclear. The present study examines the outcome of serologically positive HCV donors in HCV infected recipients.

PATIENTS AND METHODS: From1992-2001,1883 patients underwent OLTx. HCV was the indication for OLTx in 560 patients (29.7%). HCV+ donors were used in 45 HCV+ recipients while 515 HCV+ patients received HCV- donor livers. HCV+ donors were identified by standard screening using anti-HCV EIA tests. Demographics of the 45 HCV+ donors were as follows:28 male/17 female; mean age 36.5 years (range 18-66 yrs); 37 Caucasian, 7 Afro-American, 1 Asian. Pretransplant diagnosis of the 45 HCV+ recipients of HCV+ donor allografts included HCV (20), HCV /HCC (11), HCV/HBV (8), HCV/EtOH(4), HCV/HIV (2).Baseline liver biopsies were available for 31/45 (69%) HCV+ donor livers. HAI ranged from 1-5/18 and fibrosis stage 0-2/6. Patient and graft outcomes between HCV recipients receiving HCV+ and HCV- donor livers were calculated using Kaplan-Meir statistics.Mean F/U was 5.1±0.5 years.

RESULTS: One-, 3- and 5-year graft survival for HCV recipients of HCV+ donor allografts was 78%, 72% and 69%, respectively. No difference in graft survival was noted between this group and HCV recipients of HCV- donor allografts (73%, 67% and 59%, 1-, 3- and 5-year graft survival respectively, p=NS).No difference in 1-, 3- and 5-year patient survival was noted between the 2 groups (85%, 77% and 72% vs 81%, 74% and 68%, HCV+ vs HCV- donors, respectively, p=NS). Causes of graft failure in the 45 recipients of HCV+ donor allografts were HCV recurrence (6), chronic rejection (1), hepatic artery thrombosis (1) and portal vein thrombosis (1). Fourteen deaths occurred in the HCV+ donor allograft group with 2 deaths occurring less then 30 days after OLTx. The remaining causes of death included HCV recurrence (5), cardiac event (3), sepsis (2), pneumonia (1), PTLD (1). Overall HCV recurrence was 30/43(70%). Mean time to HCV recurrence was 18±3.8 months (range 1-99 months).Mean HAI score at time of recurrence was 4.9±0.5 (range 2-9) and mean fibrosis stage 1.6±0.3 (range 0-4)

CONCLUSION: The short and long term survival of HCV+ donor liver allografts with HCV+ recipients is similar to that of HCV- allografts. HCV+ allografts should be considered in HCV+ recipients as a means of increasing the pool of available organ donors.

Abstract# 1312 Poster Board #-Session: P242-III

IMPACT OF THE USE OF MARGINAL, HIGH-RISK, OR LIVING DONORS ON PATIENTS RECEIVING LIVER TRANSPLANTATION FOR HILAR CHOLANGIOCARCINOMA. Julie K. Heimbach,[1] Gregory J. Gores,[2] Scott L. Nyberg,[1] Michael B. Ishitani,[1] Steven R. Alberts,[3] Michael G. Haddock,[4] Charles B. Rosen.[1] *[1]Transplantation Surgery, Mayo Clinic, Rochester, MN; [2]Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; [3]Medical Oncology, Mayo Clinic, Rochester, MN; [4]Radiation Oncology, Mayo Clinic, Rochester, MN.*

We initiated an aggressive protocol combining neoadjuvant radiochemotherapy and orthotopic liver transplantation (OLT) for patients with unresectable stage I or II hilar cholangiocarcinoma (CCA) in 1993. Though our initial results were promising, the increasing shortage of donor livers has forced the use of sub-optimal grafts. **Purpose**: to review the impact of marginal, high-risk or living-donor grafts on outcomes for patients undergoing combined neoadjuvant chemoradiotherapy and OLT for CCA. **Methods**: We reviewed outcomes for all patients enrolled since initiation in 1993. Eligibility criteria included diagnosis of unresectable hilar CCA by intraluminal brush or biopsy, or a CA-19.9 level >100 with a radiographically malignant stricture. The protocol consisted of external beam radiation with bolus 5-FU infusion followed by Ir-192 brachytherapy and maintenance 5-FU or capecitabine, until OLT. A staging laparotomy was performed prior to OLT to rule out evidence of metastatic disease. We assessed outcomes for recipients of standard deceased donor grafts compared to those who received marginal, high-risk, or living donor grafts. In addition, we compared time from enrollment to OLT during the first and last 5 years of the protocol. **Results**: 56 patients have enrolled since 1993, of which 28 have received OLT, 6 are awaiting OLT, and 22 fell out due to disease progression. Survival of all patients recieving OLT was 82% at 5 yrs. Outcomes for recipients of standard grafts (group 1) compared to those with marginal, high-risk, or living-donor grafts (group 2) are shown in table 1

Table 1

	N	Peri-op. Mortality	Re-operation	Re-transplant	Re-admission
Group 1	15	0/15	2/15	0/15	7/15
Group 2	13	3/13*	11/13*	4/13*	6/13

*p<0.01

Grafts in group 2 included 4 living donors, 2 pediatric donors, 2 older donors, 2 donors with cancer, a donor with diabetes and prior renal transplant, a donor with a fatty liver, and an amyloid donor. Time from enrollment to OLT prior to 1997 was 4.1 months vs 9.8 months after 1997 (p<0.001). **Conclusions**: Neoadjuvant chemoradiotherapy followed by OLT provides excellent results in selected patients with hilar CCA, though the use of sub-optimal grafts does have a marked impact on outcome. Given the severe shortage of donor organs, as well as the dismal alternatives for patients with unresectable hilar CCA, the use of sub-optimal donor livers may be justified.

Abstract# 1313 Poster Board #-Session: P243-III

STATE OF RENAL TRANSPLANTATION IN OLDER AMERICANS: AN ANALYSIS OF THE UNOS DATABASE. V. Ram Peddi, Steven Katznelson, Vish Halukurike, Timothy J. Baker, Maureen A. McBride. *California Pacific Medical Center, San Francisco, CA; , UNOS, Richmond, VA.*

Patients with ESRD >65 yrs of age are a rapidly expanding group of patients who are being referred for renal transplantation. However, very little has been published/reported regarding these patients. The purpose of this study is to better understand the characteristics of this group in an attempt to improve the graft and patient survival. An analysis of the UNOS database was performed. Between April 1994 and Dec 2001, 6603 kidney alone transplants (9.7% of all transplants) were performed in this group. Their characteristics compared to that of patients 35 - 64 yrs are as follows:

Characteristic	**Recipient Age 35-64 yrs**	**Recipient Age >65 yrs**
Cadaver donor : Living donor	67.4% : 32.6%	76.6% : 23.4%
Donor age: <17 : 18-59 : 60+ : unknown	11.8% : 81.1% : 6.9% : 0.2%	10.2% : 72.5% : 17.2% : 0.1%
Cause of death is CVA	27.4%	35.9%
Donor Hypertension	12.7%	18%
Ethnicity White : Black : Other	60.2% : 23.2% : 16.6%	70.6 % : 16.4% : 13%
ESRD Cause is Diabetes	24.1%	25%
Retransplant	10.9%	3.2%
Pre-transplant Dialysis	87.7%	90.7%
H/o Angina/CAD	9.1%	17.2%
HLA 0 MM	14.1%	11.8%
Cold ischemia time > 24h	17.3%	18.4%
DGF present	18.4%	21.5%
Discharge S. Creat > 2 mg/dL	41.9%	41%
Induction therapy administered	45.4%	42.7%
Treated for rejection within 1-yr	31%	25.7%
Cause of death – Cardiac	25.9%	23%
Cause of death – Malignancy	9.7%	12.3%
Cause of death – Infection	13.1%	13.7%

Death with functioning graft was the major cause of graft loss. In recipients >65 yrs and with a functioning graft at 1-yr post-txn, the 5-yr and 8-yr patient survival rates were 73.5% and 48.8%; and graft survival rates were 68.5% and 44.4% respectively. Death censored graft survival in patients >65 yrs with functioning grafts at 1-yr post-txn when compared to that of recipients 35-64 yrs was as follows (P=<0.0001; Log-rank):

	Recipient Age 35-64 yrs	**Recipient Age > 65 yrs**
3-year	94.2%	95.0%
5-year	86.5%	89.1%
8-year	74.0%	79.2%

In conclusion, renal allograft recipients >65 yrs of age: 1) Have lower acute rejection rates compared to recipients 35-64 yrs. 2) Have lower living-donor transplantation rates 3) Better death censored graft survival and 4) 90% of patients undergo dialysis prior to transplantation.

Patient survival can be improved further by encouraging living-donor transplantation, preemptive transplantation and avoiding dialysis, and aggressive attention to cardiovascular and other risk factors seen in this age group.

Abstract# 1314 Poster Board #-Session: P244-III

FATE OF LIVERS NOBODY WANTS. Nimmi Arora,[1] Sarah Bellemare,[1] Paul Gaglio,[1] Jean Emond,[1] Lorna Dove,[1] Robert S. Brown, Jr.,[1] John Renz,[1] Milan Kinkhabwala.[1] *[1]Center for Liver Disease and Transplantation, New York Presbyterian Hospital, NY, NY.*

Both living donation and expanded criteria (EC) donor livers are increasingly utilized to treat recipients whose survival is not well reflected by conventional MELD allocation. EC is commonly due to older donor age, steatosis, underlying liver disease, or warm ischemia. EC livers are often offered to centers outside of standard MELD allocation, and the outcome after transplantation of these organs is unknown. We analyzed our center-specific experience with transplantation of expanded criteria donor livers, defined for this study as (1) livers imported from out of region, or (2) livers declined by other centers in our region. Excluded from analysis were UNOS status 1 recipients and pediatric recipients.

Results: 167 LT were performed between 1/1/02 and 12/1/03, 74% (n= 124) from deceased donor (DD) and 26% (n= 43) using living donors. Excluding status 1 and pediatric recipients, 50% of DD grafts were expanded criteria (42/84) by our definition. 3 groups were compared: DD, DD expanded criteria (DD-EC), and living donors. Results are summarized in the table below. Post transplant lab data are peak values within the first 72 hours. Mean values were compared by unpaired t-test, and survival compared by Logrank comparison of Kaplan Meier (KM) curves. **Conclusions**: EC livers offered outside of standard allocation account for half of DD transplants at our center. DD-EC livers were obtained from older age donors and had longer cold ischemia times before transplantation, but there were no appreciable differences in early graft function or survival compared to standard DD grafts or live donor grafts. Because "livers nobody wants" represent a substantial number of organs utilized and are offered out of standard allocation, improved systems are necessary to facilitate placement of locally discarded livers, to ensure that these organs are fully utilized and allocated fairly.

	Group 1 (42) DD	Group 2 (42) DD-EC	Group 3 (43) LDT	P (Gp 1 v. 2)
Donor age (range)	43 (16-64)	55 (17-90)	40	.002
CIT (h)	7.2	8.6	1	.05
Lab MELD	26	21	12.5	ns
Peak AST/ALT	2131/996	2505/1313	330/342	ns
Peak T.Bili	12	11	5	ns
Peak Protime	26	27	25	ns
ICU Days	3	4	2	ns
3mth/1y survival (%)	93 / 83	92 / 85	96 / 86	ns

Abstract# 1315 Poster Board #-Session: P245-III

SUCCESSFUL TRANSPLANTATION OF LIVERS FROM OLDER CADAVERIC DONORS. Gerald R. Stephenson,[1] Peter Abt,[1] Adam Frank,[1] Mary Kaminsky,[1] Abraham Shaked,[1] James Markmann,[1] Kim M. Olthoff.[1] *[1]Department of Surgery, University of Pennsylvania, Philadelphia, PA.*

Previous reports have implicated a negative impact on survival of advanced donor age. We propose that well-selected liver grafts from donors over age 70 that are exposed to short cold ischemia of less than 6 hours has similar short and long term function without an increase in primary non-function or delayed graft function.

Patients and methods: We retrospectively reviewed the donor and recipient records of all patients at the Hospital of the University of Pennsylvania receiving a liver allograft from a heart-beating cadaveric donor over the age of 60 between April 1996 and November 2003 (n=86), of those 22 were over 70 years of age. Graft and patient survival were analyzed by Kaplan-Meyer statistics. These results were compared to a contemporary group of patients receiving grafts < 50 years of age.

Results: Of the donor group 60 years of age and older, the mean recipient age was 55±1.0 years. 26.2% of the recipients had hepatitis C, 23.8% had alcoholic cirrhosis, and 13.1% had known hepatocellular carcinoma. The mean cold ischemic time was 6.4±0.24 hours in this group. There was a shorter mean cold ischemic time in patients who had received livers from donors over seventy years of age (5.7±0.45 v. 6.7±0.27, P=0.050). Four patients had primary non-function (donor ages 62, 64, 66 and 74). Overall graft survival was 77% at one year and 61% at three years. Stratifying the patients by donor age, there was no difference in graft survival between patients who had received livers from donors 60-65 (N=42, 1 yr survival =76%, 3 yr survival = 59.0%) and those who had received livers from donors over 66-70 (N=22, 1 year survival = 78.9%, 3 yr survival = 68.8% P=0.52), or those greater than 70 (N=22, 1 yr survival = 78.6%, 3 yr survival = 65.3%, P=0.78). This was compared to a 1 and 3 year survival of 88.0 % and 73% in recipients receiving grafts < 50 yrs (P= 0.41). Patients with hepatitis C who received older livers did not have an increased rate of graft loss in comparison to those with other diagnoses.

Conclusion: Livers from cadaveric donors over the age of 70 with short cold ischemic time can be successfully transplanted with survival rates that are similar to those from donors 60-69 years of age, albeit lower than with younger donors. These liver grafts provide an important supply of livers that can be used for recipients who otherwise may not receive a graft due to low MELD score.

Abstract# 1316 Poster Board #-Session: P246-III

DISPARATE ACCESS TO THE LIVER TRANSPLANT WAITLIST BY AGE, GENDER, RACE, AND GEOGRAPHY. Alan B. Leichtman,[1,2] Valarie B. Ashby,[1,2] Friedrich K. Port,[2] Robert M. Merion,[1,2] Roger W. Evans,[3] Robert A. Wolfe.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI; [3]Rochester, MN.*

Background: Previous studies have demonstrated disparities in access to the renal transplant waitlist based upon geography, age, gender, and race. Similar disparities may exist for liver transplantation. **Methods:** Using national mortality data from the National Center for Health Statistics (NCHS), we identified 26,300 persons aged ≤ 65 years who died in 1998 with acute (ALF) or chronic (CLF) liver failure as a primary or secondary diagnosis and compared them to the number of patients who were waitlisted for liver transplantation. For ALF, we excluded deaths due to alcoholism or cancer. For CLF, we excluded non-liver cancer deaths. Using SRTR/OPTN data, we obtained the total count of waitlist registrants in 1998 (18,097). Relative waitlisting rates (RWRs) were calculated as waitlist registrants divided by (NCHS deaths minus waitlist and post-transplant deaths). CLF subgroups of liver cancer, alcoholism, and hepatitis C were also analyzed. **Results:** In general, RWRs decreased with age, and were higher for females and for whites. RWRs varied 3.5 fold across OPTN regions for ALF and by more than 50% for CLF. Among patients with CLF from cancer, alcohol, and hepatitis C, RWRs by region ranged from 0.00 to 0.24, from 0.22 to 0.51, and from 0.50 to 0.89, respectively.

Relative Waitlisting Rates by Cause-Specific Liver Failure Pool

Group	ALF	CLF	Liver Cancer	Alcohol Related	Hepatitis C
All	0.47	0.43	0.15	0.31	0.68
Age 20-39	0.47	0.47	0.53	0.28	0.63
Age 40-59	0.35	0.42	0.12	0.34	0.68
Age 60-65	0.20	0.30	0.07	0.21	0.66
Female	0.54	0.53	0.32	0.30	0.75
Male	0.41	0.38	0.11	0.32	0.65
White	0.49	0.45	0.16	0.34	0.70
African American	0.36	0.27	0.06	0.15	0.46

Conclusions: These RWR calculations overestimate the actual ratios of the waitlisted population to the pool of potential liver transplant candidates, because patients with severe liver disease who neither died nor were waitlisted during the study period could not be captured in the denominator. However, this study is useful in demonstrating qualitative comparisons within diagnostic categories (columns). These data suggest the existence of disparate access to the liver transplant waiting list based upon age, sex, race and region. More patients may benefit from liver transplantation than are currently being waitlisted.

Abstract# 1317 Poster Board #-Session: P247-III

PREDICTORS FOR RETURNING TO WORK FOLLOWING ORTHOTOPIC LIVER TRANSPLANT BASED ON DATA FROM THE OPTN REGISTRY. Vincent P. Casingal,[1] Justin M. Burns,[1] Brent D. Matthews,[1] Daniel H. Hayes,[1] Amy E. Lincort,[1] Lon B. Eskind.[1] *[1]Department of General Surgery and Transplantation, Carolinas Medical Center, Charlotte, NC.*

The purpose of this study is to identify variables predicting return to work six months following orthotopic liver transplant (OLT).

Results are based on available data from the Organ Procurement and Transplantation Network (OPTN) database from January 1998 to October 2003. Patients who were retired and children less than 18 years of age were excluded from the study. Univariate and multiple regression analyses were performed to identify significant predictors ($p<0.05$) of patient's returning to work following OLT. Pre-transplant recipient outcome variables included age, gender, employment status, presence of ascites, dialysis, diabetes, and serum creatinine; post-transplant graft failure was also included in the analysis. Donor variables included cause of death, history of diabetes, and cadaveric vs. living donor grafts. Additional regression analysis was conducted on a subset of patients evaluating preoperative MELD scores since their introduction into the national database in October 2002.

During the 5-year period, 18,437 patients with a mean age of 50 years (65% male, 35% female) underwent OLT. At time of transplantation only 25% of patients were employed. Follow up data demonstrated a sharp increase in the percentage to 47% at 6 months following OLT. Univariate analysis demonstrated the following variables were associated with an increase in a patients likelihood to return to work within 6 months following OLT: male gender, preoperative employment, creatinine < 2 (mg/dl) at OLT, and cause of donor death (traumatic). Increasing recipient age, presence of both ascites and dialysis at transplantation, and postoperative graft failure were associated with a lower chance of returning to work. Multiple regression analysis indicated that male gender and preoperative employment were significant positive predictors that a patient would return to work within six months following OLT. Increasing patient age, presence of both ascites and dialysis at time of transplantation, and postoperative graft failure were negative predictors of this outcome. MELD scores at OLT were analyzed by groups (6-10, 11-20, 21-30, 30-40), with respect to post OLT employment, but failed to reach significance.

Certain demographic variables and pre-transplant employment are significant predictors of employment 6 months after OLT. Surprisingly, a lower pre-transplant MELD score was not a predictor that a patient would return to work after OLT.

Abstract# 1318 Poster Board #-Session: P248-III

LIVING DONOR LIVER TRANSPLANTATION IS ASSOCIATED WITH REDUCED COSTS. Richard B. Freeman,[1] Susan E. FitzMaurice,[1] Jeffery T. Cooper,[1] Richard J. Rohrer,[1] Mark Epstien,[1] Jan Smith,[1] Daniel S. Pratt.[1] *[1]Division of Transplant Surgery, Tufts-New England Medical Center, Boston, MA.*

Adult to adult living donor liver transplantation (AALDTx) remains controversial and recent reports are contradictory regarding the cost of this procedure. We compared all hospital direct costs for recipients from one month prior to transplant to one year after transplant for 75 deceased donor transplants (DDLTx) and compared these with 26 AALDTx performed over the same time period. To calculate living donor recipient procurement costs, we captured all hospital costs for every inpatient and out patient encounter the donor had at our institution from the first donor evaluation visit to one year later. Median MELD scores were 13.0 vs. 20.0 (P<0.001) for the AALDTx and DDLTx cases respectively. Overall median 1-year costs were significantly less for the AADLTx ($63,525 vs. $86,767, P=0.022) with the only significant difference in cost centers seen in lower procurement costs for the AADLTx recipients ($17,943 vs. $21,247, P< 0.0001). We did not observe significant differences among the AADLTx and DDLTx groups for other measures of resource utilization like length of stay, and other individual cost centers (Table 1). Overall one year patient (88% vs. 85%, P=0.213) and graft (81% vs. 85%, P=0.619) survival were comparable between the two groups. We conclude that AADLTx is a cost effective and efficacious procedure for properly selected donors and recipients because it can reduce donor organ costs. Severity of disease also may contribute to increased costs for the DDLTx group.

Table 1.

Median	LOS	ROOM	OR	BLOOD	PHARM	PROCURE	TOTAL
AADLTx	26.5	13773	4844	9443	6883	17947*	63525†
DDLTx	31	18327	5152	11127	9311	21247	86767

*P<0.05, †P<0.001

Abstract# 1319 Poster Board #-Session: P249-III

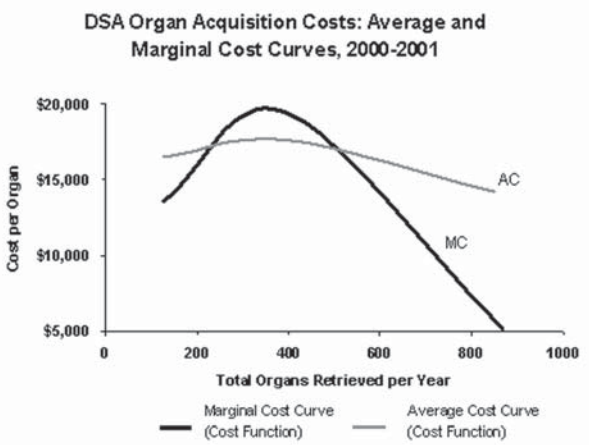
THE COST OF RECOVERING DECEASED DONOR ORGANS FOR TRANSPLANTATION. Joshua J. McGowan,[1] Akinlolu O. Ojo,[1,2] Richard E. Pietroski,[3] Friedrich K. Port,[1] Philip J. Held.[1] *[1]SRTR/URREA, Ann Arbor, MI; [2]University of Michigan, Ann Arbor, MI; [3]Transplantation Society of Michigan, Ann Arbor, MI.*

Background: We estimated the cost of retrieving deceased donor organs for transplantation using a statistical cost function (SCF) that considers labor cost, donor potential, and year.

Methods: Data consisted of annual cost reports (2000 and 2001) for 49 non-hospital Organ Procurement Organizations (DSAs) filed with the CMS fiscal intermediary. A flexible SCF was estimated: **Total DSA Costs/year = a * e^[b1(Organs retrieved) + b2O² + b3O³ + b4 (Notifiable Deaths in DSA) + b5 (CMS Wage Index (CWI) for DSA) + b6 (population/mile² (PSM) in the DSA) + b7 (year 2001 binary)].** Betas (b1-b7) were estimated via multiple linear regression. Since mean cost per organ retrieved did not differ materially by organ type, one total organ count was used per DSA. Notifiable deaths (year 1998) are an indicator of potential donors in the DSA (www.ustransplant.org). CWI and PSM are measures of resource cost.

Results: Mean costs per organ in both years approximated independent AOPO figures (2001 example: kidney $20.5K vs $18.9K; liver $19.2K vs $19.5K). The SCF had a good fit with the data (R^2 =0.96). Notifiable deaths were positively associated with higher cost (low gradient, p=0.06). All other betas were statistically significant (p<0.01). The polynomial function of average cost per organ and marginal cost curve per organ are shown (figure). 2001 costs were 7.8 % higher than in 2000. CWI was associated with higher cost; higher population density was associated with lower cost.

Conclusions: Cost reports filed by DSAs generate plausible estimates. The SCF approach provides estimates of the impact of total organs retrieved, the role of input labor costs, and changes in cost over time. Potential donor supply needs to be updated. This analysis suggests that costs per organ rise with total potential supply, though the estimated magnitude is questionable. Future analyses may offer insights into why and how some DSAs are more successful in converting potential donors.



Average Cost ($) Per Organ for Differing Levels of Cost Function Parameters* (2000-2001)

		DSA Percentiles			
Parameter	Mean	10th	50th	90th	Δ % (90th - 10th)
Organs Retrieved (Cubic Estimation)**	455	16,484	17,615	14,184	-14.0 (see figure)
CMS Wage Index	1.009	12,485	13,055	14,237	14.0
Notifiable Deaths	1741	12,500	13,143	14,193	13.5
Year (2000 vs. 2001)	0.5	12,777	---	13,779	7.8
Population per Square Mile	261	13,812	13,581	12,695	-8.1

*n=49 DSAs (98 Observations) ; r^2=0.96; p-values of all parameters <0.01 except for Notifiable Deaths (p=0.06); **all three factors in the cubic function statistically significant (p<0.001)

Abstract# 1320 Poster Board #-Session: P250-III

TRANSPLANT STAFFING RESOURCES: AN EVIDENCED-BASED ASSESSMENT AND POTENTIAL DECISION SUPPORT TOOL FOR TRANSPLANT CENTERS. E. Y. Zavala,[1] M. Strode,[2] L. B. Edwards,[3] S. Williams,[3] F. Wright,[4] C. W. Pinson.[1] *[1]Vanderbilt Transplant Center, Nashville, TN; [2]Methodist Healthcare System, San Antonio, TX; [3]United Network for Organ Sharing, Richmond, VA; [4]Texas Transplant Institute, San Antonio, TX.*

The growth of transplantation in the U.S. has shown significant volume increases in patients listed, exceeding 80,000 and active patient follow-ups exceeding 210,000 annually. Living organ donation at txp centers has increased by 13% from 2000-2002. The Txp Administrators Committee (TAC) of UNOS/OPTN, in an effort to develop a decision support tool for txp centers to substantiate appropriate staffing, conducted a national survey of txp centers staffing and work load indicators in 2003. **Methods:** A standardized staffing survey tool was developed by the TAC which surveyed the areas of: 1.) work volume indicators (txp, referrals, evaluation, living donor workups, clinic visits and listed patients); 2.) number of dedicated txp staff; 3.) identification of tasks being performed. An email notification in February 2003 was sent to 254 txp centers in the U.S. requesting voluntary participation in this survey. 127 txp centers responded representing 289 txp programs. The analysis focused on Kidney, Liver and Heart txp programs which accounted for 177 of the complete and evaluable surveys. **Results:** National averge work volume indicators and average staffing complements in full time equivelants (FTEs) were analyzed for Kidney, Liver and Heart txp programs and are shown in the table.

Average Annual Work Volume Indicators: Txps and other = referrals, pre/post txp clinic visits, listed pts, living donor wkups

Staffing Categories	Kidney Txps=90/yr Other=2753/yr	Liver Txps=43/yr Other=1973/yr	Heart Txps=48/yr Other=1166/yr
Pre Txp Coordinator	2.6	1.9	1.3
Post Txp Coordinator	2.2	2.3	1.8
Txp Assistant	1.1	1.3	0.5
Social Worker	1.1	1.1	0.7
Dietician	0.3	0.3	0.2
Financial Coordinator	0.9	1.0	0.6
Data Coordinator	0.7	0.6	0.5
Total Avg FTEs	8.9	8.5	5.6

Conclusion: This survey provides the first standardized evidenced-based txp staffing patterns in the U.S. There are substantial resources necessary to provide access to transplantation, managing listed patients and post-txp follow-up for life. Due to these increasing patient volume issues, creative staffing resources are being used to streamline and improve clinical and administrative effectiveness. Among them, txp nurse practitioners, physician assistants and txp coordinator assistants. The survey does not take into account txp administration business staff.

Abstract# 1321 Poster Board #-Session: P251-III

COMPARATIVE FINANCIAL ANALYSIS OF LIVER TRANSPLANTATION IN THE PRE & POST M.E.L.D. ERA. Dinesh Ranjan,[1] Thomas D. Johnston,[1] Hoonbae Jeon,[1] R. Craig Rogers,[2] Frank Blair.[2] *[1]Surgery/ Transplant, University of Kentucky, Lexington, KY; [2]Hospital Finance, University of Kentucky, Lexington, KY.*

Since its implementation in February 2002, the predicted financial burden of MELD implementation coupled with worsened outcome was expected to have a major impact on the survivability of liver transplant (LT) programs. There are multiple reports now showing similar clinical outcomes between the pre and post MELD era, however, there is a lack of information regarding the financial implications of MELD. Here we present a comparative analysis of LT hospital charges before and after MELD implementation at our institution.

Methods:

We tabulated the detailed hospital charges for the liver transplant admission for 125 consecutive patients transplanted between January 1,1999 and June 30, 2003. Patients dying within 72 hours of liver transplantation (n=2) were excluded, leaving 123 patients for analysis. We classified the charges in to the following major cost-centers: In-patient Room, Operating Room (including anesthesia, supplies and blood utilization), Laboratory, Pharmacy and Organ Acquisition Charge (OAC). Subsequently, the patient population was divided in to the pre and post MELD era. Median total charges, median charges for these cost centers, total median charges excluding OAC (TC-OAC) - as it was independent of MELD and clinical parameters of 1 yr patient survival and length of stay (LOS) were compared.

Results: (Table 1)

OAC increased significantly (by 16.5%) and remained the major component of total charge, accounting for 24.2% and 32.5% in pre and post MELD era respectively. Despite this, total charges only increased slightly (4.8%) in post MELD era while there was a reduction (3.9%) in total charges excluding OAC, however these differences were not significant. There was a significant reduction in OR charges and a significant increase in laboratory charges in the post MELD era. Median length of stay was comparable between the 2 groups as was 1 yr patient survival and stayed within the national norms as reported by the scientific registry of transplant recipients (SRTR).

Conclusions:

Contrary to what was expected, implementation of MELD allocation scheme resulted in neither an increased financial burden nor a decreased post-transplant survival after liver transplantation.

Table 1

	PreMELD	Post MELD	P value
N=	72	51	NA
Total charges $	91,030	95,408	NS
TC – OAC $	67,209	64,665	NS
OR $	26,175	19,530	.01
Lab $	10,105	14,706	.01
OAC $	26,650	31,045	<.0001
Pharmacy $	7,234	5,702	NS
LOS days	10	11	NS
1 yr pt survival	86.9%	91.6%	NS

Abstract# 1322 Poster Board #-Session: P252-III

COST EFFECTIVENESS OF CYCLOSPORINE (CSA), RAPAMUNE(RAPA), AND TACROLIMUS (TAC)IN COMBINATION WITH MYCOPHENOLATE MOFETIL (MMF) IN CADAVERIC RENAL TRANSPLANTS. Nauman Siddiqi,[1] Syed A. Hussain,[1] Sundaram Hariharan.[1] *[1]Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Improvement in graft survival and reduction in the rates of acute rejection have been associated with the use of newer immunosuppressive medications. With the associated increase in drug acquisition cost, a cost effective analysis is warranted to assess the different regimens.

Methods: For the purpose of this analysis three different regimens namely CsA+MMF,TAC+MMF, and RAPA+MMF were evaluated. All patients were assumed to be on 5 mg of prednisone. The cost effectiveness of these regimens was estimated using a Markov model of progression through three stages namely,1) stable post-transplant,2) acute rejection,3) graft loss.Patients were assumed to have acute rejection only in the 1st year and acute rejection to be treated with 5 days of anti-lymphocyte therapy. Progression probabilities were derived from multiple published data including United Network of Organ Sharing (UNOS). A three % discount rate was used for cost ($) and effectiveness (years). Costs were estimated from societal perspective and were obtained from published data and expert opinion. Extensive sensitivity analysis was done to test the effects of varying values of input parameters on the model results.

Results

Strategy	Cost (C)($K)	Incremental Cost ($K)	Effectiveness (E)(Yrs)	Incremental Effectiveness (Yrs)	C/E($/Yrs)	Incremental C/E($/Yrs)
CsA	302		5.88		51,329	
Rapa	425	123	8.19	2.30	51,896	53,346
TAC	506	81	9.26	1.08	54,653	75,643

Conclusions: All three regimens have similar C/E with CsA having a lower cost but also being less effective(6 discounted yrs). TAC provides better effectiveness (9.26 discounted yrs) with a much higher cost. Rapamune offers a significant advantage over both TAC and CsA with an increased effectiveness (8.19 discounted yrs) and incremental cost effectiveness of 53,346 $/yr compared to Tacrolimus of 75,643 $/yrs. Economically, Rapa/MMF offers significant advantage over CsA/MMF and TAC/MMF.

Abstract# 1323 Poster Board #-Session: P253-III

EDUCATIONAL PROGRAM TO REDUCE NONCOMPLIANCE AFTER RENAL TRANSPLANTATION. Nathalie Breu Dejean,[1] Lionel Rostaing,[2] Maryse Lapeyre Mestre,[3] Bernadette Roge,[1] Dominique Durand.[2] *[1]Center Research in Psychopathology, Mirail University, Toulouse, France; [2]Multiorgan Transplant Unit, University Hospital, Toulouse, France; [3]Clinical Pharmacology, University, Toulouse, France; [4]France.*

The objective of this randomized prospective controlled trial was to evaluate the efficacy of educational therapy for improving adherence to prescribed drugs among renal transplant (RT) patients with renal transplant and to determine the factors associated with noncompliance. The patients were transplanted between 1996 to 2001 (58 men,

52 women, mean age 49± 12 years); they were randomized for intervention group (tansplant education program and counselling; n=55) or control group(no intervention; n=55). The evaluation instruments were given at 3 times: at baseline, at the end of the educational sessions, and 3 months later. They included a specific questionnaire about compliance, quality of life (WHOQOL-26), psychological distress (Beck Depression Inventory), side effects of immunosuppressive therapy, and a questionnaire about knowledge, representation of transplantation, graft and medications. Compliance status was assessed by the specific questionnaire. The factors associated with compliance at baseline were investigated using a model of logistic regression with calculation of odds ratio (OR[95% CI]). Efficacy of the intervention program was evaluated by the comparison of compliance rate at the end of the sessions and at 3 months, using a logistic regression model including groups, baseline compliance and associated factors.Results: At baseline, there was no significant difference between intervention and control group according to socio-demographic, transplantation, medical (number of immunossuppressants and other drugs, serum creatinine) and psychological characteristics. The mean compliance score (31±7) did not differ between groups, giving a compliance rate of 47.3%. High education level (OR 2.4 [1.2-5.8]), living alone (2.7 [1.2-6.1]), negative quality of life (1.2 [1-1.5]), uncomfortable housing (1[1-1.2]), time since the date of transplantation (1.03 [1.05-1.1]), number of immunossuppressants (0.8 [0.7-1]), use of hypolipemiant (2.6[1.1-6.1]) were associated with noncompliance at baseline. Side effects of drugs were not associated with non compliance except acnea (2.7[1.1-6.9]). Compliance was significantly improved by the intervention at the end of the program sessions (69.1% versus 45.5% in controls, p=0.02) and was still improved 3 months after the education (74.5% versus 47.3%in controls, p=0.006).Our study demonstrates that a specific educational program improves compliance in patients with renal transplantation, which increased from 47.3% to 74.5%.

Abstract# 1324 Poster Board #-Session: P254-III

IMMUNOMODULATION IMPACTS SURVIVAL OF DONOR TRANSMITTED MALIGNANCIES. C. C. Rogers,[1] T. M. Beebe,[1] M. J. Hanaway,[1] M. J. Thomas,[1] T. G. Gross,[1] S. M. Rudich,[1] M. R. First,[1] R. R. Alloway,[1] J. Trofe,[1] E. S. Woodle,[1] J. F. Buell.[1] *[1]Israel Penn International Transplant Tumor Registry/Div of Transplantation, University of Cincinnati, Cincinnati, OH.*

Long-term immunosuppression has long been suspected to influence development of malignancy post transplant however, definitive data to support an influence of immunosuppression reduction on donor transmitted malignancies does not exist. The objective of this analysis is to determine if immunomodulation impacts survival in transplant recipients with donor-transmitted malignancies. **METHODS:** A retrospective analysis of all donor related tumors in our database was performed examining demographics, donor tumor histology, recipient treatment and survival. **RESULTS:** 122 donor tumor transmissions occurred. 49 patients were identified who had immunomodulation (n = 46 discontinuation of immunosuppression, n=3 immunotherapy); these were compared to 73 patients who did not receive immunomodulation (immunosuppression continued, no use of immunotherapy). A summary of their demographics, treatment and survival is presented in the table below.

	Immunomodulation(n=49)	No immunomodulation(n=73)	P value
Demographics			
Age of recipient (mean ± std dev)	42.88 ± 14.5	41.01± 15.3	0.57
Time from transplant to diagnosis			
Median (mos)	7.73	4.07	0.95
Range	0.17-42.63	0-44.73	
Time from diagnosis to death			
Median (mos)	4.13	2.07	0.245
Range	0-72.10	0-244.8	
Donot tumor histology	1 astro, 1 breast, 5 choriocarcinoma, 1 CNS Brain, 1 colon, 3 glio, 1 glio multiforme/Wilms,4 lung, 2 meduloblastoma, 15 melanoma, 2 renal cell/glioblastoma, 9 renal cell, 2 skin (karposi's), 2 unknown	1astro, 1 breast, 8 choriocarcinoma, 1 CNS brain, 4 glio, 1 glio multiforme/Whilms, 8 lung, 8 melanoma, 33 renal cell, 1 renal cell/laryngeal Ca , 2 sarcoma/spindle cell aorta/kidney, 3 unknown	
Survival			
Overall survival	41% (20/49)	45% (33/73)	0.63
Death due to malignancy	79% (23/29)	63% (25/40)	0.13
Tumor present in allograft alone	46% (6/13)	68% (25/37)	0.17
All metastatic disease	37% (13/35)	14% (4/27)	0.05
Metaststic disease alone	24% (5/21)	11% (2/18)	0.3
Metastatic disease and tumor present in allograft	57% (8/14)	22% (2/9)	0.09

In patients with metastatic disease immunomodulation was shown to significantly improve survival. However, immunosuppression cessation alone did not have a significant impact on survival in this patient population. In a subset analysis immunomodulation conveyed a survival advantage in patients whose donor histology was melanoma (40% vs. 0%, p=0.03) but not in those whose histology was choriocarcinoma or renal cell. **CONCLUSIONS:** 1) The benefit of immunomodulation is primarily seen in patients who present with metastatic disease 2) Melanoma appears to respond most favorably to immunomodulation.

Abstract# 1325 Poster Board #-Session: P255-III

SQUAMOUS CELL CARCINOMA OF THE PERINEUM AND RECTUM IN TRANSPLANT RECIPIENTS. J. F. Buell,[1] T. M. Beebe,[1] H. T. Papaconstantinou,[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.*

Squamous cell cancers (SCC) of the skin occur with higher frequency in transplant recipients than in the general population. SCC of the anorectal verge, account for 2 to 4% of all colorectal cancers. These tumors are managed varying from local excision to system chemotherapy and radiation.

Methods: Our group examined all cases of squamous cell cancer of the perineum and anus for patient demographic and tumor characteristics.

Results: 54 cases of SCC involving the perineal (n=19) and the anorectal canal (n=35). The majority (72%) of these tumors were identified in renal recipients. Mean age at diagnosis was similar as was the time to malignancy development. Interestingly the incidence of stage I disease was significantly lower in the anorectal group. Perineal SCC was managed by local resection (89%), while anorectal SCC were resected in 63% (p<0.03). Chemotherapy was then utilized in 63% of anorectal patients versus 21% of perineal tumors (p<0.05). Despite these differences in tumor stage and cancer management strategies there was no significant differences in patient survival.

	Age at Dx (yrs)	Time to CA (mos)	Stage I Disease	Follow-up (mos)	Recurrence	Survival
Perineal	46.1 ± 11.1	100.7 ± 62.8	11/19 (58%)	26.8 ± 23.5	4/19 (21%)	79%
Anorectal	49.5 ± 10.5	100.8 ± 59.6	8/35 (23%)	30.2 ± 24.8	7/35 (20%)	74%
p-value	NS	NS	<0.05	NS	NS	NS

As reported in the NCI SEER database, anal verge cancer constitutes less than 0.02% of all malignancies reported and represents 1.7% of all colorectal malignancies. Our data would suggest that squamous cell cancer of the anorectal verge represents 3.5% of all reported de novo malignancies and 26% of all colorectal malignancies compared to 1.8% identified in the general population

Conclusions: SCC of the perirectal region occurs after long intervals of immunosuppression, and comprises a higher percentage of colorectal cancers than in the general population. Interestingly, survivals are similar to the 80% 5-year survival rates reported for SCC in the general population.

Abstract# 1326 Poster Board #-Session: P256-III

EFFECT OF SITES OF PRESENTATION OF PTLD MARKEDLY INFLUENCES SURVIVAL AFTER SOLID ORGAN TRANSPLANTATION. Michael J. Hanaway,[1] Joseph F. Buell,[1] Thomas G. Gross,[1] Jennifer Trofe,[1] Thomas M. Beebe,[1] Rita R. Alloway,[1] Steven M. Rudich,[1] E. Steve Woodle.[1] *[1]Israel Penn International Transplant Tumor Registry, Division of Transplantion, University of Cincinnati, Cincinnati, OH.*

While Post-transplant Lymphoproliferative Disease (PTLD) remain a significant complication of transplantation and immunosuppression, little is known about how disease presentation influences survival. **Methods:** 871 recipients of solid organ transplants from our database were retrospectively examined. Survival analysis from time of PTLD diagnosis to death was performed by LIFEREG procedure and regression analysis along with chi square test. **Results:** Study group includes recipients of 417 kidney, 289 heart, 114 liver and 51 pancreas transplants. Median age at diagnosis of PTLD was 49.7 years. Lymph node was the most common site of presentation of PTLD in each type of organ transplant, followed by allograft involvement in kidney, liver and pancreas transplant recipients. GI Tract, liver, spleen and lung were also frequently involved sites at presentation. Survival was significantly shorter after diagnosis of PTLD with CNS involvement (in all organ tx), allograft involvement (heart and liver tx), liver involvement (kidney and heart tx). PTLD involvement of spleen, GI tract, Lymph Node and Lung did not effect survival in any type of organ transplant recipients. Multiple sites of PTLD involvement (compared to single site involvement) was associated with decreased survival in kidney, heart and lung but not pancreas transplant recipients. **Conclusions:** 1.) CNS involvement of PTLD carries a uniformly poor prognosis in all types of solid organ transplants studied here. 2.) Allograft involvement in recipients of life sustaining organ transplants (heart and liver) are associated with poor outcome compared to those with PTLD in allografts of life enhancing transplants (kidney and pancreas). 3.) While GI tract is a common site of PTLD presentation, it is not associated with a higher rate of death. 4.) PTLD involvement of non-lymphatic organs (CNS, heart allograft, liver) carries a worse survival rate than involvment of lymphatic organs (LN, spleen, GI tract).

Effect of PTLD Site of Presentation on Survival after Solid Organ Transplant

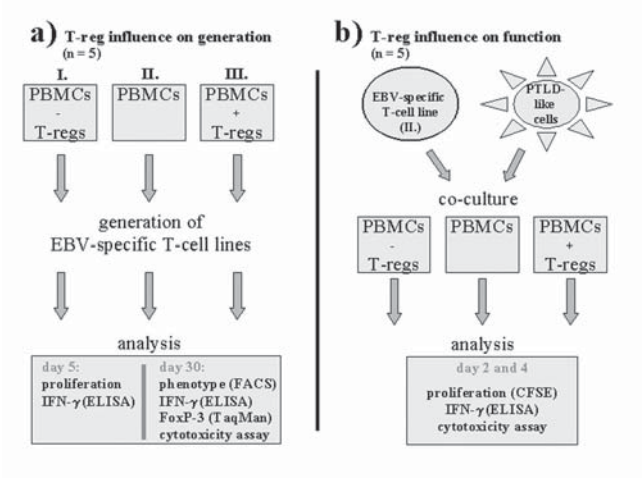
Site of PTLD	Kidney Tx	Heart Tx	Liver Tx	Pancreas Tx
CNS	p<0.0001	p<0.0001	p<0.0001	p=0.02
Allograft	NS	p=0.002	p=0.009	NS
Liver	p<0.0001	p=0.0009	NA	NS
Spleen	NS	NS	NS	NS
Lung	NS	NS	NS	NS
Lymph Node	NS	NS	NS	NS
GI Tract	NS	NS	NS	NS
Multiple Sites	p=0.0025	p=0.0002	p=0.0035	p=0.26

Abstract# 1327 Poster Board #-Session: P257-III

REGULATORY T-CELLS IN ADOPTIVE IMMUNOTHERAPY. M. H. Hammer,[1,2] L. Ang´ani,[1] H.-D. Volk,[2] P. Reinke.[1] [1]*Department of Nephrology;* [2]*Institute of Med. Immunology, Charite, Berlin, Germany.*

Infusion of EBV-specific T-cells has been proven to be a powerful treatment of EBV-associated lymphomas (PTLD) in bone marrow transplanted patients. To achieve comparable long lasting EBV-control in solid organ transplantation (SOT), higher numbers of specific T cells and more frequent cell infusions are necessary. Beside the immunosuppressive therapy in SOT, we hypothesized that negative regulatory $CD4^+CD25^+$ T cells (T-regs) could also be causative for diminished function of administered T-cells. Therefore, the influence of T-regs on the generation procedure (a) and function (b) of EBV-specific T-cell lines was determined.

experimental design



An important concern was that T-cell lines generated from patients with elevated T-reg frequencies still contain relevant numbers of these cells and therefore have impaired anti-tumour function. Indeed, T-regs influenced the generation procedure. Starting with T-reg enriched PBMCs, T-cell lines could only be generated in 2/5 volunteers, instead of 5/5 out of PBMCs or T-reg depleted PBMCs. At day 5, IFN-γ secretion was decreased in all T-reg enriched cell cultures. However, if the generation of the EBV-specific T-cell line was successful, no significant differences in phenotype, IFN-γ level, cytotoxicity or FoxP3 expression - a marker of T-reg function - were found.

To mimic the influence of T-regs on adoptively given cells, the generated T-cell lines were cultured with PTLD-like cells plus PBMCs +/- autologous T-regs. Proliferation responses were not influenced by T-regs enrichment, probably due to the activated phenotype, but IFN-γ secretion was decreased in 3/5 experiments. Interestingly, the IFN-γ decrease and the impairment in the generation procedure took place in the same 3 volunteers, whereas 2 were not affected by autologous T-regs.

Our data give evidence that T-regs can interfere with the generation process and function of specific T-cell lines. Therapeutic strategies, like depletion of T-regs with anti-CD25mabs, may benefit adoptive immunotherapy in PTLD-patients.

Abstract# 1328 Poster Board #-Session: P258-III

AGGRESSIVE SKIN CANCERS IN TRANSPLANT RECIPIENTS. J. F. Buell,[1] T. M. Beebe,[1] C. Muthiah,[1] T. G. Gross,[1] M. R. First,[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.*

In the general population, the majority of basal cell (BCC) and squamous cell cancers (SCC) of the skin are often easily managed by local excision due to their indolent tumor biology. In transplant recipients, skin cancer is denoted by a higher incidence of squamous cell and melanotic cancers compared to the general population. To detect those factors associated with aggressive squamous cell skin cancers, we examined all cases of aggressive skin cancer, defined as having lymph node involvement or distant metastases.

Methods: All aggressive non-melanoma skin cancers were examined for patient demographics, tumor characteristics and survival.

Results: Of 2018 skin cancers, 85 were identified as aggressive, with 56 patients having secondary sites of disease, and 51 with lymph node disease. Of the aggressive skin cancers, 18 were in patients with combined SCC and BCC (p=NS). A younger age at transplantation was noted in the aggressive group, while age at diagnosis was not significantly different. Exposure to immunosuppression, based on time from transplantation, was similar between groups. Recurrences were significantly more common in the aggressive group (59/85;69% vs. 479/1933;25%: $p < 0.001$). The interval from diagnosis to recurrence was shorter in the non-aggressive group, while the interval from transplant to death was similar. The aggressive skin cancer group did have a significantly higher cancer specific mortality.

	Tx Age (yrs.)	Dx Age (yrs.)	Dx Post Tx (mos.)	Recur Post Tx (mos.)	Recur Post Dx (mos.)	CA Specific Mortality	Death Post Tx (mos.)
All Skin	48.1	53.2	61.1	33.9	45.8	3.8%	106.9
Aggressive	45.6	52.1	71.2	54.1	84.7	69%	123.1
p-value	<0.07	NS	NS	<0.001	<0.001	<0.001	NS

Conclusions: Aggressive squamous cell cancers tend to occur more commonly in younger patients, while length of immunosuppression is similar to the indolent skin cancers observed in transplant recipients. Further analysis is necessary to assess whether overall intensity and/or individual immunosuppressive agents may affect the biologic nature. Despite the higher incidence of recurrences and cancer specific mortality observed in the aggressive skin malignancy group, the interval from transplant to recurrence was paradoxically shorter in the non-aggressive group. This contradictory data indicates further analysis is necessary to determine the origin of and the impact of immunosuppression on the development of post transplant skin malignancies as well as the role of immunosuppression reduction.

Abstract# 1329 Poster Board #-Session: P259-III

MALIGNANCY TRANSMISSION FROM DONORS WITH MELANOMA. J. F. Buell,[1] T. M. Beebe,[1] T. G. Gross,[1] M. J. Hanaway,[1] M. R. First,[1] C. Muthiah,[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.*

Melanoma has long been recognized as a malignancy with prolonged recurrence intervals. In several instances, the use of a single donor with metastatic melanoma has been reported to result in multiple recipient cancer transmissions.

Methods: We examined all cases of donor utilization with the diagnosis of melanoma, recognized either prior to or after donation. These cases were examined for donor and recipient demographics, tumor characteristics and recipients survival.

Results: 38 recipients of solid organ transplants from 13 donors (12 cadaveric and one living-related kidney) with melanoma were identified. Organs transplanted included 27 kidneys, 6 livers, 3 hearts, and 2 pancreases. The majority of the donors were female (12:1), with a mean donor age of 43.1 ± 8.0 yrs. Ten donors had a prior history of wide local excision of melanoma. The majority of tumors were later identified as Clark's level III lesions, with 3 donors noted to have advanced stage III disease. Mean recipient age was 42.5 ± 17.5 yrs., with an equal gender distribution. Of the 38 recipients there were 29/38 (76%) tumor transmissions confirmed, of which 7/29 (24%) involved the allograft and 28/29 (97%) developed metastatic disease. Mean time from transplantation to tumor recognition was 14.0 ± 12.7 months. Fourteen transmissions (48%) were associated with melanoma brain metastases misdiagnosis as cerebral bleed in the donor compared to two donor transmissions in ten correctly diagnosed patients ($p<0.05$). Transmission rates were not affected by recipient allograft type (extra-renal 9/11 vs. renal allograft 20/27). Donor transmitted melanoma resulted in a high mortality rate (19/28;66%). All patients with metastatic disease died from their malignancy, while 6 recipients with allograft alone disease were rendered disease free by incidental or therapeutic nephrectomy ($p<0.05$).

Conclusions: A history of metastatic melanoma should be a contraindication to organ donation, independent of disease free interval. A significant proportion of donor-transmitted melanoma arise from cases were the donor had a misdiagnosis of the etiology of their cerebral hemorrhage. This data suggests that, in instances where the etiology of cerebral hemorrhage is unclear, a limited brain autopsy should be performed. In cases where melanoma is discovered to involve the allograft, explantation should be performed, or when involving extra-renal allografts, immediate re-transplantation should be considered.

Abstract# 1330 Poster Board #-Session: P260-III

THE INFLUENCE OF ANTIGEN PRESENTING CELL (APC) MICROENVIRONMENT ON EBV MEMORY RESPONSES: TGF-β, BUT NOT IL-10, TREATMENT OF APC REDUCES IFN-γ PRODUCTION AND INHIBITS EBV-REACTIVE MEMORY CTL RESTIMULATION. Tyler C. Hoppes,[1] Navneet Cheema,[1] Anne M. VanBuskirk.[1] [1]*Surgery, The Ohio State University, Columbus, OH.*

Post-transplant lymphoproliferative disorder (PTLD) is a serious post-transplant complication often associated with Epstein-Barr Virus (EBV). Clinical studies have shown that effective cellular immunity is key to protection from PTLD, although the precise mechanisms by which the EBV memory response fails is not clear. Two important immuno-regulatory cytokines are TGF-β and IL-10. TGF-β is important in reactivation of EBV from the latent to the lytic state and IL-10 is a growth factor for EBV-transformed cells. However, both of these cytokines are also known to inhibit various aspects of cellular immunity. Whether TGF-β and IL-10 in the microenvironment have overlapping functions in inhibiting EBV-reactive immunity is not known, and could have important implications for the development of PTLD. To further understand the roles of these immuno-regulatory cytokines in the immunobiology of PTLD, we assessed the ability of TGF-β or IL-10 to inhibit two parameters of cellular immunity, IFN-γ production and memory CTL restimulation. Purified, adherent APC were pre-treated with medium, TGF-β, or IL10, overnight. The pretreated APC were then cultured with syngeneic CD3+ T

cells and irradiated syngeneic EBV-stimulators for 5 days. After the culture period, IFN-γ accumulation in the supernatant was measured and CTL activity assessed using standard methods. Supernatants from CD3+ cells cultured with APC pre-treated with either TGF-β or IL10 demonstrated significantly reduced levels of IFN-γ (p=.01, t-test) compared to CD3+ cells cultured with control APC or CD3 cells cultured with EBV-stimulators alone. Supernatants from CD3+ cells cultured with IL-10-pretreated APC demonstrated the most significant reduction. However, when CTL activity was assessed, only CD3+ cells cultured with TGF-β treated APC exhibited significantly reduced CTL activity (p=.03). CD3+ T cells cultured with IL-10 treated APC demonstrated CTL activity equivalent to controls. Our data provide two important points. First, CTL activity is separable from IFN-γ production, and second, that although memory CTL restimulation does not require APC, the APC microenvironment can influence memory CTL restimulation. Given the importance of CTL in protection from PTLD, the APC microenvironment could therefore influence clinical outcome.

Abstract# 1331 Poster Board #-Session: P261-III

SUCCESSFUL MINIMIZATION OF IMMUNOSUPPRESSION(IM) AND CONVERSION TO SIROLIMUS(SLR) IN KIDNEY TRANSPLANT RECIPIENTS WITH POST TRANSPLANT LYMPHOPROLIFERATIVE DISEASE(PTLD) AND DE NOVO NONSKIN MALIGNANCIES(DNSM). Debra Sierka,[2] Mysore S. Anil Kumar,[1] Michael Heifets,[3] Miten Parikh,[1] Michael J. Moritz,[1] Aparna Kumar.[1] *[1]Transplant/Surgery, Drexel University College of Medicine, Philadelphia, PA; [2]Transplant Pharmacy, Hahnemann University Hospital, Philadelphia, PA; [3]Nephrology/Medicine, Drexel University College of Medicine, Philadelphia, PA.*

Immunosuppression(IM) is known to cause PTLD and DNSM in kidney transplant recipients. The recommended management of these patients includes discontinuation of IM and appropriate cancer(CA) therapy with surgical ablation, chemo and/or radiotherapy.These patients are forced to return to chronic dialysis after IM is discontinued. In our center, some kidney transplant recipients with PTLD and DNSM refused to return to dialysis and continued IM on their own. Following this defying gesture from these patients we worked with them to develop a new protocol to minimize the risk of CA recurrence yet maintain function of the transplanted kidney. A multidisciplinary team approach was used including oncology, transplant and social services.The protocol included rigorous CA management, minimization of IM and conversion to SLR based IM.Whole body CAT scan or MRI at 6 month intervals and frequent surveillence by transplant and oncology teams were integral to this protocol. 13 patients have been treated in this manner; the mean age was 57±11 yrs. Two pts had PTLD, 2 lung CA, 2 thyroid CA and one each had Hodgkin's lymphoma, leukemia, mixed parotid tumor, colon, renal cell, prostate and breast CA. Mean follow-up since transplant and diagnosis of CA is 4.5±3yrs and 2.3±1.7yrs, respectively. CA was diagnosed 4.3 to 80 months after transplant and treated according to standard of care.One patient with breast CA refused chemo and radiotherapy after surgical ablation and died of metastatic disease 3 months later. All other patients remain cancer-free. Prior to diagnosis of CA, all were on calcineurin inhibitor(CNI) based immunosuppression; after CA diagnosis all patients were converted to SLR and CNI was discontinued or minimized. 12/13(92%) patients maintained a functioning graft with a mean serum creatinine of 2.2±0.9mg%. Our data indicates that minimization of IM and conversion to SLR maintains graft function and reduces recurrence of PTLD and DNSM. Patients choosing this treatment plan must be compliant with rigorous follow up protocols. Multi-center trials and long term follow up is required before this mode of therapy can be routinely recommended.

Abstract# 1332 Poster Board #-Session: P262-III

CONVERSION TO SIROLIMUS, A SUCCESSFUL TREATMENT FOR POSTTRANSPLANTATION KAPOSI'S SARCOMA. Alex Gutierrez-Dalmau,[1] Jose M. Campistol,[1] Ana Sanchez-Fructuoso,[2] Aurelio Sanz-Guajardo,[3] Auxiliadora Mazuecos,[4] Jose V. Torregrosa,[1] Federico Oppenheimer.[1] *[1]Renal Transplant Unit, Clinic Hospital, Barcelona, Spain; [2]Nephrology Department, Clinico San Carlos Hospital, Madrid, Spain; [3]Nephrology Department, La paz Hospital, Madrid, Spain; [4]Nephrology Department, Puerta del Mar Hospital, Cadiz, Spain.*

PURPOSE: The increased incidence of Kaposi's sarcoma (KS) in organ transplantation has been related to the KS herpesvirus and the permissive effect of immunosuppressive therapy. SRL has showed atitumoral and antiangiogenic activities. We postulated that conversion to SRL in renal recipients with KS could favor regression of KS lesions without increasing the risk of graft rejection.

METHODS: We performed a retrospective chart review of 5 caucasic renal transplant recipients affected of KS to determine demographics data, etiology of ESRD, immunologic risk factors, immunosuppresive treatment, KS disease follow-up, and renal function before and after SRL conversion.

RESULTS: All 5 patients were under calcineurin inhibitor treatment at the KS debut, with variable post-transplantation time (mean 21.6 months, 3-36). KS was limited to skin, without regression despite attemps to immunosuppression minimization. Conversion to SRL was performed gradually over a 2 to 5 weeks period. Renal function was stable, mean serum creatinine 1.86 ± 0.75 and 1.68±0.79 mg/dL, pre-conversion and at the end of follow up, respectively. The four patients with longer follow-up (13.7 months, 6-22) showed progressively regression of KS lesions, only hyper pigmented atrophic cutaneous lesions after a mean of 9 months (6-18 months) were identified. The fifth patient has completed 45 days of follow-up, and there is a slightly reduction in lesion sizes. Mean SRL blood level was 9.66±1.3 ng/mL. No patient has experienced acute rejection.

CONCLUSION: After SRL conversion, patients with KS showed progressive regression without an increased risk of acute rejection. SRL offers a promising approach to the management of posttransplantation KS and probably to other malignancies in organ transplant recipients.

Age	68	67	57	71	60
Gender	m	m	m	f	f
Etiology ESRD	Diabetic nephropathy	Glomerulonephritis	Tubular-Interstitial disease	Vasculitis	Polycystic Kidneys
Immunosuppressive treatment	BA, CsA, MMF, P	CsA, AZA, P	CsA, MMF, P	CsA, P	BA, FK, MMF, P
Postransplantation time to KS development (months)	3	6	36	36	27
Cutaneous involvement	Lower legs, hands, abdomen, neck and ears	Lower legs and wound site	Left lower leg	Left lower leg	Lower legs and wound site
Serum Cr before CsA withdrawal (mg/dL)	2.8	2.0	2.3	1.1	1.1
Follow-up since SRL conversion (months)	6	22	11	16	1.5
Last serum Cr (mg/dL)	2.7	1.8	2.1	0.7	1.1
Mean SRL level (ng/mL)	10.0	9.3	11.8	9.0	8.2

Abstract# 1333 Poster Board #-Session: P263-III

EBV-DNAEMIA AND PTLD IN LUNG TRANSPLANT RECIPIENTS WITH CYSTIC FIBROSIS. Robin K. Avery,[1,4] Lara Danziger-Isakov,[2] Atul C. Mehta,[3,4] Omar A. Minai,[3,4] Jeffrey T. Chapman.[3,4] *[1]Infectious Disease, Cleveland Clinic Foundation, Cleveland, OH; [2]Pediatric Infectious Disease, Cleveland Clinic Foundation, Cleveland, OH; [3]Pulmonary and Critical Care Medicine, Cleveland Clinic Foundation, Cleveland, OH; [4]The Transplant Center, Cleveland Clinic Foundation, Cleveland, OH.*

Background: It has been suggested that patients undergoing lung transplant for cystic fibrosis may have a higher risk for developing PTLD than other lung recipients, even when EBV serostatus is taken into account. It is not known whether or not recipients with CF have more frequent or higher levels of EBV-DNAemia as compared with other lung transplant recipients. Methods: Records of 253 lung recipients transplanted at one center (a predominantly adult lung transplant program) between 1996 and 2003 were reviewed for demographic and clinical information. Starting in 2001, pts had EBV DNA levels monitored, and antiviral therapy (IV ganciclovir or oral valganciclovir) was initiated with reduction of immunosuppression for positive levels. Results: Between 1996-2003, 5/12 pts who developed PTLD had CF (42%) although CF pts constituted only 36/253 (14.2%) of lung recipients overall (p=0.043). Between 2001-2003, CF pts accounted for 18% (21/120) of all transplanted pts, 6/27 (22%) of all pts with positive EBV levels, and 3/14 (21%) of all those with peak EBV DNA above 1000 copies/ml. 6/21 (29%) of CF pts and 21/99 (21%) of non-CF pts had positive EBV-DNA levels. The mean peak EBV-DNA level of CF pts with positive EBV-DNA levels was 1334 copies/ml, as compared with 2608 copies/ml for non-CF recipients. EBV-DNA levels were not available for the period before 2001. Conclusions: Lung transplant recipients with CF have a high risk of PTLD as compared with non-CF lung recipients. However, the incidence of EBV-DNAemia and the magnitude of quantitative EBV DNA viral loads in CF recipients does not appear to be greater than in other lung transplant recipients. Other factors may contribute to enhanced PTLD risk in CF patients.

Abstract# 1334

ANTIBODY RESPONSE AGAINST GLOMERULAR BASEMENT MEMBRANE AGRIN IN PATIENTS WITH TRANSPLANT GLOMERULOPATHY. Cees van Kooten,[1] Simone A. Joosten,[1] Vanessa van Ham,[1] Johan van der Vlag,[2] Bert van den Heuvel,[3] Yvo W. J. Sijpkens,[1] Leendert C. Paul.[1] *[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, University Medical Center Nijmegen, Nijmegen, Netherlands; [3]Pediatrics, University Medical Center Nijmegen, Nijmegen, Netherlands.*

Transplant glomerulopathy (TGP) is present in about 15% of patients with chronic rejection and is characterized by duplications of the glomerular basement membrane (GBM). Risk factor analysis suggested that immune responses are important in the pathogenesis. In a rat model of chronic rejection we previously identified antibodies reactive with the GBM, especially the heparan sulphate proteoglycan (HSPG) perlecan. Here we investigated whether patients with TGP have circulating antibodies reactive with GBM, and identified the antigens recognized by these antibodies.

Sera from patients with TGP were collected and tested for anti-GBM and anti-HSPG reactivity. Furthermore, normal kidney biopsies were stained with IgG purified from patients with TGP and colocalization with known HSPGs was investigated. The identity of the antigen was investigated after purification of human GBM preparation using an affinity column with IgG from a patient with TGP.

Sera of 12/16 patients with TGP contained antibodies reactive with GBM isolates but not with the Goodpasture epitope (collagen type IV), and only 50% showed presence of anti-HLA antibodies. Seven out of 12 sera reacting with GBM also reacted with highly purified HSPG, which contained both perlecan and agrin as shown with specific antibodies. Indirect immunofluorescence with patient IgG revealed a GBM staining pattern on normal human kidneys. Double staining and confocal microscopy especially showed co-localization with anti-agrin antibodies. Next we affinity purified the antigen(s) involved from human GBM using patient IgG. After elution, this resulted in an antigen(complex), that was recognized by all seven patients that recognized the HSPG. Importantly, this antigen(complex) was also recognized by specific antibodies against agrin, but not by antibodies against perlecan.

In conclusion, we identified a humoral response directed against the GBM heparan sulphate proteolgycan agrin in patients with TGP. This supports a role for humoral responses reactive with tissue specific antigens in the pathogenesis of TGP.

Abstract# 1335

IDENTIFICATION OF A NOVEL SMOOTH MUSCLE PROTEIN INVOLVED IN THE PATHOGENESIS OF CHRONIC GRAFT REJECTION. Majed M. Hamawy,[1] Jose R. Torrealba,[2] Matthew Colburn,[1] Susan Golner,[1] Zhen Davis,[1] Tara Scheunaman,[1] Huaizhong Hu,[1] Hyoung T. Kim,[1] Tausif Alam,[1] Turan Kanmaz,[1] Stuart J. Knechtle. *[1]Surgery, U. Wisconsin, Madison, WI; [2]Pathology, U. Wisconsin, Madison, WI.*

INTRODUCTION: Chronic allograft rejection is a major cause of graft loss. Vascular fibrointimal proliferation plays an important role in the pathogenesis of chronic graft rejection. We identified a smooth muscle-specific protein whose expression is strongly down regulated by TGF-β and is markedly reduced in chronic graft rejection.

METHODS: Tissues from naïve and chronically rejecting monkey renal allografts were solubilized and subjected to 2D-electrophoresis. Proteins were transferred to PVDF membranes and tyrosine-phosphorylated proteins were identified by immunoblotting. A heavily phosphorylated protein was excised and analyzed by Mass Spectrometry. Peptides corresponding to the sequenced nucleotides were used for generating polyclonal antibodies.

RESULTS: By blotting PVDF membranes with anti-phosphotyrosine antibodies we consistently observed that the level of a 57-kDa protein (isoelectric point 6) was markedly reduced, or absent, in rejecting but not in naïve or nonrejecting animals. Partial sequencing by mass spectrometry suggested that this 57-kDa protein is novel. Immunohistochemical studies showed the protein to be localized to smooth muscles. Remarkably, immunohistochemical staining of kidneys from monkeys experiencing chronic graft rejection with intimal hyperplasia failed to detect the protein in the smooth muscle layer of the vessels, confirming our in vitro studies on the disappearance of this protein from rejecting allograft tissues. Because growth factors are released in rejecting grafts, we reasoned that the absence of the protein might be due to the inhibition of its expression by a growth factor. We examined the effect of TGF-β, IFN-γ, PDGF, and TNF-α in the smooth muscle cell line CRL-1999. We found that only TGF-β blocked the expression of this protein, as there was a marked decline in the protein and its mRNA (n=6). In contrast, TGF- β induced marked increase in α-actin expression. Solubilization with detergents studies indicated that this protein is associated with the cytoskeleton.

CONCLUSION: We identified a 57-kDa cytoskeleton-associated phosphoprotein that appears to be smooth muscle-specific. Because it is heavily tyrosine phosphorylated, this protein might function as an intracellular signaling molecule. Our data implicate this protein in mediating the effects of TGF-β on smooth muscle and in playing a role in the pathogenesis of graft rejection.

Abstract# 1336

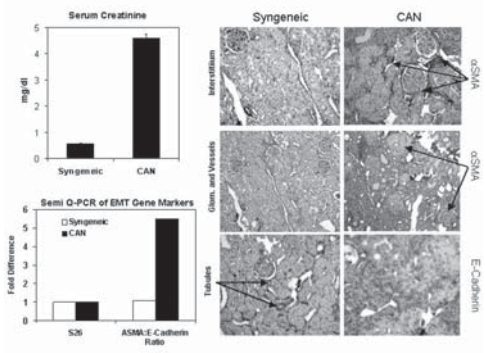
MODULATING CHRONIC GRAFT INJURY: A NEW ROLE FOR CONNECTIVE TISSUE GROWTH FACTOR (CTGF). Orlena H. Cheng,[1] Xiaojie Zhang,[1] Gary Grottendorst,[2] Roslyn B. Mannon.[1] *[1]Transplantation, NIDDK/NIH, Bethesda, MD; [2]Cell Biology, U Miami, Miami, FL.*

Despite dramatic improvements in immunosuppression, chronic allograft nephropathy (CAN) remains the leading cause of late graft loss following kidney transplantation. While TGFβ is an important contributor to the development of fibrosis in CAN, its immunoregulatory properties make it a poor target for inhibition in transplant settings. CTGF, a downstream effector of TGFβ, modulates fibrosis and angiogenesis, and represents an alternative target. Furthermore, CTGF is upregulated within the kidney in other fibrotic diseases. To further elucidate the role of CTGF in CAN, we transplanted H-2^b kidneys into H-2^d recipients (allografts) or their littermates (isografts) and evaluated graft histology, function, and cytokine expression at 1, 2, and 4 weeks post transplant prior to the development of CAN, and at 6 weeks when CAN is histologically and functionally evident. By 1w, TGFβ mRNA expression was dramatically and significantly elevated in allografts (n=8, 6.4±2.0-fold) compared to normal, non-transplanted kidneys (p=0.008), and remained significantly elevated (3-5-fold) at all timepoints studied (p<0.001). Associated with the increase in TGFβ was a rise in CTGF mRNA expression that became significant by 2w post transplant (2±0.3-fold; p=0.005), and remained elevated out to 6w (2.7-5.6-fold; p=0.02). Furthermore, nonrejecting isografts demonstrated no increase in CTGF mRNA expression. Associated with induction of CTGF mRNA was the marked expression of CTGF protein within tubular epithelial cells detected by immunohistochemistry, with a relative absence in isografts. This was accompanied by the presence of extensive myofibroblast transformation within the graft. To assess the specificity of CTGF induction in CAN, we evaluated CTGF mRNA expression in cardiac allografts. In acutely rejecting cardiac allografts, CTGF mRNA was significantly elevated as early as 2d post transplant and remained significantly elevated prior to graft loss (4-17-fold; p=0.001) compared to normal and isografted hearts. Moreover, in MHC class II mismatched allografts that develop graft arteriosclerosis over 6 weeks, CTGF expression was significantly increased by 4w (3.2±0.8-fold compared to normal hearts; p=0.022), and prior to the development of chronic injury. Our studies demonstrate a prominent expression of CTGF in the development of chronic renal injury as well as in cardiac rejection. Understanding the functional role of CTGF in immune injury will provide a new strategy for the diagnosis and management of chronic injury following organ transplantation.

Abstract# 1337

CHRONIC ALLOGRAFT NEPHROPATHY IS ASSOCIATED WITH EPITHELIAL TO MESENCHYMAL TRANSDIFFERENTIATION. Arjang Djamali,[1] Shannon Reese,[1] Joseph Yracheta,[1] Lynn Jacobson,[1] Debra Hullett,[1] Bryan Becker.[1] *[1]Medicine, Nephrology Section, University of Wisconsin, Madison, Madison, WI.*

Epithelial to Mesenchymal Transdifferentiation (EMT) is a process characterized by phenotypic transformation of renal tubule epithelial cells to myofibroblasts. Through a profibrotic process, these myofibroblasts synthesize *de novo* α-smooth muscle actin (αSMA) and downregulate the expression of E-cadherin on cell surface. Although EMT has been shown to be a common final pathway to various insults to the kidney, it remains unknown whether it is also involved in chronic allograft nephropathy (CAN), a condition associated with significant tissue fibrosis. In a transplant model of CAN, we performed Fisher 344→Lewis (n=4) and Lewis→Lewis (n=3) kidney transplants in 3 month old recipients. Allograft recipients received 1.5mg/kg/d of CsA for 10 days. Three months following transplant, blood and urine samples were collected and transplanted kidneys harvested for analysis. We measured serum creatinine and urine protein levels. We also performed semi-quantitative PCR and immunohistochemistry analysis to assess αSMA and E-Cadherin mRNA and protein levels in syngeneic and allogeneic kidneys



Allograft recipients had findings consistent with CAN: significant tubular atrophy, interstitial fibrosis and vascular wall thickening. Serum creatinine levels and proteinuria were significantly higher in the CAN group. Moreover, αSMA gene product and protein expression were higher and E-Cadherin gene and protein expression were lower in CAN compared to syngeneic transplants. There was > 5 fold increase in αSMA:E-Cadherin mRNA ratio (normalized to S26 internal control) in CAN compared to syngeneic grafts.

Our Study demonstrates for the first time that EMT and CAN are associated. Strategies to delay/arrest EMT in kidney transplantation might therefore have the potential benefit to delay the progression of CAN.

Abstract# 1338

EXTRA-CELLULAR MATRIX DEGRADATION DURING APOPTOSIS OF ENDOTHELIAL CELLS ACTIVATES NOVEL FIBROGENIC PATHWAYS. Patrick Laplante,[1] Gabrielle Gagnon,[1] Marc-Andre Raymond,[1] Anik Desormeaux,[1] Normand Vigneault,[1] Marie-Josee Hebert.[1] *[1]CRCHUM, University of Montreal, Montreal, QC, Canada.*

Persistent differentiation of fibroblasts into myofibroblasts and lack of fibroblast apoptosis have been implicated in the fibrotic response associated with various forms of solid organ chronic rejection. Yet, the mechanisms that regulate myofibroblastic differentiation and apoptosis of myofibroblasts are still poorly understood. In the present work, we tested the hypothesis that endothelial apoptosis initiates the release of fibrogenic mediators. Human embryonic fibroblasts were exposed to medium conditioned by apoptotic or non-apoptotic human umbilical vein endothelial cells (EC) in vitro. We found that medium conditioned by apoptotic EC inhibits apoptosis of human embryonic fibroblasts in vitro whereas medium conditioned by non-apoptotic human EC does not. Western analyses revealead that mediators produced by apoptotic EC induce rapid phosphorylation of AKT, followed by increased protein levels of Bcl-xl in fibroblasts in vitro whereas proteins levels of p53, cIAP-1, cIAP-2 and XIAP were not modulated. Long-term exposure (4 and 7 days) of fibroblasts to medium conditioned by apoptotic EC increased protein levels of alpha-smooth muscle actin, desmin and vimentin and induced phenotypical changes characteristic of myofibroblastic differentiation. Moreover fibroblasts exposed to mediators produced by apoptotic EC were found to produce a significantly greater amount of collagen type I, again a distinctive feature of myofibroblastic differentiation. Blockade of phosphatidylinsositol-3 (PI3) kinase activation with LY294002 in fibroblasts exposed to mediators produced by apoptotic EC inhibited overexpression of alpha-smooth muscle actin. Using mass spectrometry, we identified one of the fibrogenic mediators present in the medium conditioned by apoptotic EC as a fragment of the C-terminal domain V of the proteoglycan perlecan. We found that supplementation of serum-free medium with chondroitin-4-sulfate, a glycosaminoglycan anchored on the domain V of perlecan, induces myofibroblastic differentiation and resistance to apoptosis in fibroblasts. We propose that apoptosis of EC initiates the degradation of pericellular proteoglycans and liberation of fibrogenic fragments which in turn activate PI3-kinase-dependent fibrogenic pathways. Sustained endothelial injury could result in chronic activation of this paracrine fibrogenic loop and may represent the basis of maladaptive fibrotic responses that characterize various forms of solid organ chronic rejection.

Abstract# 1339

PROGRESSIVE ACTIVATION OF ALLORESPONSE AND TISSUE-SPECIFIC AUTOIMMUNITY IN A NOVEL MOUSE MODEL OF SPONTANEOUS GRAFT CORONARY ARTERY DISEASE. Masashi Tanaka,[1] Monika Zwierzchoniewska,[1] Golnaz K. Mokhtari,[1] Theo Kofidis,[1] Robert C. Robbins,[1] Eugenia V. Fedoseyeva.[1] *[1]Department of Cardiothoracic Surgery, Stanford University, Stanford, CA.*

The pathogenesis of graft coronary artery disease (GCAD) are still not fully understood. Accumulating evidence suggests that allo-response and tissue-specific immunity may contribute to the development of GCAD. To study this, we developed a novel minor antigen (mHC)-mismatched mouse GCAD model, and monitored the immune response to both allogeneic and tissue-specific antigens (cardiac myosin, CM). **Methods:** Hearts of FVB mice (H-2^q) were heterotopically transplanted into DBA/1 recipients (H-2^q, minor mismatch, n = 6) and C57BL/6 mice (H-2^b, MHC mismatch, n = 6). No immunosuppression was used. At 8 days, intragraft $CD4^+$ and $CD8^+$ T cell, B cells ($B220^+$), macrophages ($Mac\text{-}1^+$), and dendritic cells ($CD11c^+$) were quantified by immunostaining. In minor mismatch group, circulating allo-antibody production by flow cytometry and allo- and CM-specific T cell responses by ELISPOT were monitored at 8, 56, and 112 days. GCAD indices were assessed at 56 and 112 days. **Results:** All allografts in MHC mismatch group were acutely rejected within 9 days, whereas all allografts in minor mismatch group survived more than 112 days and developed GCAD (% of luminal narrowing, intima/media ratio, % of diseased vessels: 38 ± 11%, 0.7 ± 0.2, 81 ± 10% at 56 days, 53 ± 7%, 0.9± 0.2, 92 ± 4% at 112 days). At 8 days, infiltration of $CD4^+$, $CD8^+$ T cells, macrophages, dendritic cells but not B-cells were significantly decreased in the cardiac allografts of minor mismatch group in comparison to MHC mismatch group. Accordingly, the frequencies of IFN-γ- and IL-2- but not IL-4–producing alloreactive T cells were significantly lower than in minor mismatch group at 8 days. In minor mismatch group, allo-antibody production and the frequency of alloreactive T cells were the highest at 56 days, followed by drastic decrease at 112 days. Alternatively, the activation of CM-specific Th1-, Th2-, and B-cell responses was detected only after 56 days, and steadily increased up to 112 days. **Conclusions:** In this mHC-mismatched GCAD model, early stages of GCAD are associated with low frequency T cell alloresponse and allo-antibody production. Alloresponse diminishes later, and tissue-specific immunity dominates host anti-graft immune response. This suggests that while subthreshold alloreactivity may contribute to initiation of GCAD, the perpetuation of GCAD occurs due to progressive activation of host autoimmunity against tissue-specific antigens shared by donor and recipient.

Abstract# 1340

ADVENTITIAL GERMINAL CENTER-LIKE STRUCTURES IN A VASCULAR CHRONIC REJECTION MODEL. Olivier Thaunat,[1] Anne-Christine Field,[1] Patrick Bruneval,[1] Antonino Nicoletti,[1] Jean-Baptiste Michel,[2] Blanche Bellon.[1] *[1]U 430, INSERM, Paris, France; [2]U 460, INSERM, Paris, France.*

Chronic allograft rejection represents the main cause of long-term graft dysfunction. Excluding organ-specific manifestations, the most common histopathological characteristic is chronic vascular rejection (CVR). Adventitial inflammatory infiltration, medial smooth muscle cell (SMC) destruction and intimal thickening involving inflammatory cell infiltration are the typical CVR features. Its pathophysiology remains poorly understood however, substantial evidence suggests that anti-HLA antibodies are involved in the process. The adventitial infiltration is chronologically related to medial SMC destruction, specifically targeted by immunoglobulins, and continued as long as medial SMC persist. We hypothesize that adventitial inflammatory infiltrate may play an important role in the development of the lesions of CVR.

In the rat aortic interposition model, in which an aortic abdominal segment of a BN rat is grafted into a LEW recipient (histoincompatible rat strains), a typical chronic vascular rejection is observed. Adventitia was separated by microdissection from the rest of the graft layers at various time-points after transplantation. Systematic analysis of the microanatomy and cellular composition of the adventitial infiltrate were performed.

Over a period of 2 months, T-cell infiltrate increased, its composition changed from an equal number of CD8+ and CD4+ to a majority of CD4+ T cells, and shifted from an initial Th1 to a Th2 polarization. In the same time, percentage of CD134 positive cells, a molecule involved in T-cell help to the B cells, increased only in the subpopulation of T CD4+. An important finding was the detection of B lymphocytes in the adventitial infiltrate. Moreover, these cells exhibited a particular disposition; they were clustered in nodular aggregates located in the outer part of the graft adventitia. Staining with anti-immunoglobulin heavy-chain isotype showed that a nucleus of immature double positive IgM+ and IgD+ B-cells, was surrounded by a ring of single positive IgM+ B-lymphocytes. This structural organization suggests that adventitial B cell aggregates are germinal centre-like structure.

This study is the first to report the implication of VALT (Vascular-Associated Lymphoid Tissue) in alloimmunity, which may play a critical role in the generation of alloantibodies responsible for the development of the lesions of chronic vascular rejection.

Abstract# 1341

THE TRIALS AND TRIBULATIONS OF TRANSLATING A MURINE TOLERANCE MODEL TO THE NONHUMAN PRIMATE. Edip Akpinar,[1] Jodie M. Keary,[2] Edwin Preston,[1] He Xu,[1] Roger Kurlander,[2] Douglas A. Hale.[1] *[1]Transplantation Branch, NIDDK-NIH, Bethesda, MD; [2]Hematology DLM, CC-NIH, Bethesda, MD.*

Background: Donor bone marrow (BM) prolongs skin graft survival and generates chimerism in mice conditioned with antilymphocyte serum and sirolimus (Sir). Application of this strategy in humans requires adaptation to and testing in large animals. **Methods:** Skin grafts were performed between ABO matched, outbred cynomolgus monkeys on day 0. Treatment consisted of antithymocyte globulin (ATG, 100 mg/kg total in 5 doses on days 0,1,3,5,7) and a 30-day course of Sir and/or tacrolimus (Tac). BM, harvested from either living or cadaveric donors, was infused at day 7. Immune phenotyping and alloantibody assessment were done by flow cytometry. Blood drug levels were monitored. Chimerism was measured by genotyping using human specific Short Tandem Repeat kit. **Results:** Treatment related morbidities were observed. Initially, high Sir levels (>40ng/ml) were targeted in 10 primates and wasting(8), lethargy(4), mucocutaneous ulcerations(8), pancytopenia(8), hyperlipidemia(10), pneumonitis(4) and death(4) were encountered. Supra-therapeutic levels of Sir (>100 ng/ml) did not prolong graft survival longer than the therapeutic level (10-25 ng/ml) did. When Sir+Tac were used at 10 ng/ml levels, all monkeys had toxicity culminating in death due to systemic CMV. ATG was associated with >95% lymphocyte depletion; recovery began within 15 days and was complete by 6 weeks. Two primates succumbed to cytokine release after the first dose of ATG. Overall median graft survival was 8 days in controls (n=2) and 24, 13, 13, 67, 52 and 40 days in primates treated with ATG (n=4), Sir (n=4), Sir/BM (n=2), ATG/Sir (n=3), and ATG/Sir/BM (n=7), ATG/Tac/BM (n=2) respectively. No consistent chimerism was detected. The range of infused BM cell number was between 65 million and 2 billion cells/kg and the median graft survival in monkeys that received <200, >200 and <600, and > 600 million cells/kg was 69, 45 and 36 days respectively. The higher doses of BM were obtained from sacrificed donor and contained a higher number of progenitor cells and less T lymphocytes. All recipients developed alloantibody within 3 weeks. **Conclusion:** Efforts to translate a nonmyeloablative murine tolerance model into cynomolgus monkeys have been disappointing and emphasize the increased immunologic barrier associated with allotransplantation in larger animals and humans. Efforts to modify the treatment protocol with strategies that can facilitate donor BM engraftment and prevent sensitization in this large animal model are ongoing.

Abstract# 1342

THE G2677T BUT NOT THE C3435T POLYMORPHISM OF THE MULTIDRUG RESISTANCE GENE 1 (MDR-1) AFFECTS CYCLOSPORIN DOSE REQUIREMENTS IN STABLE ADULT HEART TRANSPLANT PATIENTS. Anne B. Taegtmeyer,[1,2,3] Jane B. Breen,[1] Panagiotis Pantelidis,[3] John D. Smith,[2] Ken I. Welsh,[3] Nicholas R. Banner,[1] Magdi H. Yacoub,[2,3] Paul J. R. Barton.[2,3] *[1]Transplant Unit, Harefield Hospital, Harefield, Middlesex, United Kingdom; [2]Heart Science Centre, Harefield Hospital, Harefield, Middlesex, United Kingdom; [3]National Heart and Lung Institute, Imperial College London, United Kingdom.*

PURPOSE P-glycoprotein, encoded by multidrug resistance gene 1 (MDR-1), is a drug efflux pump that regulates the uptake of cyclosporin (CsA) across the intestinal wall. Previous studies have shown subjects possessing the C3435T T allele of MDR-1 to have reduced uptake of p-glycoprotein substrates. We hypothesised that genetic variation of MDR-1 contributes to variation in CsA uptake and therefore dose requirements. We examined the association of both the non-coding C3435T and the coding G2677T (Ala 892 Ser) polymorphisms with CsA dose normalised trough ratios.
METHODS 92 consecutive adult heart transplant patients transplanted between 1996 and 1999 were studied retrospectively at years 1, 2 and 3 after transplantation. Target 12 hour post dose (trough) CsA level was 100 - 150 ng/ml. No patients were receiving concomitant CYP inducers or inhibitors. Genotyping for C3435T and G2677T was performed using Sequence Specific Primer PCR (SSP-PCR). Trough CsA concentrations were measured by the EMIT whole blood immunoassay and dose normalised trough ratios were calculated as trough concentration/0.5 x dose in previous 24 hours and are given as ng/ml/mg. Low dose-normalised trough values indicate high dose requirements to achieve target trough concentration.
RESULTS Genotypes were in Hardy-Weinberg equilibrium. Possession of the G2677T T allele was associated with lower mean dose-normalised trough values. Mean values (ng/ml/mg) for 2677T allele carriers *vs* non carriers were 1.43 *vs* 1.65 (n= 92 p < 0.04), 1.38 *vs* 1.59 (n=86 p <0.03) and 1.3 *vs* 1.7 (n= 68 p < 0.003) at years 1, 2 and 3 respectively. In contrast, there was no association between mean dose-normalised trough values and the C3435T polymorphism. Mean values (ng/ml/mg) for 3435T carriers *vs* non-carriers were 1.43 *vs* 1.48 (ng/ml/mg, n= 92, p = ns), 1.40 *vs* 1.43 (n = 86, p = ns) and 1.39 *vs* 1.37 (n = 68, p = ns) at years 1, 2 and 3 respectively.
CONCLUSION Possession of the G2677T T allele but not the C3435T T allele is associated with higher CsA requirements to achieve target trough concentrations. Prior knowledge of MDR-1 genotype may therefore be helpful in determining correct CsA dose and predicting the effect of concomitant p-glycoprotein substrates.

Abstract# 1343

RAPAMYCIN INHIBITS NK CELL FUNCTION. Saori Takeda,[1] Christine Hsieh,[1] Hideaki Obara,[1] Olivia M. Martinez,[1] Sheri M. Krams.[1] *[1]Department of Surgery/Transplant, Stanford University School of Medicine, Stanford, CA.*

Host-derived NK cells infiltrate solid organ allografts early after transplantation. In the DA→ Lewis, high-responder, orthotopic model of liver transplantation 56.4±6.2% of the mononuclear cells in the allograft 24 hours after transplant are NKP-P1+, TCR- NK cells. Moreover, we have determined that cyclosporine (CSA) does not prevent the infiltration of NK cells into liver allografts. However, the direct effects of immunosuppressive drugs on NK cell function remains unclear. The purpose of this study was to determine the effects of immunosuppressive drugs on primary rat NK cells. NK cells were purified from normal Lew rat spleens and primary NK cell lines were established and analyzed for proliferation in the presence of increasing doses of CSA (0-100 ng/ml), FK506 (FK, 0.1-100 ng/ml) or Rapamycin, (RAPA, 0-10 ng/ml). Proliferation was analyzed 1-7 days after initial culture. NK cells demonstrated robust proliferation both in the absence and presence of CSA. In contrast, FK significantly ($p< 0.05$) inhibited proliferation at high doses and RAPA significantly ($p< 0.05$) inhibited the proliferation of NK cells in a dose-dependent manner. In related experiments we have determined by intracellular staining and flow cytometry that NK cells are a major source of IFN-γ secretion in liver allografts. Since IFN-γ is such a pivotal cytokine in the response to alloantigen we analyzed the effects of CSA, FK and RAPA on IFN-γ production by NK cells. Primary NK cells secrete robust levels of IFN-γ. Neither CSA, FK nor RAPA inhibit IFN-γ production by NK cells. To determine if immunosuppressive drugs alters the ability of NK cells to kill, cytotoxicity against tumor targets was analyzed in the presence of absence or presence of immunosuppressive drugs (CSA, 100 ng/ml, FK,100 ng/ml and RAPA,10 ng/ml). CSA and FK did not effect NK cell–mediated killing while cytotoxicity was significantly ($p< 0.05$) inhibited in the presence of RAPA. These data indicate that activated NK cells may contribute to the rejection response in CSA and FK treated allograft recipients. Furthermore, NK cells retain effector function in the presence of CSA and FK and thus could actively contribute to the elimination of virally infected or transformed cells in transplant patients. In contrast, NK cell proliferation and killing are significantly diminished by RAPA treatment. Understanding the differential effects of these immunosuppressive drugs on NK cell effector function is important in clinical transplantation.

Abstract# 1344

MELATONIN ABROGATES ACUTE AND ACCELERATED CARDIAC REJECTION IN RATS. Florian J. Jung,[1] Lin Yang,[1] Luc Haerter,[2] Didier Schneiter,[1] Didier Lardinois,[1] Marius Keel,[2] Walter Weder,[1] Stephan Korom.[1] *[1]Division of Thoracic Surgery, University Hospital Zurich, Zurich, Switzerland; [2]Departement of Traumatology, University Hospital Zurich, Zurich, Switzerland.*

Introduction: Melatonin (5-methoxy N-acetylserotonin, MLT), the main secretory product of the pineal gland, is a multifunctional neurohormone. First, it protects tissues from damage through free radicals and attenuates ischemia/reperfusion injury in solid organ grafts. Second, it acts synergistically on a variety of cellular antioxidants. Third, it displays a complex, dose-dependent immunoenhancing and -suppressing effect in vitro and -vivo. The aim of this study is to probe for the immunoregulatory effect of MLT during the course of acute allograft rejection.
Materials and Methods: In the acute model, hearts were transplanted from LBNF1 to LEW rats and anastomosed heterotopically to the abdominal great vessels. In the accelerated model, LEW rats were sensitized with BN rat skin 7d prior to cardiac transplantation (Tx). Recipients were treated with 20(I) and 200mg MLT/kg/d (II) po. respectively; animals receiving saline served as controls. Graft survival was monitored daily through abdominal palpation. Serum samples were obtained and levels of allospecific antibodies (allo-Ab) (FACS) and total Ig (ELISA) measured. Standard MLR and cytotoxicity assays were run.
Results: High dose MLT therapy (200mg/kg/d/po) abrogated acute rejection and significantly prolonged allograft survival (mean±sd; control: 6.3±1d vs. I: 7.3±1.1d vs. II: 12.3±1d; $p<0.0001$; n=12/12/8). Circulating allo-IgM were significantly decreased at d10 and 14 post Tx in group II ($p<0.01$), whereas in group I at d10 levels were increased compared to group II ($p<0.01$). No difference in total Ig between the groups was noted. Cellular proliferative efficacy and cytotoxicity were reduced in the high dose group in comparison to group I and controls. In 4/7 recipients, 200mg/MLT/d abrogated accelerated rejection (84±27h vs. control: 28±6h; $p<0.05$).
Conclusion: Abrogation of acute and accelerated allograft rejection and significant prolongation of Tx survival have been achieved for the first time by employing the neurohormone melatonin. Our results suggest an involvement of MLT in humoral and cellular immune pathways following perfused organ transplantation. These findings may indicate a novel therapeutic immunosuppressive approach, based on modulation of the neuroendocrine/immune axis.

Abstract# 1345

MCI-186 (FREE RADICAL SCAVENGER) INDUCE PROLONGED SURVIVAL OF FULLY-ALLOGENEIC CARDIAC GRAFTS.

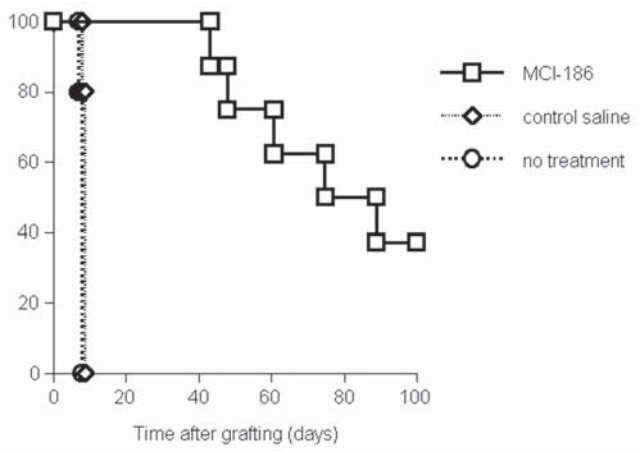
Katsunori Tanaka,[1] Nozomu Shirasugi,[2] Fumihiko Inoue,[1] Takurin Akiyoshi,[1] Osamu Aramaki,[2] Qi Zhang,[2] Kenji Matsumoto,[1] Masaki Kitajima,[1] Masanori Niimi.[2] *[1]Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan; [2]Surgery, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo, Japan.*

INTRODUCTION: MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5-one) is a newly-developed free radical scavenger which has been shown to reduce reperfusion injury in isolated perfused hearts. Here we studied the effect of MCI-186 on survival of fully-mismatched cardiac allograft in mice.

METHODS: CBA mice ($H2^k$) were given intravenous injection (single dose) of 10mg/kg MCI-186 or control saline the same day as transplantation of a heart from C57BL/10 mice ($H2^b$). A mixed leukocyte culture (MLC) assay was also performed.

RESULTS: Naive CBA mice rejected C57BL/10 cardiac grafts acutely (median survival time [MST], 9 days). The 10mg/kg of MCI-186 induced significant increase in graft survival (MST, 78 days), whereas control mice rejected their graft acutely (MST, 8 days). In MLC assay, addition of MCI-186 to MLC did not suppress cellular proliferation on alloimmune response. However, treatment of the recipients with MCI-186 inhibited the cellular proliferation, suggesting effect of MCI-186 to protect allograft without directly modulating immune systems.

CONCLUSION: Single dose of MCI-186 on the same day of transplantation induced significant prolongation of cardiac allograft survival.



Abstract# 1346

THE EXPRESSION OF APOPTOSIS GENES IS MODULATED BY THE ANTIFIBROTIC MOLECULE PIRFENIDONE IN CHRONIC CYCLOSPORINE NEPHROTOXICITY.

Fuad S. Shihab,[1] William M. Bennett,[2] Hong Yi,[1] Takeshi F. Andoh.[2] *[1]Division of Nephrology, University of Utah School of Medicine, Salt Lake City, UT; [2]Solid Organ and Cellular Transplantation Service, Legacy Medical Center, Portland, OR.*

We have previously shown that apoptosis plays a role in the progression of tubulointerstitial fibrosis (*Kidney Int* 1999; 56:2147-59) and that the antifibrotic molecule pirfenidone (PFD) ameliorates fibrosis by decreasing transforming growth factor (TGF)-β1 (*Am J Transplant* 2002; 2:111-9) in experimental chronic cyclosporine (CsA) nephrotoxicity. As a result, we decided to study the effect of PFD on the expression of apoptosis-related genes in this model. **Methods:** Salt-depleted pair-fed rats were administered vehicle (VH), VH+PFD 250 mg/kg/d, CsA 7.5 mg/kg/d or CsA+PFD. Physiologic and histologic changes were studied in addition to apoptosis by TUNEL. The mRNA expression of the pro-apoptotic genes p53 and Fas-ligand (L), and that of caspase 3 (CPP32) were studied by real-time PCR (LightCycler) and that of the anti-apoptotic gene Bcl-xL by Northern blot analysis. **Results**: Administration of PFD in CsA-treated rats significantly reduced the scores for tubulointerstitial fibrosis, tubular injury and calcinosis ($p < 0.01$ vs CsA). The number of apoptosis (+) cells was increased by CsA but significantly reduced by PFD (CsA+PFD 16±2 vs. CsA 68±19 per 20 fields at X200, $p < 0.01$ vs. CsA). The number of apoptotic cells significantly correlated with the fibrosis score ($r=0.85$, $p < 0.001$). The histologic changes and number of apoptotic cells were not affected by PFD in VH-treated rats.

	p53	Fas-L	Bcl-xL	CPP32
VH	0.73±0.06	0.55±0.03	0.33±0.01	0.31±0.01
VH+PFD	0.64±0.05	0.40±0.04	0.37±0.01	0.31±0.00
CsA	1.62±0.13*	1.08±0.19*	0.28±0.01*	0.60±0.01*
CsA+PFD	0.64±0.05#	0.32±0.02#	0.33±0.01#	0.33±0.01#

$p < 0.05$ vs *VH or #CsA, n=6/group, values are mean±SEM β actin or GAPDH corrected

The administration of PFD downregulated CsA-induced apoptosis by decreasing the expression of p53 and Fas-L (both of which were increased by CsA) and by increasing that of Bcl-xL (reduced by CsA). The net result was a decrease by PFD in the activity of caspase CPP32, previously increased by CsA. These changes were not observed in VH-treated rats. **Conclusion**: Thus, another mechanism for PFD's beneficial effect on fibrosis in chronic CsA nephrotoxicity may be through decreasing apoptosis. Whether or not this effect is related to the pro-apoptotic activity of TGF-β remains unclear and warrants further investigation.

Abstract# 1347

MYCOPHENOLATE MOFETIL SYNERGIZED WITH CD28/CD154 BLOCKADE IN PREVENTION OF SKIN ALLOGRAFT REJECTION.

Minh Diem Vu,[1] Yongsheng Li,[1] Terry B. Strom,[1] Xian C. Li.[1] *[1]Department of Medicine Harvard Medical School, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA.*

Introduction: T cells are essential in allograft rejection. Thus, blocking T cell activation and T cell proliferation remain a key strategy in preventing transplant rejection. Although CD28/CD154 costimulatory signals play an important role in T cell activation, blocking CD28 and CD154 costimulatory pathways is not effective in all transplant models. Mycophenolate mofetil (MMF) is an antagonist of the de novo pathway of purine biosynthesis, which is critical to lymphocyte proliferation. The aim of this study is to evaluate the effect of the CD28/CD154 costimulatory blockade and MMF treatment on the alloimmune response in vivo.

Materials and methods: Lethally irradiated BALB/c mice were adoptively transferred with 80-100 x 10^6 CFSE-labeled C57BL/6 spleen and lymph node cells. Recipient mice were treated with CTLA-4Ig/MR1 (0.5mg/each, i.p.) and/or MMF (100mg/kg/day, p.o.) on days 0, 1, 2, and proliferation of donor T cells in the allogeneic hosts was determined 3 days later by flow cytometry. Fully mismatched skin transplantation was performed using BALB/c ($H\text{-}2^c$) and C57BL/6 ($H\text{-}2^b$) mice as donors and recipients, respectively. CTLA-4Ig (0.25mg) and MR1 (0.2mg) costimulatory blockade were administered i.p. on days 0, 2, 4 and days 0, 2, 4, 6, respectively. MMF (100mg/kg) was given by daily gavage from day 0 to day 14. Rejection was defined as total absence of viable allograft tissue.

Results: In the in vivo allogeneic response, the CTLA-4Ig/MR1/MMF combined treatment markedly inhibited the proliferation of CFSE-labeled CD4+ and CD8+ T cells, while CD28/CD154 costimulatory blockade or MMF alone exhibited minimal effect in this regard. MMF was particularly potent in blocking CD8+ T cell proliferation in vivo. In addition, this combination significantly prolonged the skin allograft survival when compared with CD28/CD154 costimulatory blockade or MMF alone.

Treatment	Survival (days)	MST (days)
No treatment	7, 7, 7, 8, 8, 9	8
CTLA-4Ig/MR1	11, 14, 15	14
MMF	10, 12, 13	12
CTLA-4Ig/MR1/MMF	25, 43, 75, 75, 100, 105, 116	75

Conclusion: Our data demonstrate that the combined use of MMF and CD28/CD154 costimulatory blockade exhibits synergistic effect, and this protocol can inhibit both CD4+ and CD8+ T cell activation, and can induced long-term skin allograft survival.

Abstract# 1348

THYMOGLOBULIN SIGNIFICANTLY REDUCES THE RISK OF ACUTE REJECTION IN RIGHT LOBE LIVING-DONOR LIVER TRANSPLANT (LDLT) RECIPIENTS. M. S. Cattral,[1] I. McGilvray,[1] L. Adcock,[1] L. Lilly,[1] N. Girgrah,[1] G. A. Levy,[1] P. D. Greig,[1] D. R. Grant.[1] *[1]Multiorgan Transplantation Program, University Health Network, University of Toronto, Toronto, ON, Canada.*

Thymoglobulin (Thymo) has been reported to reduce the risk of rejection after transplantation, and may attenuate ischemic-reperfusion injury. The role of Thymo after LDLT, however, remains poorly defined. We compared the outcome of patients that received no polyclonal antibody induction therapy (Group 1; n=33) with those that received a course of Thymo (Group 2; n=33). **Methods:** Thymo was initiated within the first 6 hrs after LDLT and continued for 7-10 days; 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, 80%; group 2, 92%) or Neoral cyclosporine (Cya; group 1, 20%; group 2, 8%; p=NS). In group 2, initiation of Cya/Tac was routinely delayed to the 3rd -5th post-op day. Ganciclovir was given for 3 mo. to all CMV+ recipients that received Thymo and to all CMV-neg recipients of CMV+ grafts. All complications were recorded prospectively. **Results**: Demographic characteristics (age, sex, cause of liver disease) were similar in both groups. Median follow-up was 826 days in group 1 and 360 days in group 2 (P<0.01). The proportion of patients free of rejection during the first 6 months was 60% in group 1 and 94% in group 2 (p <0.001 by Log rank test). Six patients in group 1 had steroid-resistant rejection requiring OKT3 (n=5) or thymo (n=1); all 6 of these patients were receiving tacrolimus. All rejection episodes in group 1 were mild and treated successfully with steroids. Thymo was well-tolerated in all patients. There was no significant difference in the incidence of bacterial or CMV infection in the two groups. One patient in group 2 that acquired EBV from the graft developed PTLD, which has been treated successfully. Among Hepatitis C virus infected (HCV)-patients the incidence and severity of recurrent HCV was similar in group 1 and 2. Patient and graft survival at 2 years was 83% and 81% in group 1, and 83% and 83% in group 2, respectively. Causes of death in group 1 were small graft syndrome (n=2), myocardial infarction (1), and sepsis (2); and in group 2 were hemorrhage (1), recurrent hepatoma (1), and sepsis (3). **Conclusions.** Thymo significantly reduces the risk of acute rejection in LDLT recipients without increasing the risk of infectious complications. Reducing the risk of rejection may be of particular value in recipients of marginal size grafts in whom rejection may compromise function, impair regeneration, and promote graft failure.

Abstract# 1349

USE OF A HUMANIZED MONOCLONAL ANTIBODY (BASILIXIMAB) FOR INDUCTION THERAPY REDUCES THE RATE OF ACUTE REJECTION BUT DOES IT ALSO INCREASE THE RISK OF SERIOUS INFECTIONS POST LIVER TRANSPLANT IN CHILDREN? Kathleen P. Falkenstein, Stephen P. Dunn, Adela Casas, Louise Flynn. *Solid Organ Transplant, AiDupont Hospital, Wilmington, DE; Scahool of Allied Health, Drexel, Phila, PA.*

Long term graft survival is dependent on prevention of early and late graft loss from acute or chronic rejection. Children post transplant pose an enormous challenge because they often require a higher per kg dose of immunosuppression to maintain therapeutic drugs which increases their risk of acquiring viral infections (EBV, CMV, RSV). The purpose of this study was to see if the use of Basilximab induction not only reduced the rate of early acute rejection in pediatric liver transplant children but did not increase the rate of serious viral infections

We evaluated 74 children from January, 1998 to November 2003. All children received standard induction with Basilximab along with Neoral, Tacrolimus, and steroids. A retrospective chart review looked at age at transplant, disease, type of transplant, rejections, treatment of rejection, rehospitalizations, infections, immunosuppressive medications at transplant and current drug therapy. All rejections were biopsy confirmed and infections were also confirmed.

The results of our review were as follows: at time of transplant, 13/74 (17%) of children were in the ICU. Age at time of transplant was 1mth -20 yrs (mean 5.4 yrs). Reason for transplant was as follows: BA (29), Alpha I (2), Alagille's (4), Autoimmune(3), Metabolic (4), Carcinoma (7), liver/kidney (4), other (11), unknown(4) and retransplant (4). Type of transplant, living related (30), LLS (17) RL (3), whole (21) and liver/Kidney (4). Immunosuppressive at time of transplant was Tacrolimus (41) and Neoral (33) and currently, Tacrolimus (55), Neoral (8) Rapamune (3) steroids (1). Early rejection occurred in 7/74(12%). Incidence of infection was CMV 12/74 (17%), EBV 13/74(17%) and RSV (1).Post-operative complications HAT (3), PVT (4), BS (4), Bowel perforation (2) Pseudotumor cerebri (1).

In conclusion we found the use of a Basiliximab for induction therapy decreases the risk of acute rejection but increases the rate of infection especially EBV and CMV. As we enter an era of new and more potent immunosuppressive agents that are more effective in preventive early rejection and increasing long term graft survival we will face new challenges such as more effective mechanisms to diagnose and treat viral infections. Ongong surveillance utilizing PCR and other early detection methods should become routine in all transplant centers.

Abstract# 1350

COMPARISON OF ACUTE REJECTION BETWEEN WHOLE LIVER GRAFTS AND ADULT PARTIAL LIVING DONOR GRAFTS AND CADAVERIC SPLIT LIVER GRAFTS. J. Keith Melancon,[1] William D. Payne,[1] Rainer W. Gruessner,[1] Raja Kandaswamy,[1] John R. Lake,[2] Miguel Tan,[1] Angelika C. Gruessner,[1] Abhinav Humar.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN; [2]Medicine, University of Minnesota, Minneapolis, MN.*

Background: Because recent studies have shown a higher incidence of acute rejection in partial liver grafts, living donor liver grafts, and split cadaveric donor grafts, we decided to compare the incidence of acute rejection in our partial liver grafts and cadaver split grafts compared to whole liver grafts. We studied the outcome of all our liver transplants from 1999 when we began doing living donor adult partial liver transplants and adult cadaveric split liver transplants.

Results: In 4 years we have performed 137 whole liver transplants, 27 adult partial living donor grafts, and 24 adult cadaveric split liver grafts. At 4 years, patient survival for a living donor liver transplants is 96%; survival in the split cadaver graft group is 80%, and survival in the whole liver graft group and 68%. The groups were equally matched by age, gender, type of immunosuppression, and average MELD score. There were no significant differences in acute rejection (p=0.2902).

Demographics of Liver Tx Patients

Characteristic	Whole Liver (137)	Living Donor (27)	Cadaver Split (24)
Hepatitis C	25%	36%	10%
Survival	68%	96%	80%
Median Age	51.8	52.8	53.7
Gender M/F	90/47	16/11	14/10
Median MELD	17.5	130.0	16.0
Acute Rejection	32%	11%	25%

When we stratified acute rejection into subgroups of grade, early vs. late, steroid resistant, and recurrence after treatment, we noted a trend toward less severe rejection in the living donor group compared to the whole cadaver organ and split cadaver organ groups (p=0.08).

Subgroup Analysis of Acute Rejection

Outcome	Whole Liver	Living Donor	Cadaver Split
Early Rejection	77%	100%	83%
Mild Rejection	73%	100%	50%
Steroid Resistant	14%	0%	50%
Multiple Rejections	25%	0%	17%

Conclusion: In our series we found no differences between whole organ, split, or LDLTxs regarding the incidence of rejection episodes. But we noted a lower incidence of steroid resistant and multiple rejection episodes in LDLTxs. The advantage of less cold ischemia time and better immunologic matching appears to be more crucial than liver volume.

Abstract# 1351

SEQUENTIAL DETERMINATION OF PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF MYCOPHENOLATE MOFETIL (MMF) IN LIVER TRANSPLANT (LT) PATIENTS. Merce Brunet,[1] Jaume Martorell,[2] Elena Vidal,[1] Olga Jimenez,[1] Olga Millan,[2] Isabel Rojo,[2] Isabel Cirera,[3] Antoni Rimola.[3] *[1]Department of Toxicology; [2]Department of Immunology; [3]Liver Unit, Hospital Clinic, IDIBAPS, Barcelona, Spain.*

Background/Aim: MMF is increasingly used in LT patients, mainly because of adverse effects of other immunosuppressive drugs. Nevertheless, little is known about PK and PD of MMF in these patients. As a consequence, most protocols include fixed doses of MMF. Therefore, we sequentially determined the PK and PD of mycophenolic acid (MPA, the active metabolite of MMF) in LT patients to assess whether MPA monitoring could be advisable after LT. **Methods:** a) 15 LT recipients treated with MMF (1g bid, orally), tacrolimus and daclizumab were included in the study; b) 12-hour plasma MAP PK profile after one dose of MMF was determined on days 6, 10 and 16, and at months 3 and 6; c) PD (assessed by the inhibitory capacity of serum MPA on the proliferation of CEM cells, a cell line insensitive to other immunosuppressants, and expressed as the % CEM proliferation) was also determined in 7 of these patients on day 6 and at months 3 and 6, before and 1 hr after one dose of MMF. **Results:** A great interindividual variability was observed in MPA PK profiles at any time. Although there was a gradual increase in MPA AUC and C-min throughout the study, a high proportion of patients had AUC below the recommended range (30-60 µg.h/mL): 85% on day 6, 92% on day 10, 64% on day 16, 30% at month 3 and 38% at months 6, with similar percentages in relation to recommended ranges of C-min (1.0-3.5 µg/mL). An acceptable inverse correlation (r^2= -0.754). was observed between plasma MPA concentrations and % CEM proliferation. When AUC and C-min were within the recommended ranges, % CEM proliferation was low for almost all samples, but when these PK parameters were below the recommended ranges, % CEM proliferation was very variable and, therefore, unpredictable. No relationship between MPA PK and

efficacy of MMF could be established because only 1 patient developed rejection, probably due to the concomitant administration of tacrolimus and daclizumab. Gastrointestinal symptoms were the only side-effects attributed to MMF which showed a significant relationship with MPA PK. **Conclusions:** 1) MPA monitoring seems to be necessary in LT patients treated with MMF, at least during the first postoperative months, and 2) the dose of 1 g bid of MMF, habitually administered after LT, could be insufficient to maintain an adequate level of immunosuppression during the periods in which patients could not concomitantly receive other powerful immunosuppressants.

Abstract# 1352

ALTERATIONS IN CYTOCHROME P450 ACTIVITY WITH HERBALS: A NEW PARADIGM FOR THE MANAGEMENT OF IMMUNOSUPPRESSION. Kenneth D. Chavin,[1] G. Mark Baillie,[1] Jennifer L. Donovan,[1] C. Lindsay DeVane,[1] Robin M. Taylor,[1] Ying Ruan,[1] Jun-Sheng Wang,[1] John S. Markowitz.[1] *[1]Department of Surgery, Division of Transplant, Medical University of South Carolina, Charleston, SC.*

Herbal preparations are increasingly used concomitantly with conventional prescription and over-the counter medications. Numerous studies and case reports have documented the importance of phytochemical-drug interactions.

CYP3A4 and CYP2D6 account for the oxidative metabolism of >80% of all presently marketed medications including many antimicrobials, and immunosupressants. Inhibitory effects on CYP system can lead to adverse events or toxicity. Conversely, CYP induction increases drug clearance and can lead to therapeutic failure. Our objective was to develop a screening tool to determine the potential of popular herbal preparations for their ability to inhibit or induce the activity of CYP3A4 and CYP2D6: St. John's wort (Hypercium perforatum), Siberian ginseng (Eleutherococcus senticosus), Ginkgo biloba and Saw Palmetto (Serno repens).

METHODS: Herbal products were characterized for their major phytochemical constituents prior to study initiation and doses were within generally recommended ranges. Each herbal product was assessed in 12-14 normal volunteers. Specific probe drug substrates for CYP3A4 (alprazolam (APZ); 2 mg) and CYP2D6 (dextromethorphan (DM) 100 mg) were administered at baseline and after 14 days of treatment with the herbal product. The plasma pharmacokinetics and urine concentrations of APZ and DM were determined to indicate degree of enzyme inhibition or induction. RESULTS: Saw Palmetto and Siberian ginseng did not have signficant effects on CYP3A4 or CYP2D6 activity. Ginkgo biloba had marginal, but unlikely clinically significant, effects on CYP3A4 activity. Ginkgo biloba did not significantly affect CYP2D6 activity. St. John's wort significantly decreased the area under the alprazolam plasma concentration vs. time curve ($p<0.0001$) and decreased the elimination half-life from 12.4 ±2.4 hours ($p<0.0001$). CONCLUSION: These results suggest that of the 4 herbal preparations assessed to date, none will result in interactions with CYP2D6 substrates. Only St. John's wort, due to its strong inductive effects on CYP3A4 activity, is likely to result in diminished clinical effectiveness of immunosupression or increased dosage requirements for calcineurin inhibitor-based immunosupression.

Abstract# 1353

THE ROLE OF BONE MARROW INFUSIONS IN WITHDRAWAL OF IMMUNOSUPPRESSION IN ADULT LIVER ALLOTRANSPLANTATION. Panagiotis Tryphonopoulos,[1] Debbie Weppler,[1] Tomoaki Kato,[1] Juan R. Madariaga,[1] David M. Levi,[1] Seigo Nishida,[1] Jang Moon,[1] Gennaro Selvaggi,[1] Arie Regev,[3] Pablo Bejarano,[2] Amr Khaled,[2] Joshua Miller,[1] Phillip Ruiz,[1,2] Camillo Ricordi,[1] Andreas G. Tzakis.[1] *[1]Surgery-Transplant, University of Miami School of Medicine, Miami, FL; [2]Pathology, University of Miami School of Medicine, Miami, FL; [3]Hepatology, University of Miami School of Medicine, Miami, FL.*

Introduction: We infused unmodified cadaveric donor bone marrow at the time of transplantation in order to increase chimerism and hopefully induce tolerance in liver recipients.

Materials and methods: We attempted withdrawal of immunosuppression in patients that were at least 3 years post-transplantation, without history of autoimmune disease and no rejection for at least a year before entering the study. Patients were accepted irrespective of BM infusions. Immunosuppression was reduced by 1/3 upon enrollment in the study, an additional 1/3 at 12 months and completely at 24 months. Patients with rejection, disease recurrence, or non-compliance with the follow-up were removed from the study. In addition to routine follow up a liver biopsy and chimeric studies were performed at every phase of the study and 6 months following complete withdrawal.

Results: One hundrend and seven patients entered the study. Forty six had donor bone marrow infusions (5X108 cells/kg) at the time of transplantation (group A) and 61 had not (group B).

Follow-up is till November '03: Four patients died (group A: n=3, group B: n=1) due to cardiac death (n=1), complications of biliary surgery (n=1), dialysis (n=1), and chronic rejection (n=1).

Withdrawal of immunosuppression was stopped in 84 patients: (34 (74%) from group A and 50 (82%) from group B) due to rejection (n=75), non-compliance (n=7), or hepatitis-C recurrence (n=2). Two patients (group A) developed chronic rejection. One died as previously mentioned and the second lost her graft and was retransplanted. Twenty one patients, 11 (24%) from group A and 10 (16.5%) from group B, remain immunosuppression-free for 3 to 34 months (average 21.8 months).

Conclusion: Successful withdrawal of immunosuppression is possible in adult liver allotransplantation in approximately 20% of the patients. Donor bone marrow infusion does not seem to induce tolerance in this setting.

Abstract# 1354

ADJUSTING CYCLOSPORINE DOSE TO ACHIEVE C_2 TARGET RANGE: EVIDENCE FROM A PROSPECTIVE, MULTICENTER TRIAL IN LIVER TRANSPLANTATION. Stephen Pollard,[1] Federico Villamil.[2] *[1]Department of Liver Transplantation, St James's University Hospital, Leeds, United Kingdom; [2]Liver Unit, Fundacion Favoloro, Buenos Aires, Argentina.*

C_2 monitoring of cyclosporine microemulsion (CsA-ME) provides clinical benefits compared to C_0 monitoring in de novo liver transplant recipients. However, CsA-ME absorption after liver transplantation can be impaired by factors such as paralytic ileus, cholestasis and clamping of external biliary drainage that do not affect renal transplant patients. This analysis compared CsA-ME absorption following liver and renal transplantation. **Methods:** Data was obtained from two multicenter, prospective, randomized trials. The LIS2T study included 499 de novo liver transplant patients randomized to CsA-ME C_2 monitoring (n=250; C_2 target for months 1-3: 0.8-1.2µg/mL) or tacrolimus C_0 monitoring (n=245), with or without azathioprine. All patients received steroids and were followed for 6 months. The MO2ART study included 299 de novo renal transplant patients managed by CsA-ME C_2 monitoring (C_2 target for month 1, 1.6-2.0µg/mL; month 2, 1.4-1.6µg/mL; month 3, 1.2-1.4g/mL). Absorption of CsA-ME was evaluated by C_2 level per 1mg/kg/day CsA-ME at different time points. The number and cause of dose changes in the LIS2T study was also analyzed. **Results:** The ratios of C_2 per 1 mg/kg/day in liver and renal transplant recipients are described in the table. The mean number of dose changes per patient in the CsA-ME group of the LIS2T study was 19 (median 17, range 1-62) and in the tacrolimus group it was 16 (median 15, range 1-58), with no difference in proportion of patients receiving one or more dose changes to achieve target level or due to adverse events, unsatisfactory effect, drug intolerance or other reasons. In the CsA-ME and tacrolimus cohorts in LIS2T, 6.4% and 2.0% of dose changes were <5% of total dose, respectively. **Conclusions:** CsA-ME absorption in the early post-transplant phase is markedly lower in liver transplant patients compared to renal transplant recipients. Absorption of CsA-ME continues to improve until the end of month 2 following liver transplantation, whereas in renal transplantation absorption plateaus by the end of month 1. Dose changes should take this increase in absorption into account in order to avoid overshooting the pre-defined C_2 target range. Use of C_2 monitoring does not affect the number or cause of dose changes compared to tacrolimus C_0 monitoring. A greater proportion of dose changes <5% total dose were performed in the CsA-ME group and may be clinically irrelevant.

C_2 per 1mg/kg/day CsA-ME

	Day 2	Day 3	Day 5	Month 1	Month 2	Month 3
Liver	29	36	50	137	193	196
Kidney	95	132	156	248	251	272

Abstract# 1355

MATHEMATICAL MODELING TO PREDICT RENAL FUNCTION AND GRAFT SURVIVAL BASED ON 5 YEAR DATA OF PEDIATRIC RENAL TRANSPLANT PATIENTS WITH MYCOPHENOLATE MOFETIL (MMF) VERSUS AZATHIOPRIN (AZA) BASED IMMUNOSUPPRESSION. Therese Christina Jungraithmayr,[1] Burkhard Tönshoff,[2] Lothar Bernd Zimmerhackl,[1] for the German MMF Study Group.[1,2] *[1]Pediatrics, Leopold-Franzens-University, Innsbruck, Austria; [2]Pediatrics, Ruprecht-Karls-University, Heidelberg, Germany.*

Introduction: Short term results for graft survival after renal transplantation (RTx) are improving with new immunosuppression protocols and quality measures. MMF has been shown to reduce acute rejection episodes (ARE) within 3 years compared to an AZA based protocol (Staskewitz 2001; Jungraithmayr 2003). How to best predict long term graft survival and to differentiate a good from a minor good functioning graft and thus quality of life is unclear.

Patients and Methods: In an open prospective multicenter study 86 pediatric patients (P) transplanted between 1996-9 using MMF, neoral and prednisone as immunosuppression (MMF-group) were compared retrospectively to 54 P transplanted between 1990-6 treated with AZA (AZA-group) without induction therapy. Demographic data and number of HLA mismatches were comparable.

Results: Five years after RTx graft survival was 89.8% in the MMF and 73.1% in the AZA group ($p<0.05$). Half lives of graft survival according to Kaplan-Maier were 29,4 years in the MMF compared to 23,6 in the AZA group. Glomerular filtration rate (GFR) as parameter of renal function was calculated by the Schwartz formula. For the prediction of the quality of graft function in the long term a mathematical model was used including an exponential, a linear and a 1/ creatinine calculation. A GFR of 10ml/min/1.73sqm and 1/creatinine of 0.1 mg/dl, respectively were assumed as loss of renal function. A GFR of ≥50ml/min/sqm respectively ≥0.5 mg/dl was defined as good renal function without the need of supportive medication. Loss of function occurred after 13.5 (linear model)/29.5 years (exponential model) in the MMF group and after 10.3 /21.8 years in the AZA group, respectively. Living with a good functioning graft also lasted longer in the MMF group compared to the AZA group. According to 1/creatinine function was lost after 14.5 years in the MMF and after 6 years in the AZA group.

Conclusion: Compared to the half life calculation according to Kaplan Maier these calculation models seem to be more realistic and allow to differentiate between a graft survival with good renal function and a graft survival close to dialysis. However, instead of graft survival, survival with graft function with a GFR≥50 ml/min/1.73sqm should be calculated. This value would be a better predictor for long term quality of life, at least in pediatric patients.

Abstract# 1356

SUCCESSFUL WITHDRAWAL OF STEROIDS IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS ON CYCLOSPORIN A AND MYCOPHENOLATE MOFETIL - 4-YEAR FOLLOW-UP. Britta Höcker,[1] Ulrike John,[2] Christian Plank,[3] Elke Wühl,[1] Lutz T. Weber,[1] Joachim Misselwitz,[2] Wolfgang Rascher,[3] Otto Mehls,[1] Burkhard Tönshoff.[1] *[1]Department of Pediatrics, University Hospital, Heidelberg, Germany; [2]Department of Pediatrics, University Hospital, Jena, Germany; [3]Department of Pediatrics, University Hospital, Erlangen, Germany.*

The addition of mycophenolate mofetil (MMF) to a cyclosporin A (CsA)-based immunosuppressive regimen after renal transplantation may allow steroid withdrawal and, hence, amelioration or avoidance of steroid-specific side effects. We therefore analyzed in a retrospective case control study over a mean follow-up period of 45.6 ± 2.3 months the safety and efficacy of steroid withdrawal in 40 children after renal transplantation (RTx) (aged 11.4 ± 4.9 years) on CsA, MMF and prednisone (mean dose 4.3 ± 0.9 mg/m²); a matching concurrent group (criteria: age at RTx, age and Tx function) served as control. Both cohorts were comparable regarding gender, number of RTx from living or cadaveric donors, and HLA mismatches.

Parameter	Steroid withdrawal (n=20)		Control group (n=20)	
	baseline	last follow-up	baseline	last follow-up
CCr (ml/min/1.73 m²)	94.5 +/- 6.2	87.4 + /- 6.5**	97.5 +/- 6.1	80.0 +/- 6.7
MAP SDS	1.62 +/- 0.33	0.74 +/- 0.33*##	1.56 +/- 0.40	2.31 +/- 0.36
Antihypertensive drugs (N)	2.00 +/- 0.33	1.35 +/- 0.33*	1.50 +/- 0.26	1.40 +/- 0.29
Height SDS§	-2.24 +/- 0.38	-0.77 +/- 0.38**#	-2.37 +/- 0.38	-2.11 +/- 0.48
Height SDS$	-1.33 +/- 0.38	-1.09 +/- 0.40	-0.90 +/- 0.34	-0.90 +/- 0.35
BMI SDS	0.87 +/- 0.31	0.45 +/- 0.30*	0.18 +/- 0.21	0.34 +/- 0.30

CCr, calculated creatinine clearance; MAP, mean arterial blood pressure (BP); §only in pre-pub. pat. (n = 12); $only in pub. pat. (n = 28); BMI, body mass index; SDS, standard deviation score. *P<0.05 vs. baseline; **vs. control; #P<0.01 vs. baseline; ##vs. control.

No acute rejections (AR) occurred in the steroid withdrawal group, 3 borderline ARs and 1 AR grade I (Banff classification) in the control group. *Conclusions:* This study suggests that the addition of MMF to a CsA-based immunosuppressive regimen in pediatric renal transplant recipients has increased significantly the safety of steroid withdrawal in this patient population. Steroid withdrawal was associated with a marked catch-up growth in the presence of stable graft function, a significant reduction in cushingoid appearance and a reduced need for hypertensive medication.

Abstract# 1357

RECURRENCE OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS) FOLLOWING PEDIATRIC KIDNEY TRANSPLANTATION IN THE MODERN IMMUNOSUPPRESSION ERA: A REPORT FROM THE NORTH AMERICAN PEDIATRIC RENAL TRANSPLANT COOPERATIVE STUDY (NAPRTCS). Sharon M. Bartosh,[1] Donald M. Stablein,[2] Richard N. Fine.[3] *[1]Pediatrics, University of Wisconsin, Madison, WI; [2]EMMES Corporation, Rockville, MD; [3]Pediatrics, State University of New York at Stony Brook, Stony Brook, NY.*

FSGS is the etiology of 10-15% of pediatric end stage renal failure. Following transplantation, recurrence of FSGS accounts for a significant percentage of graft loss. In a report from NAPRTCS over a decade ago, prior to the use of mycophenolate mofetil or IL-2 receptor antibodies, the recurrence rate was found to be 20%. The aim of this study was to determine whether newer immunosuppression regimens have had an impact on recurrence rates. 218 children have been identified in the NAPRTCS database since 1998 who have been transplanted with modern immunosuppression regimens. In a preliminary report of 72 of these patients, 29% had recurrence. The recurrence rates did not differ by prior dialysis status, gender or donor source (38% LD vs 23% CAD) but recurrence did tend to decrease with increasing recipient age. The recurrence rates were 44%, 41% and 19% for the < 6, 6-12 and ≥ 13 yr olds respectively ($p = 0.1$ by logistic regression). Recurrence rates were 35%, 22% and 17% for white, black and hispanic patients respectively. Recurrence was not significantly affected by choice of calcineurin inhibitor (CyA 35%, tacrolimus 26%) or choice of induction agent (polyclonal antibody 31%, IL 2 receptor antibody 32%). Graft failure rates were 14% in patients without recurrence and 29% in patients with recurrence (P=0.14). 5 of 6 graft failures in the patients with recurrence were attributed to recurrence (6th attributed to non adherence). In conclusion, it does not appear that current immunosuppressive regimens have altered the rate of recurrence of FSGS after pediatric kidney transplantation. Although not statistically significant, a trend was seen toward increased recurrence in white patients, in recipients of living donor grafts, in those patients treated with CyA and in younger patients.

Abstract# 1358

CORRELATES OF NON-ADHERENCE FOR ADOLESCENTS ONE YEAR AFTER A KIDNEY TRANSPLANT. Nataliya Zelikovsky,[1] Aileen Walsh,[2] Kevin Meyers.[1] *[1]Department of Pediatrics, Nephrology Division, The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA; [2]Department of Psychology, The Children's Hospital of Philadelphia, Philadelphia, PA.*

A. To examine the demographic, treatment related, and regimen factors associated with non-adherence for adolescent kidney transplant recipients one year after their transplant.

B. Participants completed the Medication Adherence Measure (MAM), a semi-structured interview assessesing self-reported medication adherence. Subjects were asked to describe their medication regimen and to report the number of doses taken, taken late, or missed the week prior to the interview to obtain a comprehensive adherence profile. Non-adherence scores were calculated by dividing the number of missed doses by the number of doses the patient was prescribed for each medicine and an average across all medicines in the regimen. Adherence scores for doses taken and taken late were similarly calculated.

In addition to self-report, a Biomedical Marker Score was obtained. A nephrologist rated adherence by comparing blood serum levels of immunosuppressant medications to the expected range. When the actual lab value fell within 80 to 120 percent of the expected range, an adherence score of 0 was assigned. Lab values 40 to 80% and 0 to 40% of the expected range were assigned scores of –1 and –2, respectively.

C. 21 adolescents (mean age = 16, 86% male, 71% Caucasian) participated in this study. 46% of this sample reported at least some non-adherence; 19% reported missing more than 10% of their prescribed doses during the referent week. Medication non-adherence was positively correlated with patient age, with older patients more likely to be non-adherent. Non-adherence was also associated with patient ethnicity, with Caucasian patients being more adherent than minority patients. For treatment related variables, non-adherence was associated with being older at the time of transplant and with having been on dialysis during prior to transplant. Regimen variables associated with non-adherence included being on more medications and having a higher number of prescribed doses per week. Self-reported non-adherence was positively correlated with nephrologist rated non-adherence based on Tacrolimus serum levels, but patient report revealed more non-adherence than did ratings based on blood serum levels.

D. The results suggest several risk factors for adolescent non-adherence after transplant

including demographic, treatment related, and regimen variables. These results also indicate that adolescents may be fairly accurate reporters of their own non-adherence if questions are posed in the right way. Implications for interviewing patients about adherence are discussed.

Abstract# 1359

LYMPHOPENIA AS A PREDICTOR OF RISK OF VIRAL DISEASE VERSUS SUBCLINICAL INFECTION. Jodi Smith,[1] Carrie Gordon,[1] Connie Davis,[3] Patrick Healey,[2] Ruth McDonald.[1] *[1]Pediatric Nephrology, University of Washington, Seattle, WA; [2]Surgery, University of Washington, Seattle, WA; [3]Nephrology, University of Washington, Seattle, WA.*

Background: More potent immunosuppressive agents are associated with an increased risk of viral-associated morbidity. Post-transplant viral surveillance identifies viral load but its ability to predict likelihood of developing viral disease *versus* remaining asymptomatic with subclinical infection is limited. Such a tool could guide use of pre-emptive therapy. In the HIV/AIDS population, lymphopenia is a well-established marker of infection risk.

Methods: We undertook a retrospective case-control study of pediatric renal transplant recipients to evaluate the temporal relationship between lymphopenia (absolute lymphocyte count < 1000 cells/mm³) and subsequent development of viral disease vs. subclinical infection. Cases were patients with primary CMV or EBV disease, defined as new detection of viral DNA by real-time quantitative PCR (DNAemia) associated with clinical symptoms. Controls were patients with subclinical CMV or EBV infection, defined as asymptomatic DNAemia. Cases and controls were matched by donor and recipient EBV and CMV status and time post-transplant. Only subjects with monthly viral surveillance for the 4 months prior to diagnosis were included to reduce the possibility that lymphopenia was viral-mediated. Measures of all absolute lymphocyte counts (ALC) available in the 8 weeks prior to viral diagnosis were used.

Results: Four cases and 8 controls were available for analysis. Characteristics of cases and controls were similar with respect to time post-transplant (mean: 8 m vs. 10 m) and CMV and EBV donor and recipient status (CMV D+/R-: 3/4 vs. 6/8 and EBV D+/R-: 3/4 vs. 7/8). The mean ALC in the 8 weeks prior to diagnosis of viral disease was 915 cells/mm³ (± 184) compared to 2350 cells/mm³ (± 450) in those with subclinical infection ($p<0.001$).

Conclusion: Patients with viral disease were significantly more likely to have lymphopenia in the 8 weeks prior to diagnosis compared to those with subclinical infection. We conclude that lymphopenia may be an important predictor of a patient's risk of developing viral disease.

Absolute lymphocyte count and viral infection/disease

Case	Mean ALC (cells/mm³) in 8 wks prior to dx (SD)	Viral disease
1	690 (245)	Primary CMV
2	982 (438)	Primary EBV
3	863 (159)	Primary EBV
4	1125 (538)	Primary CMV
Control		**Subclinical infection**
1	1753 (309)	Primary CMV
2	2352 (611)	Primary CMV
3	3102 (345)	CMV reactivation
4	2813 (405)	EBV reactivation
5	2467 (299)	Primary CMV
6	1890 (178)	Primary EBV
7	2109 (375)	CMV reactivation
8	2312 (487)	Primary CMV

Abstract# 1360

UNEXPECTEDLY HIGH PREVALENCE OF PRE-TRANSPLANT ABNORMAL GLUCOSE TOLERANCE IN PEDIATRIC KIDNEY TRANSPLANT RECIPIENTS. Seiichiro Shishido,[1] Hiroyuki Satoh,[1] Hiroshi Asanuma,[1] Hiroshi Hataya,[1] Kenji Ishikura,[1] Masahiro Ikeda,[1] Akira Hasegawa.[2] *[1]Urology and Kidney Transplantation, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose, Tokyo, Japan; [2]Nephrology, Toho University, Ohmori, Tokyo, Japan.*

Background. New-onset diabetes has been described in pediatric transplant recipients, with reported incidence of up to 20%. In children, tacrolimus appears to be particularly associated with an increased risk for diabetes after transplantation. However, limited information is available regarding the incidence and features of pre-transplant status of abnormal glucose tolerance in pediatric kidney transplant recipients.

Methods. To determine a patient's risk for developing post-transplant diabetes mellitus (PTDM) in children, an oral glucose tolerance tests (OGTT) was performed in 53 non-diabetic pediatric recipients before transplantation between August 1999 and January 2003. The subjects were classified into three groups by World Health Organization criteria on the basis of OGTT. These patients received either a ciclospoline (CsA)-based or a tacrolimus (FK)-based immunosuppressive regimen post-transplantation. However, the recipients who showed abnormal glucose tolerance in the pre-transplant OGTT chose the CsA-based regimen.

Results. The mean age of 53 patients was 9.7 ± 5.4 years (range 2.5-18 years), with 35 males and 18 females. The mean post-transplant follow-up was 27 ± 14 months. Fasting plasma glucose level before transplantation was within normal limit (<110 mg/dl) in all patients. The mean BMI was 16.5 ± 3.3 kg/m². However, 14 of 53 patients (26.4%) revealed abnormal glucose tolerance in the pre-transplant OGTT: 10 (18.9%) with impaired glucose tolerance (IGT), and 4 (7.5%) with DM. Thirty-one patients (58.4%) received the CsA-based immunosuppression, while 22 recipients (41.5%) had the FK-based regimen post-transplantation. PTDM defined as >2 weeks of insulin therapy after renal transplant developed in 2 patients. OGTT of the 2 patients showed normal glucose tolerance before transplantation. On the contrary, 14 patients with abnormal glucose tolerance did not develop PTDM under CsA-based immunosuppression.

Conclusion. Our results demonstrated that the unexpectedly high prevalence of abnormal glucose tolerance was observed in the pre-transplant OGTT even in a pediatric population. Identification of patients with pre-transplant risks might allow modification of post-transplant immunosuppression with non-diabetogenic agents.

Abstract# 1361

GRAFT THROMBOTIC RISK FACTORS IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS. Laura Massella,[1] Antonia Legato,[1] Luca Dello Strologo,[1] Erica De Candia,[2] Raffaele Landolfi,[2] Gianfranco Rizzoni.[1] *[1]Division of Nephrology and Dialysis, Bambino Gesù Children's Hospital and Research Institute, Rome, Italy; [2]Center of Hemorragic and Thrombotic Diseases, A.Gemelli, Catholic University of Sacred Heartt, Rome, Italy.*

Acquired or inherited thrombophilic factors may predispose renal transplant to thrombotic events with graft loss. In addition, inherited thrombophilia was found significantly associated with acute rejection and transplant loss within the first year after transplantation. To our knowledge, pediatric data are not reported. AIM To evaluate the contribution of clinical and laboratory risk factors to acute vascular graft thrombosis. PATIENTS and METHODS We studied 101 pts. who underwent 109 renal transplants between 1993 and 2003. Up to now, we have investigated 78/101 pts. (73.5%) for protein C and protein S deficiencies, factor V Leiden, prothrombin G20210A mutations, antiphospholipids antibodies (APAs) and prolonged aPTT-LAC dependent. About clinical risk factors, we evaluated the presence of early onset (within 1st year of life) or prolonged nephrotic syndrome (NS), previous arterious-venous fistula (FAV) thrombosis, multiple graft vessels and donor/recipient body weight (BW) ratio, considering a value < 0.8 as index of inadequate donor-recipient size matching (M. Cardillo, ESOT 2003). Donor/recipient BW ratios are available only in 64/101 pts. at the moment. Statistical analysis: X-square test. RESULTS Thrombofilic screening showed 8/78 (10.2%) abnormalities (3 protein S deficiences – 1 acquired, 2 factor V Leiden, 1 G20210A mutation, 2 prolonged aPTT- LAC dependent, 1 APAs).

Graft Thrombosis	N	Previous AVF thrombosis	Early onset NS	Multiple graft vessels	Don. BW/Rec. BW<0.8	Thromboph. . screening abnorm.
Yes	8	3/8(38%)	3/8(38%)	2/8(25%)	6/8(75%)	3/8(38%)
No	101	6/101(6%)	7/101(6.9%)	10/101(10%)	10/64(16%)	5/78(6.4%)
		p<0.01	p<0.01	n.s.	p<0.001	p<0.01

CONCLUSIONS Our data demonstrate a significative association between graft thrombosis and all the clinical and laboratory risk factors considered, except graft multiple vessels. The identification of risk factors might be of value to start individualized anticoagulation strategies.

ADULT LIVING DONOR AND SPLIT LIVER TRANSPLANT: OUTCOMES AND INNOVATIONS

Abstract# 1362

MELD SCORE AND PREOPERATIVE VARIABLES AS PREDICTORS OF THE OUTCOMES AFTER ADULT-TO-ADULT LIVING DONOR LIVER TRANSPLANTATION. Ichiro Tsunematsu,[1] Yasuhiro Ogura,[2] Koichi Kozaki,[2] Kayoko Inoue,[3] Akio Koizumi,[3] Koichi Tanaka.[2] *[1]Department of General and Gastroenterological Surgery, Osaka Medical College, Osaka, Japan; [2]Department of Transplantation and Immunology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Department of Health and Environmental Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan.*

Background:
MELD scores have been used as indicator for cadaveric liver allocation in the United States. Recently, the applicability of MELD score as predictor of the outcomes after liver transplantation was reported. It is, however, controversial whether MELD score can be applied as indicators for the outcomes after adult-to-adult living donor liver transplantation (ALDLT). In this study, we tested whether MELD score and other preoperative variables can predict short term mortality after ALDLT.
Methods:
Between November 1994 and April 2003, 319 patients underwent ALDLT at our institute. A total of 274 recipients were enrolled, after excluding the cases requiring special transplant procedures, such as auxiliary partial orthotopic liver transplantation, Domino transplantation, Dual liver transplantation and re-transplantation. We defined a case who died within 90 days after ALDLT. All recipients were categorized into three groups according to MELD score, which were calculated by laboratory data at the time of ALDLT: Group A (n=103): MELD score ≤18; Group B (n=71): 19≤MELD≤24; and Group C (n=100): 25≤MELD. MELD groups, as well as 21 preoperative variables, such as etiology, blood mismatch, ascites, encephalopathy, and laboratory data, were analyzed by Cox proportional hazard regression method.
Results:
A total of 55 patients (20.1%) died within 90 days after ALDLT. Twelve patients (11.7%) died in Group A, 12 patients (16.9%) in Group B, and 31 patients (31.0%) in Group C. Cox proportional hazard regression demonstrated that MELD score was significant determinant for short term mortality (Table). Among preoperative variables, leukocytosis (p=0.012) and ascites (p=0.058) were significantly associated with short term mortality in Group B, and urgent status (p=0.014) and positive culture of fungus (p=0.071) were significantly associated in Group C.
Conclusion:
The present study demonstrated that MELD score is a good predictor for 90-day mortality in our ALDLT series. However, additional risk factors also affect the short term survivals of the recipients with moderate or high MELD scores. This suggests that a special model in addition to MELD score may be required to predict the outcomes after ALDLT.

Mortality univariate analysis of specific groups of MELD scores

Group	MELD score	n=	Hazard Ratio	95% CI	p value
Group A	≤18	103	1.00		
Group B	19-24	71	1.54	0.67-3.53	0.305
Group C	25≤	100	2.96	1.51-5.77	0.002

Trend P<0.001

Abstract# 1363

ADULT LIVING DONOR VERSUS DECEASED DONOR LIVER TRANSPLANTATION: A SIX YEAR SINGLE CENTER EXPERIENCE. Daniel G. Maluf,[1] Adrian H. Cotterell,[1] Marc P. Posner,[1] Mitsuru Nakatsuka,[1] Richard T. Stravitz,[1] Martha K. Behnke,[1] Mitchell L. Shiffman,[1] Richard K. Sterling,[1] Velimer A. Luketic,[1] Robert A. Fisher.[1] *[1]Virginia Commonwealth University, Medical College of Virginia Hospitals, Richmond, VA.*

The use of adult-to-adult living donor liver transplantation (A2ALLTx) is an accepted technique. No > 3 years prospective comparison of A2ALLTx to adult deceased donor liver transplantation (ADDLTx) has been reported.
Patients and Methods: This is a prospective, IRB approved comparison of A2ALLTx to ADDLTx. The data includes: age, sex, race, primary liver disease, waiting time, Pre-transplant CTP/MELD score, peri-operative mortality, acute and chronic rejection, post-transplant complications, charges, graft survival, and patient survival.
Results: In six years 284 liver transplants were performed in 273 pts. 209 Ltx from ADDLTx (73.6%) and 75 A2ALLTx (26.4%). Data comparing ADDLTx vs A2ALLTx showed no difference in sex, race, primary disease, presence of HCC, peri-operative mortality or immunosuppression. Hepatitis C (HCV) was the most common reason for transplantation in both groups (46.4% vs 39%). Data regarding overall patient/ graft survival, rejection, pre-transplant MELD score, charges, biliary complication rate and re-transplantation rate are shown in Table 1. Comparison of patient/graft survival, re-transplantation rate in patients with and without HCV is shown in Table 2.
Conclusion: Overall, the A2ALLTx, in a lower risk, younger recipient group, is as durable a liver replacement technique as the ADDLTx. Patients with A2ALLTx presented less acute rejection and less histologic HCV recurrence with no difference in histologic severity or re-transplant rates. Biliary complications were more common in A2ALLTx but were not associated with increased graft loss compared to ADDLTx.

Table 1

Patient/Graft Survival

	ADDLTx (%)	A2ALLTx (%)	P value
1 y	84.4% / 82 %	77.4% / 73.9%	p>.05
3 y	79.9% / 76.4%	72.1% / 70.3%	p>.05
5 y	70.1% / 65.0%	72.1% / 70.3%	p>.05
	Acute Cellular Rejection (1 year) Biopsy	Proven	
	n=44/184, 23.9%	n=8/75,10.7%	p<.025
	MELD Score (Mean)		
	20.7+/-6.1	13.4 +/- 3.1	p=.001
	Charges (Donor evaluation to 6 m post-t)		
	$321,236.00	$374,248.00	p>.05
	Biliary complications		
	11.10%	25.00%	p<.05
	Re-transplantation Rate		
	4.00%	5.30%	p>.05

Table 2

	Patient Survival		Graft Survival		
	ADDLTx (%)	A2ALLTx (%)	ADDLTx (%)	A2ALLTx (%)	P Value
HCV+ 1 y	87.2	75.4	83.4	68.5	p>.05
HCV+ 3 y	82.8	67.1	81	64.2	p>.05
HCV+ 5 y	70.6	67.1	68.9	64.2	p>.05
Re-tx(%)	5.2	10.3			p>.05
HCV- 1 y	82.1	78.1	81.6	78.1	p>.05
HCV- 3 y	76.8	75.2	75.2	75.2	p>.05
HCV- 5 y	70.5	75.2	69.1	75.2	p>.05
Re-tx(%)	2.9	2.2			p>.05

Abstract# 1364

POST-OPERATIVE LIVER DYSFUNCTION FOLLOWING DONATION OF SEGMENTAL LIVER GRAFTS. Mark S. Orloff,[1] Adel Bozorgzadeh,[1] Kerrie E. Lansing,[1] Jackie Cullen,[1] Charlotte Ryan,[2] Ashok Jain,[1] David Waldman.[3] *[1]Surgery, University of Rochester Medical Center, Rochester, NY; [2]Pathology, University of Rochester, Rochester, NY; [3]Radiology, University of Rochester, Rochester, NY.*

Segmental liver grafts from live donors continues to supplement the severe shortage of cadaver organs. Grafts need to be of adequate size to support the recipient in liver failure, yet sufficient mass must remain to prevent donor liver failure. Few studies have elucidated how small this remnant can be. Most centers accept 30% remant liver volume as being sufficient, however there is little data to define a safe minimal size. We evaluated post-donation graft dysfunction in living donors and correlated it with remnant size and pre-transplant histology.
Methods: Ninety five consecutive adult donors of right lobe grafts were evaluated for remnant size and abnormal histologic features on pre-donation biopsy and correlated to peak post-operative serum Bilirubin, AST, ALT, Alkaline Phosphatase INR, post-operative complications, and lengths of stay. Remnant liver size was based on estimation by measurement of relative CT volumes. Data was analyzed using SPSS software and compared using 2-tailed t tests.
Results: Remant volumes varied from 20% to 50% estimated total liver volumes. There was no statistical difference in post-operative complications, or lengths of stay in comparing the groups of 20%-25% remant volume,or 26%-30% remnant volume to the larger remnants. However there was a significantly higher peak in serum bilirubin, AST, and ALT in the 20%-25% remnant group compared to the others (p<0.01, p<0.01, and p<0.01 respectively). There was no difference in peak INR or alkaline phospatase related to small size. Of histologic abnormalities only grafts with macrosteatosis estimations of greater than 10% showed significant dysfunction compared to the other grafts. Dysfunction was detected in elevated bilirubin, AST, and ALT (p<0.014, p<0.009, and p<0.005 respectively) in the mildly steatotic livers. No difference in INR elevation was detected in the steatotic group.
Conclusion: Donors could be safely left with estimated remnant volumes of 20% without increases in complications or lengths of stay. There is a slight trend toward liver dysfunction as evidenced by significant increases in bilirubin and transaminases, but not INR in donors with small remnant volumes. Macrosteatosis greater than 10% in donors behaved similarly. While it is tempting to be assured that patients with small remnants do well, it is cautionary that there is a measurable difference in liver dysfunction in small and macrosteatotic remnants.

Abstract# 1365

IGF-1 AND GH IN LIVING-DONOR LIVER TRANSPLANTATION.

Martin Stockmann,[1] Diana Hünerbein,[1] Sabine Nolting,[1] Klaus-Jürgen Gräf,[2] Thomas Konrad,[3] Thomas Steinmüller,[1] Peter Neuhaus.[1] *[1]Charité - Campus Virchow Klinikum, Transplantation Surgery, Berlin, Germany; [2]Charité - Campus Virchow Klinikum, Dep. of Endocrinology, Berlin, Germany; [3]Institute for Metabolic Research, Frankfurt, Germany.*

Introduction: The relevancy of insulin-like growth factor 1 (IGF-1) and growth hormone (GH) in liver regeneration and metabolism after liver resection or liver transplantation is not well understood. The aim of this study was to analyze IGF-1 and GH in donors and recipients before and after living-donor liver transplantation of the right hepatic lobe.

Methods: 16 donors and 15 recipients were investigated before and on day 1, 2, 3, 5, 7, and 10 as well as 6 months after right lobectomy or liver transplantation of the right lobe, respectively. GH was measured by immuno-radiometric assay (IRMA, BioSource), IGF-1 by RIA of Mediagnost.

Results: Before transplantation GH of recipients was elevated (8,03 ± 2,46 ng/ml), IGF-1 was reduced (73,20 ± 12,82 ng/ml) compared to healthy donors before lobectomy (GH: 0,60 ± 0,06 ng/ml, IGF-1: 157,07 ± 18,88 ng/ml), p<0,01. After transplantation IGF-1 of recipients continuously increased until 6 months and reached donor IGF-1 before operation on POD 7. GH decreased and was bisected already an POD 2 (3,49 ± 0,77 ng/ml), p<0,05. There was no significant difference after POD 3 to donor GH before operation. GH of the donors increased until POD 3 (3,29 ± 0,79 ng/ml, p<0,01) and was still increased after 6 months. IGF-1 of the donors strongly decreased by 60% after lobectomy until POD 5 (63,67 ± 9,67 ng/ml, p<0,01) and was still slightly decreased after 6 months (141,87 ± 14,38 ng/ml).

Discussion: Postoperative changes in GH and IGF-1 in donors and recipients after living-donor liver transplantation of the right hepatic lobe are completely different. Right lobectomy of healthy donors induced a strong decrease of IGF-1 and as a consequence an increased secretion of GH. In contrast, recipients show continuously increasing IGF-1 and decreasing GH after living-donor liver transplantation in parallel to improved liver function. Thus, liver function seems to have an important influence on the GH/IGF-1 axis. Further investigations are necessary to elucidate the meaning of these changes.

Abstract# 1366

FEASIBILITY OF LEFT-LOBE LIVING DONOR LIVER TRANSPLANTATIONS BETWEEN ADULTS: SINGLE CENTER EXPERIENCE OF 89 CASES.

Yuji Soejima,[1] Mitsuo Shimada,[1] Tomoharu Yoshizumi,[1] Yoshihiko Maehara.[1] *[1]Department of Surgery and Science, Kyushu University, Fukuoka, Japan.*

[Background] Living donor liver transplantation (LDLT) between adults has now become a legitimate alternative to cadaveric LT. Right lobe grafts have been predominantly used between adults with acceptable results. However, operative mortality for the right-lobe donor is realistic and estimated to be as high as 1%, due to a smaller remnant liver. To minimize the risk for the donor, left-lobe grafting could be an ideal option in adult-to-adult LDLT. The purpose of this study was to assess the feasibility of left-lobe LDLT between adults based on a single-center experience.

[Methods] Between October 1996 and November 30 2003, 110 adult-to-adult LDLTs using either a left-lobe graft (n=89) or a right-lobe graft (n=21) were performed at our institution. Among 89 left-lobe donors, 72 donors were blood-type identical, while16 were compatible and 1 was imcompatible to the recipient. Indications for LDLT were liver cirrhosis in 35 cases (27 cases with HCC), fulminant hepatic failure in 26, cholestatic liver disease in 22, retransplantation in 3 and others in 3. Twenty-eight patients were ICU-bound. The actual graft weight (GV) to the recipient standard liver volume (SLV) ratio (GV/SLV) was used for graft size evaluation. [Results] The mean graft weight was 452g (range 220-750g), which resulted in 40.3% (range 21-66%) of the recipient SLV. Nine grafts were extremely small (GV/SLV<30%). The overall 1-, 3- and 5-year patients survival were 79.3%, 77.1% and 71.6%, respectively, which were not different from those of right-lobe LDLTs. Twenty-two grafts (24.7%) were lost to chronic rejection (n=2), hepatic infarction (n=4), disease recurrence(n=3), GVHD (n=1), hepatic artery thrombosis (n=2) and other causes (n=10). Small-for-size syndrome occurred in 20 cases (22.5%), which led to only one direct graft loss. Graft size and donor age did not affect the patient survival. Major donor morbidity included biliary stricture (n=2), bile leak from cutting surface (n=2), gastric stasis (n=4), MRSA infection (n=1) and transient arm paralysis (n=3) with zero mortality. [Conclusion] Left-lobe LDLTs were feasible between adults without affecting patient and graft survival.

Abstract# 1367

LIVER TRANSPLANTATION USING CAVO-PORTAL TRANSPOSITION: AN EFFECTIVE TREATMENT IN PATIENTS WITH COMPLETE SPLANCHNIC VENOUS THROMBOSIS.

J. Pirenne,[1] B. Ceulemans,[1] R. Aerts,[1] D. Monbaliu,[1] W. Coosemans,[1] C. Verslype,[1] W. Van Steenbergen,[1] P. Yap,[1] J. Fevery,[1] F. Nevens.[1] *[1]UZ Gasthuisberg, University Hospital, Leuven, Belgium.*

A decade ago, segmental portal vein thrombosis was a contraindication to liver transplantation (LTx), but development of techniques such as thrombectomy and vascular interposition graft make it possible to offer LTx to these patients. Complete/extensive thrombosis of the venous splanchnic system, however, is not manageable by conventionnal LTx techniques and is associated with a very poor prognosis. Multivisceral Tx including the intestine is done for this indication but remains a high-risk procedure. One alternative is the performance of a Cavo-Portal-Transposition (CPT) e.g. perfusion of the liver by recipient caval flow, but data on this procedure are scarce (<30 case-reports). Therefore, results of LTx using CPT at a single-center were analyzed.

Methods/Results. Of 116 LTx done in a 2-year-period (05/2001-05/2003), 5 (4.3%) {2females/3males; mean age: 53 (41-71); 3 Budd-chiari, 1 reTx/chronic rejection, 1 cryptogenic cirrhosis} undergone CPT. All had complete chronic splanchnic thrombosis (intraoperatively-confirmed) associated to advanced liver failure (child C), major portal hypertension and moribund condition. CPT was done by completely diverting the inferior recipient vena caval flow into the graft vena porta directly (2) or via an interposition iliac vein (3). Normal anatomical infrahepatic caval flow was completely interrupted (5). Graft function was adequate immediately postTx. Portal hypertension (ascites/varices/hypersplenism) substantially improved postTx but this was a slow process associated to a long hospital stay: 77days (17-145). Graft and patient survival are 100% at 3 mths; 1 patient (71yo) died >3 mths due to biliary leak/sepsis unrelated to CPT. At last follow-up (8mth-2yr) liver synthesis capacity and gut function are satisfactory in the 4 survivors.

Conclusion: CPT represents a salvage procedure which provides adequate survival (80%) in moribund patients with diffuse splanchnic thrombosis not amenable to conventionnal LTx, and who would otherwise rapidly succumb. CPT avoids technically/immunologically more challenging multivisceral Tx. The intriguing observation that liver and gut can function satisfactorily in absence of direct anatomical portal drainage from the gut into the liver probably reflects the spontaneous development of extra-anatomical portocaval collaterals. One penalty of CPT, however, is the persistance of some degree of portal hypertension.

Abstract# 1368

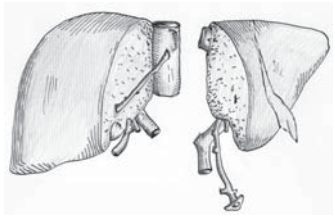
SPLIT LIVER TRANSPLANT (SLT) FOR 2 ADULT RECIPIENTS – EVOLUTION OF THE SURGICAL PROCEDURE.

Abhinav Humar,[1] Timothy D. Sielaff,[1] John R. Lake,[2] William D. Payne.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN; [2]Medicine, University of Minnesota, Minneapolis, MN.*

Background: SLT for 2 adult recipients is a procedure that is slowly developing as issues regarding donor and recipient selection are better defined. It remains a technically demanding procedure and optimizing the surgical technique of the split will play an important role in its success and ability to expand the donor pool.

Results: Since 1999, we have performed 28 right lobe/left lobe splits (transplanted into 28 adult recipients) from 14 deceased donors. In the first 9 donors, the split was performed exactly similar to the way we perform adult living donor procedures. The inferior vena cava was preserved with the left lobe graft, along with the middle hepatic vein and all the main hilar structures, including the common hepatic artery, portal vein, and common bile duct (CBD). In the last 5 donors, we have modified the procedure to include the cava with the right lobe graft. This had the advantage of maximizing outflow of the right lobe graft (by preserving all short haptic veins draining into the cava) and allowed back-table reconstruction of large middle hepatic vein (MHV) tributaries using vein grafts from the donor (thus minimizing warm ischemic time for the right graft at time of implantation). Outflow of the left lobe graft was then based on the common confluence of the MHV and left HV. In the last 3 donors, we have modified the split further to preserve the CBD with the right lobe graft (Fig). Biliary drainage of both grafts is then possible with a single anastomosis in both recipients (right graft via CBD, left graft via single left hepatic duct). Prior to this modification, 9 of the 11 right grafts required 2 separate biliary anastomosis; since then, all 3 right lobe grafts have had a single biliary anastomosis. All 14 left lobe grafts have had a single biliary anastamiosis.

Conclusions: The surgical procedure of adult SLT continues to undergo modifications. Optimizing the surgical technique will play a crucial part in the success of this procedure.



Abstract# 1369

SPLIT LIVER TRANSPLANTATION: A UNOS ANALYSIS. Tse-Ling Fong,[1,2] Yong W. Cho,[1,2] John Donovan,[1,2] Hassan Zaghla,[1,2] Linda Sher,[1,2] Yuichi Iwaki,[1,2,3] Rick Selby.[1,2] *[1]Liver Transplant Program and Center for Liver Disease, USC Keck School of Medicine, Los Angeles, CA; [2]Dept. of Surgery, USC Keck School of Medicine, Los Angeles, CA; [3]Dept. of Nephrology, USC Keck School of Medicine, Los Angeles, CA.*

Background: The chronic shortage of cadaveric liver grafts has stimulated alternative surgical techniques such as the splitting of the cadaveric liver for two recipients to expand the donor pool.

Objectives: This study evaluates the split-liver transplants (SLT) and the impact of this procedure on the donor pool.

Methods: From January 1, 1994 to December 31, 2002, 38,741 cadaveric and 1,877 living donor liver transplants were reported to the United Network for Organ Sharing (UNOS). Among 38,741 cadaveric donor liver transplants, 37,064 whole-, and 1,014 split-liver transplants (468 ex vivo, 484 in situ, and 62 unknown) were identified. The outcome of split-liver transplants was compared with those of whole-liver (WLT), and living donor liver transplants (LDLT). The log-rank test was used for comparison of two survival curves.

Results: Patient survival rates of SLT (85% at 1- and 79% 3-yr) were similar to those of WLT (85% at 1- and 78% 3-yr, P=0.60). Graft survival rates of SLT (72% at 1- and 64% 3-yr) were statistically significantly inferior than those of WLT (79% at 1- and 71% 3-yr, P<0.001) and LDLT (78% at 1- and 71% 3-yr, P<0.001). Graft survival rates reported for in situ SLT (73% at 1- and 65% 3-yr) were not significantly higher than those of ex vivo SLT (71% at 1- and 62% 3-yr, P=N.S.). Incidence of primary graft failure was 10% for SLT compared with 4% for WLT (P<0.001) and 4% for LDLT (P<0.001). Incidence of vascular thrombosis was 6% for SLT compared with 3% for WLT (P<0.001) and 5% for LDLT (P=0.48). Incidence of biliary tract complication observed in recipients of SLTs (2%) was not significantly higher than those of WLT (1%) and LD (1%). Thirty percent of split-livers were transplanted to UNOS status 1 patients, whereas 15% of whole livers (P<0.001) and 8% living donor liver (P<0.001) to emergent patients. After excluding patients who died within a month after transplantation, graft survival rates of SLT (88% at 1- and 78% 3-yr) were not significantly inferior to those of WLT (88% at 1-yr and 79% 3-yr, P=0.52) and LDLT (88% at 1- and 80% 3-yr, P=0.08).

Discussion: Early patient death is a major confounding factor in this study. SLT, if careful patient selection is applied, can result in patient and graft survival comparable to those of WLT and LDLT, despite higher incidences of primary graft failure and vascular thrombosis.

Conclusion: Split liver transplantation can increase the cadaveric donor liver pool.

Abstract# 1370

SPLIT LIVER TRANSPLANTATION USING THE LEFT LATERAL SEGMENT: A MULTI-CENTER STATEWIDE EXPERIENCE. T. C. Lee,[1] N. R. Barshes,[1] W. K. Washburn,[2] G. A. Halff,[2] B. A. Carter,[3] L. J. Bristow,[1] J. D. Scott,[1] L. Nguyen,[1] J. A. Goss.[1] *[1]Dept. of Surgery, Baylor College of Medicine, Houston, TX; [2]Dept. of Surgery, University of Texas at San Antonio, San Antonio, TX; [3]Dept. of Pediatrics, Baylor College of Medicine, Houston, TX.*

Split liver transplantation (SLT) increases the pool of available organs for pediatric orthotopic liver transplantation (pOLT). With increased cooperation and communication between transplant centers, SLT can further increase the number of donor allografts. Herein, we report a multi-center experience of 56 pOLT using a left lateral segment (LLS) allograft.

Methods: The current study consists of a retrospective review of 56 pediatric LLS transplants performed at 2 collaborating pOLT centers between 9/1997 and 10/2003. Median patient follow-up was 20 months.

Results: A summary of donor demographics is presented in Table 1. Liver splitting was performed *in-situ* and *ex-vivo*, 64% and 36% of the time respectively. The celiac axis was maintained with 60% of the LLS, and arterial reconstruction was required in 16 (29%) of the grafts. Recipient data is summarized in Table 2. The most common indication for pOLT was biliary atresia. 23 recipients were UNOS status 1 at time of pOLT and the remaining 33 had a median PELD score of 16. The overall allograft and patient survival rate at 1 year was 82% and 89%, respectively. Comparative UNOS averages for pediatric allograft and patient survival at 1 year were 79% and 87%. There was an 11% biliary complication rate: 1 bile duct stricture and 5 biliary leaks. Vascular complications included 5 HAT and 5 PVT accounting for an 18% vascular complication rate.

Conclusion: SLT increases the number of allografts available for pOLT and does not have the safety concerns of living donor transplantation. With increased program collaborations, the number of available allografts is increased even further. While SLT is technically demanding, and has a significant learning curve, patient and allograft survival rates compare favorably with UNOS averages. Therefore, SLT should be considered as an option for pediatric liver transplant recipients.

Table 1 (Median)

Age	19 yrs
BMI	23
Peak AST	89
Peak NA	153
Creatinine	1.3

Table 2 (Median)

Age	14 mos
PELD	16
1st Tx	94%
Status 1	41%

CMV AND NON-ORGAN SPECIFIC IMMUNOSUPPRESSION

Abstract# 1371

AN ASSESSMENT OF THE CLINICAL UTILITY OF CMV SEROLOGY TESTING IN A LARGE COHORT OF HIGH RISK CMV D+/R- 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 Disease, University of Toronto, Toronto, ON, Canada; [2]Infectious Disease, Mayo Clinic, Rochester, MN; [3]Roche Products Limited, Welwyn Garden City, United Kingdom.*

Background: Organ transplant recipients who are CMV D+/R- remain at risk for CMV disease despite anti-viral prophylaxis, with late onset disease often occurring beyond 6 months. The clinical utility of serology testing in these patients has not been well established. We assessed CMV IgG and IgM testing in a large cohort of D+/R- SOT recipients.

Methods: 352 CMV D+/R- organ transplant recipients (liver, kidney, heart, pancreas) participating in an international trial comparing 100 days of oral ganciclovir vs. valganciclovir prophylaxis were evaluated. CMV serology (IgG and IgM) was tested on day 28, 56, 100, 6 months and 12 months post-transplant and correlated with outcomes.

Results: A total of 352 patients (232 valganciclovir and 120 ganciclovir) were studied. All patients were seronegative pre-transplant. IgG seroconversion occurred in 16.2% patients by day 28 post-transplant, 19.6% by day 56, 26.9% by day 100, 63.4% by 6 months, and 75.3% by 12 months. IgM detection was 8.3% by day 100, 41.8% by 6 months, and 54.9% by 12 months. CMV IgG seroconversion rates by end of prophylaxis were 33.3% in ganciclovir treated patients vs. 23.6% in valganciclovir treated patients (p=0.06). The highest end of prophylaxis seroconversion rates were observed in liver recipients (40.5%) and the lowest in kidney recipients (9.0%). Predictive value of IgG seroconversion was assessed. Seroconversion by day 100 (end of prophylaxis) was not predictive of subsequent onset of CMV disease or viraemia (rate of CMV disease: 13.3% in seropositive patients vs. 17.8% in seronegative patients (p=NS); rate of viraemia: 44.8% in seropositive patients vs. in 49.8% seronegative patients (p=NS)). However, at 6 months post-transplant, IgG serostatus was highly predictive of subsequent CMV disease between month 6-12 (CMV disease rate 1.3% in IgG seropositive patients vs. 10.0% in IgG seronegative patients (p=0.002)). IgM and IgG results during prophylaxis (day 28 and 56) were not predictive of CMV disease or viraemia.

Conclusions: In D+/R- patients receiving 100 days of antiviral prophylaxis, the clinical utility of IgG or IgM measurements while on prophylaxis or at end of prophylaxis is limited in terms of predicting subsequent disease. However, a single IgG measurement at 6 months post-transplant may be very useful for stratifying patients in terms of risk of late onset CMV disease.

Abstract# 1372

A CONTRAST IN THE INCIDENCE AND PREVALENCE OF HUMAN HERPESVIRUS (HHV)-6 AND HHV-7 REACTIVATION IN CYTOMEGALOVIRUS (CMV) D+/R- SOLID ORGAN TRANSPLANT (SOT) PATIENTS RECEIVING ORAL GANCICLOVIR OR VALGANCICLOVIR PROPHYLAXIS. 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 direct and indirect role of HHV-6 and HHV-7 in post-SOT clinical syndromes is increasingly recognized. Studies suggest that their pathogenicity is exerted through their interaction with CMV. Indeed, CMV D+/R- SOT patients have the highest incidence of HHV-6 and -7 reactivation. In this study, we determined whether oral ganciclovir (OGCV) or valganciclovir (VGCV) prophylaxis, which reduced the incidence of CMV disease, is also effective in suppressing HHV-6 and HHV-7 reactivation.

METHODS: 2,232 blood samples were obtained from 263 CMV D+/R- SOT patients who received OGCV (n=95) or VGCV (n=168) prophylaxis for the prevention of CMV disease. Samples collected prior to (baseline) and during (days 14, 42, 70, and 100)

anti-CMV prophylaxis, and at months 4, 4.5, 6, 8, and 12 post-SOT were quantified for HHV-6A, HHV-6B, and HHV-7 DNA using a LightCycler-based PCR assay (lowest limit of detection, 1 copy). Significant HHV-6 and HHV-7 DNAemia was defined as ≥1000 copies/mL.

RESULTS: HHV-6A DNAemia was not observed at any time point. Four of 263 patients (1.5%) had significant HHV-6B DNAemia (peak level, 1096-91050 copies/mL). The prevalence of significant HHV-6B DNAemia was relatively constant before, during, and after prophylaxis (range: 0.6-1.4%). In contrast, the prevalence of significant HHV-7 DNAemia was higher (2.3-7.8%). Significant HHV-7 DNAemia occurred in 48 patients (18.3%). For these patients, the mean (median; range) for significant HHV-7 DNA levels were 3455 (1645; 1382-9211) prior to, 4904 (1672; 1007-80350) during, and 3868 (1981; 1045-48540) copies/mL after prophylaxis.

CONCLUSION: The incidence of significant HHV-6 and HHV-7 DNAemia in this large cohort of CMV D+/R- SOT patients appears lower than the reported rates in a historical cohort of CMV D+/R- SOT patients who were not receiving anti-CMV prophylaxis [HHV-6 (27%); HHV-7 (45%)]. Nonetheless, the contrasting incidence and prevalence in HHV-6 and HHV-7 reactivation highlights the differential *in vivo* activity of GCV on β-herpesviruses: HHV-7 is least susceptible, compared to HHV-6 (and CMV), to prophylactic doses of OGCV and VGCV. We are assessing the clinical relevance of this finding, including a potential role for HHV-7 (and breakthrough HHV-6) in the pathogenesis of late CMV disease.

Abstract# 1373

KINETICS OF CMV LOAD REDUCTIONS FOLLOWING PRE-EMPTIVE THERAPY WITH VALGANCICLOVIR OR INTRAVENOUS GANCICLOVIR IN ADULT SOLID ORGAN TRANSPLANT PATIENTS.

Frank M. Mattes,[1] Emma G. Hainsworth,[1] Aycan F. Hassan-Walker,[1] Andrew K. Burroughs,[2] Paul Sweny,[3] Paul D. Griffiths,[1] Vincent C. Emery.[1] *[1]Department of Virology, Royal Free and University College Medical School, London, UK, United Kingdom; [2]Centre for Hepatology, Department of Medicine, Royal Free and University College Medical School, London, UK, United Kingdom; [3]Centre for Nephrology, Royal Free and University College Medical School, London, UK, United Kingdom.*

Background:

In solid organ transplant patients, both primary infection and re-infection can cause CMV disease with high morbidity. Pre-emptive therapy with intravenous (iv) ganciclovir has shown to be effective in preventing CMV end organ disease in solid organ transplant patients. Valganciclovir, given 900 mg daily, gives similar ganciclovir plasma levels to iv ganciclovir (5mg/kg od), and has been recently licensed for prophylaxis against CMV disease in solid organ transplant patients. We report here the results of a retrospective study, comparing the kinetics of decline in CMV load following either iv ganciclovir (GCV; 5mg/kg bd) or valganciclovir (VGCV; 900mg bd) in adult solid organ transplant patients receiving pre-emptive therapy.

Methods:

CMV viremia was monitored twice weekly on whole blood by CMV PCR. Patients with two consecutive positive CMV PCR results were treated with iv ganciclovir (5 mg/kg bd) or with Valganciclovir (oral 900 mg bd) at the discretion of the physician, until becoming CMV PCR negative (<200 genomes/mL blood). Primary study outcome was defined as time to become CMV negative and rate of decline in viral load after initiating antiviral therapy.

Results:

In total, 45 patients were included in the study. Twenty three patients were treated with iv GCV compared to 22 treated withVGCV. Mean baseline CMV loads and replication rate (r) prior to antiviral therapy were similar between both treatment arms (iv GCV: 3.85 log10 gen/ml; VGCV 3.81 log10 gen/ml; p=0.78, replication rate: iv GCV 0.4 ge day-1 vs 0.49 ge day-1, p=0.44). The time between the first positive CMV PCR sample and starting antiviral therapy was 9 days in both groups. Median time to become CMV PCR negative was 14 days in the ivGCV group compared to 15.2 days within the VGCV group (p=0.88). The half-life of decline in the ivGCV and VGCV treated groups was 1.73 days and 2.16 days respectively (p=0.7). None of the patients experienced symptomatic CMV disease.

Conclusion:

In solid organ transplant recipients, the decline rate of CMV load following pre-emptive therapy with valganciclovir (900mg bd) is not significantly different to that observed following iv ganciclovir therapy (5mg/kg bd).

Abstract# 1374

RANDOMIZED TRIAL OF ORAL GANCICLOVIR PLUS CYTOMEGALOVIRUS (CMV) IMMUNE GLOBULIN (Ig) VERSUS ORAL GANCICLOVIR ALONE FOR CMV PROPHYLAXIS IN LIVER AND KIDNEY TRANSPLANT RECIPIENTS.

Mark Johnson,[1] Steven Zacks,[2] Paula McIver,[1] Mark Russo,[2] Robert Dupuis,[3] Kenneth Andreoni,[1] David Gerber,[1] Greg Malat,[3] Jane Salm,[1] Roshan Shrestha,[2] Jeffrey Fair.[1] *[1]Departments of Surgery; [2]Medicine; [3]Pharmacy, Division of Transplantation, University of North Carolina, Chapel Hill, NC.*

Introduction: CMV infection effects morbidity, mortality, and cost post-transplant and the most effective regimen remains controversial.

Objective: To compare the efficacy of oral ganciclovir alone (Group 1) vs CMV Ig plus oral ganciclovir (Group 2) in liver and kidney transplant recipients.

Methods: An open-label randomized study. Dosing: oral ganciclovir 1 g TID for 3 months and CMV Ig 150 mg/kg IV within 48 hours, 150 mg/kg (livers) or 100 mg/kg (kidneys) IV at 2, 4, 6, and 8, and 100 mg/kg (livers) or 50 mg/kg (kidneys) IV at 12 weeks post-transplant. Primary outcomes included CMV disease, syndrome or infection, graft rejection, patient/graft survival, and adverse events. Data were analyzed using standard statistical methods. Immunosuppression was organ specific and comparable.

Results: Between 9/1997 and 10/2002, 200 Patients were enrolled, 194 were included in this review.

RESULTS

	Group 1 (no cytogam)	Group 2 (yes cytogam)	p Value
N	99	95	
Age ± SEM	43.9 ± 1.25	50.59 ± 1.15	0.000124
Female (%)	41	43	0.7778
Liver* (%)	49	48	0.889
Kidney ** (%)	50	47	0.6760
CMV status (Donor/Recipient)			
+/+ (%)	44	49	0.4852
+/- (%)	23	21	0.7368
-/+ (%)	22	20	0.7325
-/- (%)	10	5	0.1876
Overall CMV Incidence (%)	10.1	13.7	0.4382
CMV Infection (%)	4	6.3	0.4677
CMV Syndrome (%)	3	4.2	0.6533
CMV Disease (%)	3	3.2	0.9359
Average time to CMV (mos) ± SEM	4.3 ± 0.6	6.9 ± 0.86	0.028
1 Yr Graft Survival (%)	97	97	1.0000
1 yr Patient Survival (%)	94.9	92.6	0.5075
Rejection (%)	29.3	40	0.1172
Opportunistic Infections# (%)	10.1	9.5	0.8883

* 1 pt in Grp 2 had Liver-Kidney tx. ** 1 pt in Grp 1 and 4 pts in Grp 2 had K-P tx. # OI's include yeast, B-K, EBV infections

Over-all incidence of CMV was 11.9% (n=23) with 13 (56.5%) in the high risk D+/R- category (7 in Group1, 6 in Grp2), 9 (41%) in the D+/R+ category (2 in Grp 1, 7 in Grp 2), and one in the D-/R- category (Grp1). Five patients in Group 1, and 2 patients in Group 2 developed CMV while on prophylaxis.

Conclusions: Donor positivity is the greatest risk factor for developing CMV infection post transplant accounting for 97.5% of CMV post-transplant. Although CMV infection rates were similar in both groups, patients who received CMV Ig had fewer prophylaxis failures (2 vs 5 pts) and a significant delay in onset of CMV over patients who received oral Ganciclovir alone.

Abstract# 1375

ABROGATION OF THE ALLOREACTIVE RESPONSES OF CADAVER DONOR INTESTINAL LYMPHOCYTES BY SHORT-TERM CAMPATH-1H EXPOSURE. James M. Mathew,[1,2] Werviston De Faria,[1] Tomoaki Kato,[1] Teresa Vallone,[1] Silvia Alvarez,[1] Manuel Carreno,[1] Bonnie Blomberg,[1] Laphalle Fuller,[1,2] Violet Esquenazi,[1,2] Joshua Miller,[1,2] Andreas G. Tzakis.[1] *[1]Surgery - Division of Transplantation; [2]Microbiology & Immunology, University of Miami and the VA Medical Center, Miami, FL.*

Introduction: Donor passenger gastrointestinal lymphocytes have been implicated in the development of graft versus host (GvH) reactivity that in turn may contribute to post-transplant morbidity in intestinal transplantation. We asked whether Alemtuzumab or Campath-1H (C-1H) treatment could alter this activity.

Methods: Pieces of cadaver donor intestines and draining lymph nodes were obtained before and ~ 6 hours after revascularization (pre- and post- R) of donor organs with the recipients who had been either treated with or without C-1H immediately pre-operatively or from donors treated with C-1H 2-8 hours prior to organ harvesting. Lymphocytes from these tissues were isolated and tested as responders in mixed lymphocyte reactions (MLR).

Results: Cells isolated from intestinal mucosa, lamina propria and lymph nodes harvested pre-R reacted to both the recipient and third party lymphocytes with stimulation indices (SI) ranging from 8.6 to 194 (n=10). Post-R preparations in C-1H treated recipients reacted at significantly lower magnitude giving SI of 2.0 56.6 ($p<0.01$). Such reduction was not observed with post-R samples from patients not treated with C-1H. Lymphocytes from both proximal (jejunal) and distal (colic) regions gave similar alloreactive responses. Both the pre- and post-R samples had similar cellular yields, initial viability and flow cytometric profiles, suggesting that despite the functional changes there was no cellular or receptor loss by the short-term (6 hour) systemic exposure to C-1H in the intestinal graft or its lymph nodes once revascularization has occurred. Attempts at performing similar experiments from cadaver donors who were treated with C-1H 6-8 hours prior to organ harvesting were not successful as very few lymphocytes could be obtained from equivalent quantities of intestinal tissues (n=3). However, cadaver donors who were treated with C-1H for <3 hours before organ harvesting gave similar results as untreated donors (n=3) described above.

Conclusions: Campath-1H treatment of the donor and/or recipient might bring about a drastic reduction in potential GvH reactivity by the donor intestinal lymphoid cells.

Effect of Camapth-1H Exposure

	Pre-R	Post-R
Recipient PBL	22.0	
Donor Intestinal Lymph Node Cells	194.0	45.7
Donor Intestinal Intraepithelial Lymphocytes	143.5	23.1

SI to third party stimulations (Representative Experiment)

Abstract# 1376

CALCINERUIN INHIBITOR (CI) AND STEROID-FREE IMMUNOSUPPRESSION (ImmSx) FOR PANCREAS (Px) AND Px-KIDNEY (KD) RECIPIENTS USING CAMPATH FOR BOTH INDUCTION AND MAINTENANCE (maint.). R. Kandaswamy,[1] R. Gruessner,[1] A. Gruessner,[1] A. Humar,[1] D. Sutherland.[1] *[1]Surgery, Univ of MN, Mpls., MN.*

Introduction: There is an increasing emphasis on using either a CI or steroid-free regimen in combination with either mycophenolate mofetil (MMF) or rapamcyin (Rap). Most desirable would be a maint. regimen that is both CI and steroid-free to minimize non-immunosuppressive side effects. We used an anti-T-cell antibody (Campath) for maint. ImmSx in a series of Px and KD Tx recips; early results are described.

Patients and methods: From 1/03 to 11/03, 46 diabetic recips of either a simultaneous Px/KD (SPK) (n=11), a Px after KD (PAK) (n=16), a PxTx alone (PTA) (n=13) or a KD after PxTx (KAP) (n=6) were given Campath 30 mg IV x 3 for induction ImmSx and thereafter 30 mg whenever the absolute lymphocyte count exceed 200mm^3 (usually required at 2-4 month intervals). The only oral immunosuppressant was MMF (if enterically tolerated at target 2-3 gm/day), adjusted by neutrophil count, or Rap (target level 5-8). A concurrent group of patients (n=34) were given Campath only for induction with tacrolimus (target level 5-8) or cyclosporine (target level 150-200) and MMF as the intention to treat maint. ImmSx (SPK n=7, PAK n=15, PTA n=10, KAP n=2). Patient and graft survival and rejn episode rates were calculated for all cases with death with a functioning graft (DWFG) counted as a graft failure. Mean followup was 5 mos (range 1-10).

Results: In the Campath maint. group, there were 3 technical failures (TFs), 1 SPK, 2 PTA, 2 DWFG (1 SPK, 1 PAK), 2 rejn graft losses (both PTA) and 6 reversible rejn episodes (1 SPK, 3 PAK, 1 PTA, and 1 KAP). In the CI maintenance group, there were no TFs, 2 DWFGs (both PTA), no rejn losses, and 6 reversible rejn episodes (1 SPK, 2 PAK, 2 PTA, and 1 KAP). Cytomegalovirus occurred in 1 Campath (PTA) and 2 CI (both PAK) maint. patients. There were no PTLD occurrences and 1 in the CI (a PTA) maint. group. In the SPK and PAK categories, there were no significant differences in actuarial pt graft survival and rejn episode rates. In the PTA category, the rejn episode rate (reversible and nonreversible combined) was higher in the Campath maint. group ($p=0.02$)

Conclusion: Early results with a Campath maint. protocol in Px Tx recips are at least as good with those with CI maint., but so far there have more rejns in the PTA category. Nevertheless, the advantages of a CI-free and steroid-free protocol is attractive. Anti-T-cell maint. therapy in organ allograft recips should be considered as an alternative to conventional ImmSx.

Abstract# 1377

RABBIT ANTI-THYMOCYTE (RATG) PRECONDITIONING AND INDUCTION FOR PEDIATRIC INTESTINE TRANSPLANTATION. Jorge Reyes,[1] George V. Mazariegos,[1] Geoffrey J. Bond,[2] Rakesh Sindhi,[1] Amy Smith,[1] William McGhee,[3] Camila Macedo,[1] John J. Fung,[2] Kareem Abu-Elmagd.[2] *[1]University of Pittsburgh Medical Center, Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]University of Pittsburgh Medical Center, Thomas E. Starzl Transplantation Institute, Pittsburgh, PA; [3]Department of Pharmacy, Children's Hospital of Pittsburgh, Pittsburgh, PA.*

BACKGROUND: Improvement of rejection after intestinal transplantation (ITx) led to the use of a steroid free protocol based on rATG preconditioning and induction with low dose tacrolimus (TAC).

METHODS: 36 children 5 months to 20 years received pre-transplant rATG 2-3mg/kg, followed by ITx (liver/intestine n=12, intestine n=15, multivisceral n=6, modified/multivisceral n=1, liver/intestine/pancreas n=1, intestine/pancreas n=1). Post-transplant rATG 2-3mg/kg (total of 5 mg/kg), with TAC started within 48 hours. Target trough concentrations were of 15-20 ng/dl for 2 weeks, then 10-15ng/dl thereafter. Follow-up ranged from 1-20months (mean of 9 months).

RESULTS: The first and second year patient/graft survivals were 100% and 97% respectively. 18/36 children experienced rejection (50%) which occurred at 3 to 30 days after ITx. 18 children remain free of maintenance steroids; 2 received prednisone for liver allograft rejection (3/9, 33%). Maintenance TAC monotherapy is as follows: 2 receive a single daily dose 3 days/week, 1 receives a single daily dose every other day, 12 receive a single daily dose 7 days/week (levels mean 9.8ng/dl), and 18 are receiving a 2 times/day dosing (levels mean 10.2ng/dl). Two patients are on rapamycin. Two patients developed EBV infection documented by EBV-PCR which resolved with antiviral therapy; 1 developed CMV enteritis.

CONCLUSION: The use of rATG preconditioning induction has decreased the incidence and severity of rejection after ITx with improved patient and graft survivals. We speculate that the "steroid free" strategy with consequent improvement in infectious and toxic complications of immunosuppression may result in better short and long term survival.

Abstract# 1378

TYPE 1 PROGENITOR DENDRITIC CELL (pDC1) MAY CHARACTERIZE IMMUNOLOGICAL EVENTS AFTER STEROID-FREE PEDIATRIC ABDOMINAL TRANSPLANTATION WITH THYMOGLOBULIN (rATG). Ali Abdullah,[1] Joseph Seward,[1] Amy Magill,[1] Jorge Reyes,[1] Adrianna Zeevi,[1] Rakesh Sindhi.[1] *[1]Transplantation, University of Pittsburgh, Pittsburgh, PA.*

Background: pDC types 1 and 2 prime TH1 and TH2 responses, respectively. Cross-sectional studies link rejection risk to relative TH1/pDC1 preponderance. Longitudinal validation during rejection, opportunistic infections (INF), or rejection-free state is unavailable. *Methods*. Frequencies (%) of lineage negative, HLADR^{hi} cells, which were either CD123+ (pDC2) or CD11c+ (pDC1), were measured by flow cytometry in peripheral blood from 19 pediatric liver (n=10) and intestine (n=9) recipients. Samples were obtained before and within 90 days after transplantation (Tx) , and during rejection (REJ) and INF. Results: Median age was 3 years (0.5-20), time to pDC analysis was 41 days (7-71) in 6 subjects with no rejection, and 13 subjects with REJ. pDC were also measured in 3/13 REJ experiencing enteritis (INF) due to CMV, rotavirus and adenovirus respectively, after treatment of REJ. *Results*: pDC1 decreased significantly after Tx in Non-rejectors, and increased significantly with INF (Table 1). Although pDC subtypes were altered minimally during rejection, their relative frequencies resulted in a significantly decreased pDC2/pDC1 ratio, suggesting TH1 preponderance. pDC2 were not affected in any clinical state. *Conclusion*: Early after Tx, clinical states of immune activation, e.g. rejection and opportunistic viral infection, may be characterized by a relative or absolute preponderance of the TH1-priming pDC1, while a rejection-free state may demonstrate a paucity of this phenotype. The role of pDC subsets in post-transplant immune monitoring needs additional study.

Table 1

Clinical State	Days between pDC analysis	Timing of pDC analysis	%pDC2	%pDC1	%pDC2 +%pDC1	%pDC2 /%pDC1
Non-Rejectors (n=6)	34±22	Pre-Tx vs Post-Tx, p value	0.17±0.1 vs 0.11 ±0.16,p=NS	0.32±0.1 vs 0.17 ±0.17,p=0.05	0.49±0.17 vs 0.28±0.29, p=0.02	0.53±0.27 vs 0.59 ±0.68,p=NS
Rejectors (n=13)	42±16	Pre-Tx vs Post-Tx, p value	0.11±0.11 vs 0.1±0.1, p=NS	0.16±0.08 vs 0.14±0.1, p=NS	0.27±0.15 vs 0.25±0.2, p=NS	0.8±0.7 vs 0.62 ±0.45,p=0.05
Infections (n=3)	101±50	Pre-Tx vs Post-Tx, p value	0.1±0.06 vs0.15±0.16, p=NS	0.1±0.02 vs 0.26±0.1, p=0.05	0.15±0.08 vs 0.41±0.26, p=NS	1.1±0.7, 0.51±0.42, p=NS

Abstract# 1379

THE MANAGEMENT OF END STAGE RENAL DISEASE IN THE RECIPIENTS OF EXTRARENAL TRANSPLANTS. Bradley H. Collins,[1] Carlos E. Marroquin,[1] Janet E. Tuttle-Newhall,[1] Dev M. Desai,[1] Stephen R. Smith,[2] David W. Butterly,[2] R. Randal Bollinger,[1] Paul C. Kuo.[1] *[1]Department of Surgery, Duke University Medical Center, Durham, NC; [2]Department of Medicine, Duke University Medical Center, Durham, NC.*

INTRODUCTION: Patients with end stage heart, lung, or liver disease occasionally develop renal failure. Often, these patients are not considered for transplantation of the primary organ system because of increased morbidity and mortality associated with renal failure. Concurrent kidney transplantation has been performed successfully in this population of patients. Renal failure can also develop in the recipients of heart, lung, or liver transplants, further complicating their management. Kidneys from living or cadaveric donors have been transplanted into these groups of patients with success. The purpose of this retrospective analysis was to evaluate the outcome of extrarenal transplant recipients who received a kidney transplant at the time of or subsequent to heart, lung, or liver transplantation.

METHODS: The entire heart, lung, and liver transplant databases of a single institution were reviewed and all patients who received a simultaneous kidney transplant or underwent renal transplantation subsequent to the extrarenal transplant were selected for the analysis summarized below.

RESULTS: Twenty kidney transplants were performed in 19 extrarenal transplant recipients. This cohort of patients included 5 bilateral lung, 5 heart, and 9 liver recipients. Each of the 7 patients who underwent *concurrent* renal transplantation received the kidney from the cadaveric, extrarenal donor. Of the 13 kidney transplants performed *after* extrarenal transplantation, 10 were from living donors. The etiologies of renal failure in patients who received concurrent kidney transplants were multiple. The indication for transplantation in 9 of the 13 extrarenal recipients who subsequently received a kidney transplant was calcineurin inhibitor nephrotoxicity. Patient survival remains 100% to date. Of the 12 kidney transplants performed greater than year ago, 1 year allograft survival is 100%.

CONCLUSION: Kidney transplantation represents an excellent form of renal replacement therapy in the recipients of extrarenal transplants. It can be performed safely at the time of extrarenal transplantation or remotely when renal failure develops with excellent short-term results. The leading indication for late renal transplantation is nephrotoxicity secondary to the use of calcineurin inhibitors. Immunosuppression protocols that limit exposure to these agents may decrease the incidence of renal failure following heart, lung, or liver transplantation.

Cytokines and Alloreactivity

Abstract# 1380

T-BET DEFICIENT RECIPIENTS EXHIBIT A TH2 PHENOTYPE, DEVELOP ACCELERATED ACUTE AND CHRONIC HUMORAL VASCULARIZED ALLOGRAFT REJECTION, AND ARE RESISTANT TO IMMUNOSUPPRESSION AND TOLERANCE. Xueli Yuan,[1] Micheal R. Clarkson,[1] Isabela Schmitt,[1] Alan D. Salama,[1] Hugh Auchincloss, Jr.,[1] Laurie H. Glimcher,[2] Mohamed H. Sayegh.[1] *[1]Transplantation Research Center, Bigham and Women's Hospital and Children's Hospital, Boston, MA; [2]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA.*

T helper cell polarization into Th1 or Th2 subsets has significant effects on the outcome of experimental solid organ transplantation. Th2 cytokines have been associated with alloimmune hyporesponsiveness in vivo, however, in several transplant models Th1 cytokines are required for tolerance induction. T-bet, a T box transcription factor, is critical for the development of Th1 responses. We investigated the role of T-bet in allograft rejection and tolerance using full thickness skin and vascularized cardiac transplant models. Survival of fully mismatched cardiac grafts in T-bet knockout (KO) recipients was slightly but significantly shorter than in wild type (WT) controls (6.1±0.3 days vs. 7.3±0.5 days, $p<0.05$). This accelerated rejection time-course was accompanied by a Th2-predominant alloimmune response and histologic and immunohistologic evidence of an antibody-mediated rejection process similar to that seen in human recipients with acute humoral rejection (AHR). Interestingly, and mimicking human transplant recipients with AHR, T-bet KO recipients were resistant to immunosuppression with either cyclosporine or rapamycin. In addition, administration of either CTLA4Ig (500ug IP on day 0, 250ug days 2,4 and 6) and/or anti-CD40L mAb (MR1, 500ug IP on day 0, 250ug days 2,4 and 6) to WT mice yields long-term cardiac allograft survival, however, in T-bet KO recipients treated with combined costimulatory blockade no allograft survived beyond 18 days. In contrast to IFN-g KO recipients, administration of exogenous recombinant INF-g does not reverse resistance to tolerance induction. Furthermore, in a class II mismatched model, BM12 skin grafts transplanted into T-bet KO recipients are acutely rejected similar to WT recipients, while cardiac grafts develop accelerated and more severe cardiac allograft vasculopathy (CAV) as compared to WT recipients. Interestingly, in this model IFN-g KO recipients accept skin grafts and are protected from CAV. Therefore, this suggests that the acute humoral and accelerated chronic rejection and resistance to tolerance in T-bet KO recipients are independent of IFN-g deficiency. We conclude that T-bet deficient mice provide a novel model for understanding mechanisms and developing novel therapies for AHR and chronic rejection in vascularized allografts.

Abstract# 1381

ALLOANTIBODY PRODUCTION REQUIRES COGNATE INTERACTIONS BETWEEN INDIRECTLY PRIMED CD4 T CELLS AND PEPTIDE/MHC COMPLEXES EXPRESSED ON B CELLS. 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.*

CD4 T cells responding through the indirect pathway may provide requisite helper signals for induction of an isotype switch and IgG alloantibody production. To provide further insight into the mechanisms of this phenomenon we first showed that in the absence of CD4 T cell help, B6 (H-2^b) TCR KO mice transplanted with allogeneic (H-2^k) heart grafts, and B6 CD4 KO mice transplanted with allogeneic heart grafts, did not produce anti-donor IgG alloantibodies (n=3 per group) 30-60 days posttransplant. Control WT recipients of allogeneic heart grafts had detectable IgG alloantibodies in their sera within 7-14 days of the transplant. We next made use of an H-2^b-restricted TCR transgenic CD4 T cell, Mar, specific for an indirectly presented peptide derived from allogeneic male C3H heart grafts. Adoptive transfers of Mar T cells into B6 TCR KO or CD4 KO recipients of allogeneic C3H male heart grafts specifically led to activation of the Mar T cells via the indirect pathway (as assessed by flow cytometry and ELISPOT assays) and induced a high titer of specific, anti-donor IgG alloantibodies by 2-3 weeks posttransplant (n=3-4 per group) as assessed by flow cytometry. This result shows that CD4 T cells responding via the indirect pathway can provide the helper signals required to initiate an alloantibody isotype switch. We then asked whether the recipient B cells must have cognate interactions with the CD4 T cells responding via the indirect pathway in order to produce the alloantibodies. To assess this we transferred Mar T cells into recipients lacking MHC class II on B cells, followed by transplantation with an allogeneic C3H male heart graft. Notably, the recipients (n=4) rejected the heart grafts and the Mar T cells were indirectly primed to become Th1 effector cells. Nonetheless, we were not able to detect anti-donor IgG alloantibody in these animals by flow cytometry. Alloantibody was detected in the serum of control animals with MHC class II expression on B cells. The findings demonstrate that alloreactive CD4 T cells must have cognate interactions via their TCR with peptide/MHC complexes on recipient B cells in order to initiate an antibody isotype switch. Designing therapies aimed at blocking such specific interactions regardless of the activation state of the responding CD4 T cells may be effective strategies for limiting alloantibody production in allograft recipients.

Abstract# 1382

TREATMENT OF PRE-DIABETIC NOD MICE WITH FLT3-LIGAND *IN VIVO* DELAYS THE DEVELOPMENT OF DIABETES AND RESTORES THE GENERATION OF MYELOID DENDRITIC CELLS. Isabelle J. Fugier-Vivier,[1] Paula M. Chilton,[1] Leslie A. Weeter,[2] Hong Xu,[1] Yimimg Huang,[1] Murkunda B. Ray,[3] Francine Rezzoug,[1] Suzanne T. Ildstad.[1] *[1]Institute for Cellular Therapeutics, University of Louisville, Louisville, KY; [2]Department of Physiology and Biophysics, University of Louisville, Louisville, KY; [3]Department of Pathology, University of Louisville, Louisville, KY.*

Type I diabetes is a systemic autoimmune disease. In the human and mouse, a number of abnormalities in the development of antigen presenting cells has been defined and proposed to be contributing to the autoimmune process. Mixed chimerism reverses the autoimmune process in nonobese diabetic (NOD) mice and prevents the development of diabetes. The goal of this study was to determine the mechanism by which this occurs. It has been known that NOD mice lack $HSA^+/Ly6C^+$ myeloid progenitor cells. We addressed if mixed chimerism restores this myeloid cell population and if these cells are required for the preservation of peripheral tolerance. We generated NOD/B10.BR mixed chimeras and found that the induction of mixed chimerism resulted in an increase in the $HSA^+/Ly6C^+$ population to levels similar to those observed in the donor strain. Interestingly, the $HSA^+/Ly6C^+$ cells in the chimeric animals were donor as well as NOD-derived. Similarly, we found that co-culture of NOD BMC *in vitro* with Flt3-ligand (FL) developed an $HSA^+/Ly6C^+$ phenotype that was primarily comprised of myeloid dendritic cells (DC). To determine whether FL was a key factor in diabetes progression, pre-diabetic NOD mice were treated with a 10-day course of FL. Strikingly, there was a highly significant delay in the development of diabetes, which correlated with reduced insulitis and restored production of myeloid DC. Moreover, there was a significant increase in the level of $CD4^+/CD25^+$ T_{reg} cells in pancreatic lymph nodes. These data indicate that mixed chimerism as well as FL treatment of NOD mice *in vivo* (and treatment of marrow *in vitro*) results in production of myeloid DC. This increase is correlated with an increase in tolerance and a decreased incidence of disease. In conclusion, these data suggest that the underlying myeloid abnormality in NOD mice is due to a block in development of myeloid DC, which is restored through treatment with FL and may provide a benign and novel approach to treat autoimmune diabetes.

Abstract# 1383

TNFR2 (P75) SIGNAL TRANSDUCTION IS REQUIRED FOR TRANSCRIPTIONAL ACTIVATION OF HCMV IE GENE EXPRESSION IN ALLOGENEIC TRANSPLANTATION. Soo Jung Kim,[1] Zheng J. Zhang,[1] Thomas K. Varghese,[1] Mary A. Hummel,[1] Gail Thomas,[1] Michael Abecassis.[1] [1]*Division of Organ Transplantation, The Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Reactivation of latent Human Cytomegalovirus (HCMV) results in infection and disease, causing both morbidity and mortality in immunosuppressed patients. However, the molecular mechanism which leads to reactivation from latency is unknown. Recently, we have shown that allogeneic transplantation induces IE gene expression in MCMV latently infected mice and in MIEP-lacZ mice carrying a comparing b-reporter gene under the control of the HCMV Major Immediate Early Promoter (MIEP) and that TNF can substitute for allogeneic transplantation in inducing IE gene expression in some organs. Since induction of IE gene expression is an essential first step in CMV reactivation, identification of factors leading to induction of IE gene expression would be important in preventing CMV reactivation. **Aim**: To study the role of TNF receptor-mediated signaling on induction of HCMV IE gene expression. **Methods**: MIEP-lacZ transgenic mice were cross-bred with TNFR1, TNFR2, and TNFR1,2 knockout mice. Kidneys from these mice were transplanted into allogeneic recipient mice and removed 2 days after transplant. The contralateral donor kidneys were removed at the time of the transplant and were analyzed as controls. The extent of transgene expression was measured using a quantitative chemiluminescence assay for b-galactosidase activity and the level of induction was determined by comparing b-galactosidase activity in transplanted kidneys with that of the contralateral control kidney. **Results**: Genetic deficiency in the Type 1 TNF receptor has no effect on induction of IE gene expression, but genetic deficiency in both the Type1 and Type2 TNF receptors significantly decreases the induction of IE gene expression in an allogeneic transplant. Studies with TNFR2-/- MIEP-lacZ mice demonstrate that TNFR2 alone mediates MIEP-lacZ induction in an allogeneic transplant. **Conclusions**: TNF signaling is important for induction of IE gene expression in an allogeneic transplant and the Type 2 TNF receptor plays the major role in this induction. Given that Type 1 TNF receptor mediates induction of IE gene expression through soluble TNF in our system and that TNFR2 is known to mediate signal transduction of membrane-bound TNF, our results suggest that signaling of membrane-bound TNF mediates induction of IE gene expression through TNFR2 in an allogeneic transplant.

Abstract# 1384

STAT5a/b TRANSCRIPTION FACTORS ARE IMPORTANT FOR T BUT NOT B CELL FUNCTIONS DURING ALLOGRAFT REJECTION. Ye Zhang,[1] Robert Kirken,[2] Lucrezia Furian,[1] Slawa Janczewska,[1] Xiumei Qu,[1] Mouer Wang,[1] Ronald Kerman,[1] Stanislaw M. Stepkowski.[1] [1]*Surgery, UT-Medical School, Houston, TX;* [2]*Integrative Biology and Pharmacology, UT-Medical School, Houston, TX.*

Objective: The T-cell-growth factors signaling through Janus kinases (Jak1 and 3) and signal transducers and activators of transcription (Stat1, 3, 5a/b and 6) induce full T cell response. Using the Stat5a/b double knockout, we examined the role of both proteins in acute allograft rejection. Methods/Results: Normal C57BL/6 Stat5a/b+/+ (H-2b) recipients acutely rejected C3H (H-2k) heart allografts (7.3±0.5 days; n=4). In contrast, Stat5a/b-/- mice rejected heart allografts in a delayed fashion (19.3±7.6 days; n=6; p<0.01). In vitro, T cells from Stat5a/b-/- mice failed to proliferate in response to conconavalin A (ConA) or alloantigens and at 24 hrs and 48 hrs displayed 30% and 90% apoptosis, respectively, as shown by TUNEL assay; T cells from Stat5a/b+/+ mice proliferated in response to ConA or alloantigen with only 5% apoptosis. Microarray analysis showed that at 24 hrs after activation Stat5a/b-/- purified T cells expressed predominantly pro-apoptotic mRNAs (ATM, bok, CIDE-A, CIDE-B, Bax, Nip3, CARD, Myd88, and blk) in comparison to activated Stat5a/b+/+ T cells. Western blot confirmed that after 24 hrs and 48 hrs of activation Stat5a/b-/- T cells expressed elevated level of pro-apototic Bax and no anti-apoptotic Bcl-2 proteins in contrast to Stat5a/b+/+ T cells. However, PCR method showed that (at 24 hrs) ConA-stimulated T cells from Stat5a/b+/+ or Stat5a/b-/- mice produced similar amounts of interleukin (IL)2, IL4, IL10 and interferon- mRNA. Purified B cells from both Stat5a/b+/+ and Stat5a/b-/- mice proliferated in similar fashion after stimulation with lipoplysacharides (LPS) and showed no apoptosis. As shown by FACS, sera from both Stat5a/b+/+ and Stat5a/b-/- rejectors had identical concentrations of donor-specific IgM, IgG1, IgG2a and IgG2b alloantibodies. Conclusions: Although Stat5a/b are not required for cytokine production by T cells, Stat5a/b are important for T cell survival after activation. In contrast, Stat5a/b deficiency does not affect B-cell function, including proliferation, differentiation and immunoglobulin class switching. Thus, Stat5a/b-deficient recipients may mediate rejection of allografts following T-B cell collaboration by donor-specific IgM and IgG alloantibodies.

Abstract# 1385

TOLL-LIKE RECEPTOR 4 (TLR4) EXPRESSION AFTER IL-13 GENE TRANSFER IS STAT6-DEPENDENT. Bibo Ke,[1] Xiu-Da Shen,[1] Feng Gao,[1] Douglas G. Farmer,[1] Ronald W. Busuttil,[1] Jerzy W. Kupiec-Weglinski.[1] [1]*Dumont-UCLA Transplant Ctr, David Geffen School of Medicine, Los Angeles, CA.*

Background: Activation of host toll-like receptor (TLR) system, which controls innate immunity, may progress into adaptive T cell immune responses. Our previous studies have shown that TLR4 plays a key role in triggering inflammatory response associated with Ag-independent hepatic ischemia/reperfusion (I/R) injury. This study was designed to analyze putative role of TLR4 in regulating liver inflammation following IL-13 gene transfer. **Methods:** Using a partial lobar liver warm ischemia model, groups of Balb/c wide-type (WT) and Stat6-deficient (KO) mice were assessed for the extent and severity of hepatic I/R injury (n=4/gr). Separate groups were injected with Ad-IL-13 or Ad-β-gal reporter gene ($2.5x10^9$ pfu I.P.). 48 h later, a midline laparotomy was performed, mice were heparinized, and an atraumatic clip was used to interrupt blood supply to the cephalad lobes of the liver. After 90 min of partial hepatic ischemia, the clip was removed, initiating hepatic reperfusion. Sham control WT mice underwent the same procedure, but without vascular occlusion. Mice were sacrificed after 6 h of reperfusion; liver tissue/peripheral blood samples were collected for future analysis. The hepatic I/R injury was assessed by histological Suzuki's grading. **Results:** WT, Ad-β-gal controls, as well as Stat6 KO and Stat6 KO mice treated with Ad-IL-13/Ad-β-gal showed significant edema, sinusoidal congestion/cytoplasmic vacuolization, and severe hepatocellular necrosis (30-50%). In contrast, WT mice treated with Ad-IL-13 revealed minimal sinusoidal congestion without edema/vacuolization or necrosis. These correlated with decreased sGOT levels and local neutrophil accumulation, selectively in WT mice treated with Ad-IL-13. In addition, WT mice treated with Ad-IL-13 revealed markedly depressed TLR4 expression, yet augmented expression of Stat6 and HO-1, as compared with WT, Stat6 KO, or Stat6 KO + Ad-IL-13/Ad-β-gal groups. 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 selectively in WT+ Ad-IL-13 group. **Conclusion:** This study documents that IL-13 gene transfer prevents hepatic I/R injury by downregulating TLR4 expression. Stat6 and HO-1 induction by IL-13 gene transfer exerts synergistic cytoprotective effect against hepatic inflammatory injury. Hence, activation of Stat6 is required for IL-13-induced depression of TLR4 signaling during Ag-independent pro-inflammatory response induced by hepatic I/R.

Abstract# 1386

MONOCYTES ACT AS A BRIDGE BETWEEN T CELLS AND ALLOGENEIC ENDOTHELIAL CELLS VIA IFNγ-DEPENDENT PROCESSES. He Xu,[1] Kiran K. Dhanireddy,[1] Allan D. Kirk.[1] [1]*Transplantation Branch, NIDDK/NIH, Bethesda, MD.*

Monocytes up-regulate many co-stimulatory molecules when combined with T-cells in allogeneic mixed lymphocyte-endothelial cell (EC) reactions, but purified monocytes fail to express CD40 when co-incubated with EC alone. This suggests that host T-cells, monocytes, and EC are all required for optimal monocyte-mediated antigen presentation. In this study, we have hypothesized that monocytes act as a bridge between EC and T-cells, and that a 3 cell model more closely approximates the in vivo situation. To assess the interdependence of these cells, transwell co-cultures were established using purified human monocytes, T-cells and allogeneic EC. EC-conditioned monocytes were also co-incubated with purified T-cells to assess the order in which these interactions could occur. Antibodies against interferon-γ (IFNγ) receptor-1, IFNγ receptor-2, and IFNγ were added to assess the role of the IFNγ pathway in these interactions. Cells were analyzed by FACS for monocyte-derived CD40, a indicator of optimal monocyte activation. Co-incubation of purified monocytes with EC did not result in CD40 expression, but did result in EC membrane uptake as assessed by PKH EC membrane staining. In contrast, monocytes co-incubated with both EC and T-cells up-regulated surface CD40, indicating that T-cells were required for optimal monocyte activation. EC-conditioned monocytes also up-regulated CD40 but only after incubation with T-cells, indicating that the exposure to EC could be remote from the exposure to T-cells. When T cells were separated from monocyte-EC interaction by a transwell, monocytes did not express CD40. Furthermore, IFNγ receptor-1 blockade markly reduced CD40 expression during the monocyte-EC-T-cell interactions. The presence of anti-IFNγ also prevented monocyte-derived CD40 expression. In contrast, IFNγ receptor-2 blockade did not inhibit monocyte-derived CD40 up-regulation. These data demonstrate that both T-cells and EC provide stimulation for responding monocytes and that these interactions are contact and IFNγ dependent. These data suggest that monocytes can act as bridge between EC and T cells, facilitating membrane uptake in the periphery, but reserving full activation and costimulation until exposed to antigen activated T-cells. One mechanism by which the T-cells provide final monocyte costimulatory capacity is via the IFNγ receptor-1 pathway. This provides a mechanism by which antigen can be engulfed and transported to secondary lymphoid tissue without risking T-cell activation of non-antigen specific cells during transit.

Abstract# 1387

INTERFERON REGULATORY FACTOR 1(IRF-1) PLAYS A KEY ROLE IN LIVER ISCHEMIA/REPERFUSION INJURY. Yuan Zhai,[1] Feng Gao,[1] Ryan O;Connell,[2] Xiu-Da Shen,[1] Bo Qiao,[1] Genhong Cheng,[2] Ronald W. Busuttil,[1] Jerzy W. Kupiec-Weglinski.[1] *[1]The Dumont-UCLA Transplant Center, Department of Surgery, David Geffen School of Medicine-UCLA, Los Angeles, CA; [2]Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine-UCLA, Los Angeles, CA.*

While employing global genetic profiling (microarray) to study the molecular mechanisms of hepatic ischemia/reperfusion (IR) injury in a rat syngeneic orthotopic liver transplant (OLT) model (LEW to LEW), we have focused on the expression of interferon regulatory factor-1 (IRF-1). OLT survival after 24h cold (4C) preservation in UW solution was 100%. In contrast, OLT survival after 36h cold preservation was 0%. Compared with native livers (1088±99) and normal OLT without cold preservation (716±162), IRF-1 was significantly induced only in 36h (16798±1207), but not 24h (1412±381) cold-preserved OLT. To test the functional significance of IRF-1 induction in liver IRI, we utilized a mouse partial hepatic ischemia model. Indeed, 90 min of warm ischemia, followed by 6h reperfusion markedly increased intrahepatic IRF-1 expression, as measured by quantitative RT-PCR. It correlated with the hepatocellular damage (sALT levels/histology Suzuki criteria). IRI was ameliorated in IRF-1 KO mice, as compared with WT controls, and evidenced by lower sALT levels (691±294 vs. 4467±1737) and preserved liver architecture. To further dissect the mechanisms of IRF-1 mediated liver IRI, we then examined putative interactions between IRF-1 and the recently established TLR4-mediated proinflammatory pathway. Indeed, although IR-triggered intrahepatic IRF-1 induction was abolished in TLR4 KO mice, it was readily maintained in TLR2 KO and MyD88 KO mice. Furthermore, studies in IFNAR KO mice have documented that intact type-1 interferon pathway was required for IRF-1 induction. We have recently shown that liver IR activates TLR4, and that IP-10 is the key downstream effector mediating liver IRI. Thus, we measured intrahepatic IP-10 production in IRF-1 KO mice. Indeed, quantitative RT-PCR showed that IP-10 expression was completely prevented in IRF-1 KO livers subjected to IR. The level of TNF-α, one of the potential effector molecules in liver IRI, was also decreased in IRF-1 KO livers. Hence, IRF-1 expression is one of the critical elements in TLR4-initiated liver IRI. IRF-1 induction is mediated by TLR4, but not TLR2 activation, and it remains MyD88-independent. The requirement for IFNAR indicates that IRF-1 induction results from secondary response of TLR4 activation, and might be mediated by IFN-β produced from the primary response. Most importantly, IRF-1 is required for IP-10 production, which may explain why IRF-1 KO mice remain resistant against liver IRI.

Abstract# 1388

ACTIVATION OF TLR4 IS CRITICAL IN THE MECHANISM OF EXTENDED COLD ISCHEMIA/REPERFUSION INJURY AFTER LIVER TRANSPLANTATION. Xiu-Da Shen,[1] Bibo Ke,[1] Yuan Zhai,[1] Feng Gao,[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 Medical Center, UCLA Medical Center, Los Angeles, CA; [2]Pathology, UCLA Medical Center, Los Angeles, CA.*

Badkground:: Ischemia/reperfusion injury (IRI) remains an important clinical problem after liver transplantation. Recent studies have documented that toll-like receptor (TLR) system plays an important role in the activation of innate immunity, and in controlling adaptive host immune responses. This study was designed to address the putative role of TLR4 signaling in the mechanism of antigen-independent prolonged cold hepatic IRI. **Methods and Result**s: Isogeneic orthotopic liver transplants (OLT) with rearterialization were performed in groups of wild-type (WT) Balb/c and TLR4-deficient (C.C3 TLR4^{lps-d}; KO) mice. Donor livers transplanted from WT to WT mice were preserved in UW solution at 4°C for either 1h (Gr. 1) or 24h (Gr. 2). In Gr. 3, donor livers transplanted between TLR4-deficient mice were stored in UW solution for 24h. The sALT, TNF-α, IL-2, and IFN-γ levels in Gr. 2 recipients were markedly increased at 1h, 6h, 1d and 3d after reperfusion, as compared with Gr 1 OLTs. However, unlike in WT group, the cytokine expression levels were dramatically reduced throughout in TLR4 KO OLTs. Similarly, disruption of TLR4 signaling significantly diminished OLT level of IFN-γ-inducible protein 10 (IP-10), a chemoattractant for activated T cells. The well-functioning OLTs from TLR4 KO donors revealed diminished expression of capase-3, along with decreased levels of apoptotic endothelial cells/hepatocytes, as compared with relevant WT controls. This data was consistent with histological Suzuki's criteria of hepatic IRI. Thus, WT livers revealed notable sinusoidal congestion/hepatocyte vacuolization, with hepatocellular necrosis (10-20%) in d1 and d3 OLTs. In contrast, TLR4 KO livers showed only mild hepatocyte ballooning and 2-4% hepatocellular necrosis.**Conclusion**: This study documents, for the first time, the critical role of the TLR4 system in the mechanism of extended cold hepatic IRI after OLT in mice. Indeed, disruption of TLR4 signaling diminished the early proinflammatory responses and ameliorated hepatic IRI, indicating that this antigen-independent hepatic insult might be initiated by the activation of the sentinel TLR4 system. Thus, based on new concepts of hepatic IRI injury, these results provide the rationale for the development of novel therapeutic approaches in OLT patients.

Abstract# 1389

SHOULD HEPATOCELLULAR CARCINOMA BE TREATED DURING THE WAITING LIST PERIOD? Jean F. Botha,[1] B. Daniel Campos,[1] Wendy J. Grant,[1] Debra L. Sudan,[1] Byers W. Shaw, Jr.,[1] Fedja Rochling,[1] Daniel F. Schafer,[1] Alan N. Langnas.[1] *[1]Transplantation, University of Nebraska Medical Center, Omaha, NE.*

Background: In order to prevent tumor progression while waiting for a donor, adjuvant loco regional therapy has been recommended for patients with hepatocellular carcinoma (HCC) who are listed for liver transplantation. The aim of this study was to assess the efficacy of adjuvant therapy in patients with HCC while waiting for liver transplantation.
Methods: We reviewed 77 patients with HCC and cirrhosis who were evaluated and listed for liver transplantation. We compared 36 patients (Group 1) who received pretransplant loco regional therapy with 41 patients (Group 2) who did not receive any pretransplant therapy. Treatments included 22 trans arterial chemo embolizations (TACE), 9 percutaneous ethanol injections (PEI), 4 radiofrequency ablations (RFA) and 7 liver resections. Groups were compared with respect to overall survival, recurrence free survival and dropout from the waiting list. In addition, all explants were examined for viable tumor, grade and vascular invasion.
Results: There were no differences between the two groups with respect to age, gender, underlying liver disease, tumor stage (AJCC), time on the waiting list, immunosuppression, and number of patients exceeding the Milan criteria. Sixty-two patients underwent liver transplant, 29 of whom received pre transplant loco regional therapy and 33 who did not. There were no differences in survival between the two groups at 1 year (84% vs. 75%) and at 4 years (59% vs. 51%) (p=0.88). Recurrence free survival at 1 year (86% vs. 94%) and at 4 years (69% vs. 63%) was also not significantly different (p=0.08). Dropout from the waiting list due to tumor progression occurred with similar frequency in both groups (11% vs. 12%) (p=0.9). Viable tumor was found in 26/29 explants in group 1 and in all explants in group 2.
Conclusions: These data suggest that pretransplant loco regional adjuvant therapy administered to patients with HCC while on the waiting list does not confer an advantage in terms of overall survival and disease free survival after transplantation. Furthermore, adjuvant therapy does not appear to prevent patient dropout from the waiting list.

Abstract# 1390

RADIOFREQUENCY ABLATION OF LIVER TUMORS BEFORE LIVER TRANSPLANTATION-A CLINICAL AND HISTOLOGICAL EXAMINATION. Adrian P. Martin,[1] Robert M. Goldstein,[1] George Netto,[2] Nora Katabi,[2] Takehisa Ueno,[1] Nicholas Onaca,[1] Edmund Q. Sanchez,[1] Srinath Chinnakotla,[1] Henry Randall,[1] Sherfield Dawson,[1] Marlon F. Levy,[1] Goran B. Klintmalm.[1] *[1]Transplant Surgery, Baylor Regional Transplant Institute, Dallas, TX; [2]Pathology, Baylor University Medical Center, Dallas, TX.*

Background: Radiofrequency ablation (RFA) of liver tumors is offered to patients (pts.) awaiting liver transplantation (LTX) in order to decrease their tumor load. The study evaluates the efficiency of RFA by analyzing peri-ablation findings, and examining the explanted native liver.
Material and Method: Retrospective collected data on 33 pts. who underwent RFA prior to LTX between January 1998 and May 2003 were reviewed. All pts. met T1 or T2 UNOS criteria. Post-ablation MRI's were obtained every 3 months until LTX. Gross and H&E stains of explants were reviewed. Percentage of viable to necrotic hepatoma at the ablation site was calculated by surface area mapping.
Results: 25 pts. were male and 8 female. Liver disease was related to Hepatitis C (n=25), Hepatitis B (n=3), alcohol (n=3), cryptogenic (n=1), PBC (n=1). The approach was laparoscopic (n=29), open (n=5) or percutaneous (n=2). 3 pts. had 2 separate RFA sessions. A total of 41 tumors were ablated (6 pts. had 2 tumors ablated and 1 pt. had 3). Ultrasound was used to localize and assess intraoperative ablation.
3 pts. had residual tumors after ablation (2 were re-ablated and 1 was embolized). 1 pt. had a new tumor on follow-up immediate after RFA and it was ablated.
6 pts. (18%) had new tumors on pathology. 2 of these were not seen on MRI. No patient developed extrahepatic or port side spread of tumor.
On the explanted specimens, 36 ablation sites were found, with dimensions between 1.2 and 6 cm (mean 3.1). Necrosis by mapping was: 100% n=15, 90-99% n=9, 70-80% n=3, 60-65% n=2, 45-50% n=4, 35% n=2, 10% n=1). The 3 re-ablated pts. had 95-100% necrosis on pathology. The pt. who had embolization developed multifocal disease and 35% necrosis at the ablation site.
Mean follow-up was 21.7 months after LTX (0-57). 30 pts. (91%) are currently alive, free of recurrence. 3 pts. (9%) died after LTX, 1 of them (3%) with recurrent HCC (sternal metastasis).
Conclusions: Extensive tumor necrosis was obtained in the majority of pts. (over 90% in 73%). 18% of pts. had new tumors found in explants, which emphasized close follow-up of all pts. who have had a diagnosis of HCC. The recurrence rate of HCC after LTX was minimal and survival was excellent. RFA appears to be highly efficient in reducing the tumor load before LTX and decreasing the risk of recurrence after LTX.

Abstract# 1391

ROLE FOR LIVING DONOR TRANSPLANTION IN PATIENTS WITH HEPATIC MALIGNANCY. David Axelrod,[1] Alan Koffron,[1] Laura Kulik,[2] Elena Crisan,[2] Patrice Al-Saden,[1] Mary Mulcahy,[2] Jonathan Fryer,[1] Michael Abecassis.[1] *[1]Division of Transplantation, Department of Surgery, Northwestern University, Chicago, IL; [2]Department of Medicine, Northwestern University, Chicago, IL.*

Background: The role of LDLT in patients with hepatic malignancy, especially HCC that exceeds Milan criteria and cholangiocarcinoma, remains unclear.

Aims: The current study will review a single center experience with LDLT for patients with hepatic malignancy to assess the potential role for LDLT in patients with HCC or cholangiocarcinoma.

Materials and Methods: 38 adult-to-adult LDLT were performed at our center between 1999 and 2003. Of these, 18 patients were transplanted for hepatic malignancy (47%). Twelve patients had HCC, of which 5 had T1/T2 disease, 6 had T3 disease and one had T4a disease. Of the 6 patients with T3 disease, 5/6 exceeded UCSF criteria. Interestingly, the patient with T4a disease did not exceed UCSF criteria. No patients who underwent LDLT for HCC received adjuvant therapy. All six patients with cholangiocarcinoma had either T1or T2 disease. All patients have at least 6 months follow-up.

Results: Among the six patients with ≥T3 **HCC**, 2 patients died 18 months following LDLT of recurrent HCC. One patient with T3 HCC is alive 3 years after LDLT but has a bony recurrence and one patient is recurrence-free at 9 months. All patients with recurrent HCC exceeded UCSF criteria. The remaining two patients died of other causes (HUS-1; recurrent HCV-1). The patient with T4a is recurrence free 7 months post LDLT. Of the 5 patients with T1/T2 HCC, only one patient had locally recurrent HCC. He was treated with local excision 4 months post LDLT and is alive and disease-free one year following LDLT. One patient died 11 months after LDLT of recurrent HCV. The others are alive and disease-free 26, 23 and 6 months following LDLT.

Of the patients with **cholangiocarcinoma**, 2/6 received no adjuvant therapy. One patient died of recurrent disease 26 months after LDLT and the other is alive without disease 45 months after LDLT. 4/6 patients underwent pre-transplant treatment with combined chemotherapy and radiotherapy. All of these patients are alive and disease-free 15, 12, 11 and 6 months post LDLT.

Conclusion: LDLT for HCC within Milan and UCSF criteria results in significant disease-free survival that is compromised primarily by recurrence of HCV. Where as in patients who exceed both Milan and UCSF criteria, recurrence is high despite the short waiting time for LDLT. Finally, despite the preliminary nature of our data, LDLT appears to be an effective therapy for patients with cholangiocarcinoma who receive pre-transplant adjuvant therapy.

Abstract# 1392

LIVING DONOR LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA IN A SINGLE CENTER. Mikiko Ueda,[1] Yasutsugu Takada,[1] Hironori Haga,[1] Kenji Uryuhara,[1] Kohei Ogawa,[1] Yasuhiro Fujimoto,[1] Mureo Kasahara,[1] Yasuhiro Ogura,[1] Koichi Kozaki,[1] Hiroto Egawa,[1] Koichi Tanaka.[1] *[1]Department of Transplantation and Immunology, Kyoto University Faculty of Medicine, Kyoto, Kyoto, Japan.*

Aim. Long-term survival has not been clarified for living-donor liver transplantation (LDLT) for hepatocellular carcinoma (HCC) so far. The purpose of the present study is to analyze survival and risk factors affecting tumor recurrence in LDLT for HCC in a single center.

Method. From February 1999, 69 patients with unresectable HCCs with no extrahepatic lesions and no major vessel invasion and 8 patients with incidental HCCs received LDLT (male: 55, female: 22). There were no exclusion criteria of LDLT for the number and the size of the tumors. The age of the patients ranged from 21 to 69 years with a median age of 54 years. The number of the patients with the UICC tumor Stage I was 10, Stage II 13, Stage III 6 and Stage IVA 40. The tumor spread was within the Milano criteria in 21 patients and out of Milano criteria in 48 patients. Forty-eight patients had received transarterial chemoembolization, 34 patients had received cytoablation therapy for HCCs and 10 patients had underwent hepatic resection for HCC previously. Serum levels of alpha-phetoprotein at the time of transplantation ranged from 0.9 to 20.362 ng/ml with the median level of 29 ng/ml.

Results. Sixteen patients died within the first three months after LDLT due to tumor-unrelated causes. Patient survival and recurrence -free patient survival was 74.8% and 89.3% at 1y, 63.5% and 84.1% at 2y, and 63.7% and 80.7%, respectively. Recurrence-free patient survival with HCCs within the Milano criteria was 100% at 1y, 2y and 3y and that with HCCs out of Milano criteria was 80.9%, 71.9% and 66.7% at 1y, 2y and 3y. There was no significant recurrence-free patient survival between patients with HCCs within the Milano criteria and HCCs out of the Milano criteria. The risk factors of the histological findings with HCCs in the explanted livers which significantly affects the tumor recurrence were poorly differentiated HCC, tumor number greater than 4, maximum diameter of the tumor greater than 5 cm and microscopic vascular invasion ($P < 0.05$).

Conclusion. Although tumor recurrence rate was higher in the patients with HCC out of Milano criteria, there was no significant difference in recurrence-free patient survival between patients with HCCs within the Milano criteria and patients with HCCs out of the Milano criteria. Overall recurrence-free patient survival of 80.6% at 3y was thought acceptable in LDLT for HCCs.

Abstract# 1393

IMPACT OF MELD EXCEPTIONS ON TRANSPLANT AND DEATH RATES IN LIVER CANDIDATES. Ann M. Harper,[1] Erick B. Edwards,[1] David C. Mulligan,[2] Hugo E. Vargas,[2] Hector Rodriguez-Luna,[2] Kunam S. Reddy,[2] Aydr A. Moss,[2] Richard B. Freeman.[3] *[1]Research Department, United Network for Organ Sharing, Richmond, VA; [2]Division of Transplant Medicine, Mayo Clinic, Phoenix, AZ; [3]Division of Transplant Medicine, Tufts University School of Medicine/New England Medical Center, Boston, MA.*

OPTN liver allocation policy allows centers to apply for increased MELD/PELD scores for patients with exceptional diagnoses for whom their calculated MELD/PELD score may not represent their need for transplant. For the 12,981 adult candidates added to the OPTN liver waiting list between 2/27/02 and 8/27/03, 14.8% (1917) requests for exceptions were made, of which 67.1% (1287) were for candidates with hepatocellular cancer (HCC) and the remainder (630) were for other indications. For the 1688 candidates with approved MELD exceptions, the diagnosis and narrative text fields were categorized and we used a Kaplan-Meier method with competing risks to determine the rates of transplant, removal for death/too sick, removal for other reason, and continuation on the list as of November 29, 2003 within 90 days of initial listing or exception. Approximately 23% of patients with no exception received a transplant within 90 days, compared with 56.4% for patients with exceptional case applications. Death rates were 7.9% for standard cases, versus 5.0% for exceptions ($p<0.05$). Patients initially listed with low MELD scores (6-15) with exceptions experienced greater increases in the transplant rate (55.6 vs. 10.2, $p<0.05$) with little effect on death rates. Of patients with an initial MELD score of 16-25, transplant and death rates were 32.3 and 9.6 with no exception, versus 60.3% and 10.2% for exceptions. In the higher standard MELD categories (26-35), patients with exceptions had lower rates of transplantation (43.3% vs. 61.0%) but increased death rates (30.0% vs. 25.0). Patients with HAT had the highest rates of transplantation at 90 days (72.2%), followed by cryptogenic (69.2%), HPS (64.2%), and PSC (63.6%). The death rates for exceptional cases varied by diagnosis, ranging from 3.0% (PSC) to 23.3% (CAHC) and 27.8 (other). The most common reasons for upgrade requests and the associated transplant and death rates are as follows: ascites (42.6% and 19.1%, n=68), biliary complications (73.3% and 6.7%, n=45), cholangitis (64.5% and 6.5%, n=31), encephalopathy (35.7% and 28.6%, n=14) and portal hypertensive bleeding (57.9% and 15.8 %, n=19). Patients granted exceptional MELD scores have higher rates of transplantation and lower rates of death compared to standard cases with similar MELD scores. Standardization of upgrade criteria and continued scrutiny of the exception process will be necessary for continued improvement of the liver allocation system.

Abstract# 1394

DECREASED SURVIVAL IN LIVER TRANSPLANT PATIENTS REQUIRING CHRONIC DIALYSIS: A CANADIAN EXPERIENCE. M. Cantarovich,[1] F. Ivis,[2] K. Badovinac.[2] *[1]Medicine, McGill University Health Center, Montreal, QC, Canada; [2]Canadian Organ Replacement Register, Canadian Institute for Health Information, Toronto, ON, Canada.*

End-stage renal disease (ESRD) requiring chronic dialysis constitutes a serious complication in 2%-13% of liver transplant (LTx) pts. Purpose: To describe the prevalence of ESRD and the survival of LTx pts requiring chronic dialysis in Canada. Methods: We studied 3,820 pts who received a LTx between Jan 1, 1981 and Dec 31, 2001. Data was extracted from the Canadian Organ Replacement Register, managed by the Canadian Institute for Health Information. An attempt was made to match each LTx pt on dialysis with 2 dialysis controls (n=195) without a LTx (No LTx-Dialysis group). Results: 103 LTx pts (2.8%) developed ESRD requiring chronic dialysis. (LTx-Dialysis group) and 3,717 pts did not develop ESRD (LTx-No Dialysis group). Pts in the LTx-Dialysis group were older than pts in the LTx-No Dialysis group (48.5±13 vs. 43±19, P=0.0024). There was no difference in the primary disease. Chronic dialysis was initiated at 61.5±44.5 months (median 60) post-LTx. Pt's years post-LTx in the LTx-Dialysis group was 7.5±4 and in the LTx-No Dialysis group was 5±4 (P<0.0001)

Survival post-LTx

Survival	0-1 yr	1-3 yrs	3-6 yrs	6-9 yrs	9-12 yrs	12-15 yrs	>15 yrs
LTx-Dialysis group	100%	94%	89%	72%	60%	41%	34%
LTx-No Dialysis group	100%	83%	78%	74%	69%	66%	63%

The mean time elapsed between the initiation of chronic dialysis and death was 19±15 months (median 15) in LTx pts. Causes of death in the LTx-Dialysis group and in LTx-No Dialysis group included: cardiac (21% vs. 10%, P=0.02), vascular (2% vs. 8%, P=NS), infection (17% vs. 18%, P=NS), social (8% vs. 0.3%, P=0.0002), other (27% vs. 37%, P=NS) and not reported (25% vs. 27%, P=NS).

Survival after the initiation of dialysis

Survival	0-1 yr	1-2 yrs	2-3 yrs	3-4 yrs	4-5 yrs	>5 yrs
LTx-Dialysis group	100%	77%	60%	48%	18%	0%*
No LTx-Dialysis group	100%	85%	76%	64%	53%	43%

*P=0.0027

When compared to the LTx-Dialysis group, the causes of death in the No LTx-Dialysis group included: included: cardiac (40%, P=0.04), vascular (6%, P=NS), infection (9%, P=NS), social (19%, P=NS), other (19%, P=NS) and not reported (7%, P=0.01). Conclusion: The incidence of ESRD after LTx in Canada is low. LTx pts requiring chronic dialysis lived longer vs. LTx pts not experiencing ESRD. Survival post-dialysis initiation was lower for LTx pts vs. dialysis controls without a LTx. Cardiac and social causes are the main etiologies of death. Future studies should focus on renal protective strategies to prevent the development of ESRD after LTx.

Abstract# 1395

LIFE EXPECTANCY AFTER SUCCESSFUL LIVER TRANSPLANTATION. Arie P. van den Berg,[1] Wim J. Sluiter,[2] Els B. Haagsma,[1] Paul M. J. G. Peeters,[3] Maarten J. H. Slooff.[3]
[1]Gastroenterology and Hepatology, University Hospital, Groningen, Netherlands; [2]Internal Medicine, University Hospital, Groningen, Netherlands; [3]Hepatobiliary Surgery and Liver Transplantation, University Hospital, Groningen, Netherlands.

Backgrounds: Although the long-term prognosis after liver transplantation is generally reported as excellent, most series are uncontrolled, and there is limited information available about the survival after the 10th year post-transplant. It is unknown whether, or to what extent, liver transplant recipients regain a normal life expectancy. We therefore analyzed the survival and its determinants in all adult patients who were transplanted in our center between 1979 and 2001 and survived the first post-operative year.

Methods: The hospital and outpatient charts of all patients were reviewed. For each patient we determined the life expectancy at the time of transplantation, based on age-, sex- and era-matched dutch population controls. We determined the patient survival rates and compared them with the expected survival. We also determined the number of expected years of life that were lost due to premature death, and the fraction of remaining life expectancy after transplantation that was actually achieved. The loss of expected years was related to specific causes of death.

Results: 317 of all 402 (77%) patients survived the first year after transplantation, and constitute the study population. The median follow-up was 6.2 (range, 1.0-22.7) years. The 10- and 20-year survival rates of these 317 patients were 83% and 64%, respectively. Increasing age, original disease (HCV *vs.* all other diagnoses) and re-transplantation were independent risk factors for death. Patients died a median 10 years earlier than expected on the basis of the life expectancy of population controls. They survived for a median 54% (95% CI 4-78) of their life expectancy at the time of transplantation. This was independent of the age at transplantation.

Graft failure due to *de-novo* or recurrent disease or infection occurred in a cumulative 15% of patients; these deaths tended to occur within a few years after transplantation and led to a median loss of 25 expected years of life. Death due cardiovascular disease and cancer occurred later, but reached cumulative incidences as high as 37 and 16%, respectively, causing a loss of about 10 years of life.

Conclusions: The life expectancy of patients who have survived the first year after liver transplantation is about 10 years shorter than that of matched population controls; these patients achieved a median 54% of their life expectancy at the time of transplantation.

Abstract# 1396

PARAMETERS FOR PATIERNT SELECTION FOR LIVER RE-TRANSPLANTATION BASED ON MELD AND ALLOGRAFT CHARACTERISTICS. Rafik M. Ghobrial,[1] Yue Ming Huang,[2] Randolph Steadman,[2] Jeffrey A. Gornbein,[3] Ronald W. Busuttil.[1]
[1]Department of Surgery, David Geffen School of Medicine, Los Angeles, CA; [2]Department of Anesthesiology, David Geffen School of Medicine, Los Angeles, CA; [3]Department of Biomathematics, David Geffen School of Medicine, Los Angeles, CA.

Objective: Liver retransplantation (Re-OLT) based on Model for End Stage Liver Disease (MELD) has been associated with exceedingly poor outcomes. This study determined applicable pre-transplant parameters that accurately predict post-transplant survival, for appropriate selection of re-OLT candidates.

Methods: Multivariate Cox proportional hazard regression analyzed multiple donor and recipient variables that may impact patient survival after re-OLT. This cohort included 32.909 primary OLT and 3,276 re-OLT recipients with complete data sets obtained from the United Network for Organ Sharing (UNOS) over the last 10 years.

Results: Eight recipient and donor-related factors were identified to impact survival following re-OLT. The first three recipient-related factors were similar to the MELD parameters: log creatinine, log bilirubin, and prothrombin time with hazard ratios (HR) of 2.132, 1.06, and 1.009, respectively. Additionally, recipient age had a significant HR of 1.018. HR of Warm (W) and cold ischemia times (IT) were 1.001 and 1.007 with median levels of 51 min and 9 hrs, respectively. Re-OLT within the first 7 days, after the first transplant, markedly increased (HR 1.53); while re-OLT within 7-30 or >30 days doubled (HR 2.153 and 2.131) the risk of death following transplantation. When WIT and CIT were held at median levels, post-re-OLT survival can be accurately predicted based on the MELD score, recipient and donor ages. One-survival outcomes are propotionately worsened with advanced recipient age, increased donor age or rising MELD scores. Thus, based on the model, a 50 year-old re-OLT candidate with a MELD score of 28 exhibits a 1-yr survival of 75%, which drops to 64%, if a 30 or a 50 yr-old donor organs were to be utilized, respectively. Further, the same 50-yr old patient, if re-transplanted at a MELD score of 36 would exhibit a 59.8% survival estimate at one year. Worst patient survival outcomes (<60% at one-year) are predicted by the combination of MELD scores >24, recipient age >50 and donor age >50.

Conclusions: Survival after re-OLT can be accurately predicted by combination of the MELD scores with recipient and donor ages. This application of this re-OLT model, that incorporates the MELD and donor organ and recipient criteria, for retransplantation organ allocation would efficiently utilize a scarce donor-organ pool and maximize re-OLT survival benefis.

Abstract# 1397

CONTRIBUTION OF TRANSJUGULAR LIVER BIOPSY (TJLB) IN PATIENTS WITH ACUTE LIVER FAILURE (ALF) CLINICAL PRESENTATION IN DECIDING PATIENT SELECTION AND TIMING FOR ORTHOTOPIC LIVER TRANSPLANTATION (OLT). Angelo Luca,[1] Marta Ida Minervini,[2] Roberto Miraglia,[1] Salvatore Gruttadauria,[3] Giovanni Vizzini, Antonio Arcadipane, Bruno Gridelli.[3]
[1]Radiology, ISMETT, Palermo, Italy; [2]Pathology, ISMETT, Palermo, Italy; [3]Surgery, ISMETT, Palermo, Italy.

PURPOSE: ALF treated with conservative therapy has a poor prognosis, although individual survival varies greatly. In these patients, the eligibility for OLT must be quickly established. The aim of this study was to assess the role of TJLB in the management of patients with ALF clinical presentation.

MATERIALS AND METHODS: Eighteen patients with ALF clinical presentation were referred at a single liver transplant center during a 52-month period. In all patients the TJLB was performed using a Quick-Core needle. Specimens were immediately evaluated at the frozen section examination and than submitted for permanent histology. Clinical data, and follow up were analyzed.

RESULTS: The mean age was 45 years (range 22-66); women were the most affected (67%). At the admission prothrombin activity was 29±15%, INR 2.7±1.2, AST 2193±1956 UI, ALT 2940±2467 UI, bilirubin 15.5±8.4 mg/dl, lactate 5.3±3.9 mmol/l. Etiology was VHB in 7 cases, Nimesulide (NSAID) toxicity in 4, herbal toxicity in 1, Amanita phalloides mushroom poisoning in 1, Wilson's disease in 1, and unknown in 4. TJLB was technically successful in all patients. There were no procedure-related complications. The mean number of cores was 1.9 (range = 1-4). Tissue specimens were satisfactory for diagnosis in all cases. In 15 patients, the preliminary clinical diagnosis of ALF was confirmed by TJLB; in 3 patients the initial diagnosis of ALF was modified for the presence of unknown chronic liver disease ("acute on chronic disease"). During the follow up seven patients with necrosis < 60% were successfully treated with medical therapy; seven patients with necrosis > 70% required OLT; four patients not eligible for OLT died, three of them had "acute on chronic disease" and one a submassive necrosis. All transplanted patients are still alive. There was a strict correlation ($r=0,972$, $p<0.0001$) between necrosis at the frozen section examination and necrosis at the histology. The average time for TJLB and frozen section examination was 80 minutes.

CONCLUSION: In patients with ALF clinical presentation, the presence of submassive or massive liver necrosis and chronic liver disease are predictors of poor prognosis. TJLB and frozen section examination can be a quick and effective tool in clinical decision making, especially in deciding patient selection and the best timing for OLT.

Abstract# 1398

LIVER NK CELLS EXPRESSING TNF-RELATED APOPTOSIS-INDUCING LIGAND HAVE TOXICITY AGAINST TRANSPLANTED AUTOLOGOUS HEPATOCYTES. Hiroshi Mitsuta, Hideki Ohdan, Makoto Ochi, Takashi Onoe, Daisuke Tokita, Hidetaka Hara, Kohei Ishiyama, Wendy Zhou, Yuka Tanaka, Kentaro Ide, Toshimasa Asahara. *Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, , Hiroshima University, Hiroshima, Hiroshima, Japan.*

Activated liver NK cells target not only infected or transformed hepatocytes but also intact hepatocytes in a nonspecific fashion. But the mechanisms underlying NK cell-induced killing of self-hepatocytes are not clear. In the present study, we demonstrated that liver NK cells have cytotoxicity against normal syngeneic hepatocytes in mice. Poly I:C treatment enhanced hepatocyte toxicity of liver NK cells but not that of spleen NK cells. Unlike NK cells in other tissues, approximately 30-40% of liver NK cells constitutively express TRAIL. An *in vitro* NK cell cytotoxic assay revealed that hepatocyte toxicity of liver NK cells from both naïve and poly I:C-treated mice was inhibited partially by an anti-TRAIL mAb alone and completely by the combination with an anti-FasL mAb and perforin inhibitor, concanamycin A, indicating contribution of TRAIL to NK cell-mediated hepatocyte toxicity. Liver TRAIL$^+$ NK cells expressed high levels of CD69, indicating their naturally activating state. The majority of TRAIL$^+$ NK cells lacked expression of Ly-49 inhibitory receptors (Ly-49A, Ly-49C/I and Ly-49G2) recognizing self-MHC class I, indicating a propensity to targeting self-hepatocytes. Poly I:C treatment significantly upregulated the expression of Ly-49 inhibitory receptors on TRAIL$^-$ NK cells. However, such compensatory alteration was not seen at all in the TRAIL$^+$ NK cell fraction. Thus, TRAIL$^+$ liver NK cells have less capacity for self-recognition, and this might be involved in NK cell-dependent self-hepatocyte toxicity.In the case of hepatocyte transplantation, NK cell-mediated cytotoxicity would be a major obstacle to engraftement of autologous hepatocytes, because direct interaction of the hepatocytes transplanted via the portal vein with liver NK cells is inevitable. To address this possibility, CFSE-labeled syngeneic hepatocytes were infused into the liver via the portal vein in normal B6 mice. Seven days after hepatocyte transplantation, the fluorescence of the CFSE-labeled hepatocytes was undetectable in the liver. In contrast, when the recipient mice were treated with anti-AsGM1 sera prior to transplantation, hepatocyte grafts were clearly detected in the perisinusoidal area of the liver. These findings indicate that liver NK cell-mediated hepatocyte toxicity causes insufficient intrahepatic engraftment of transplanted hepatocytes.

Abstract# 1399

ADAPTOR PROTEINS AND HOST ALLORESPONSES: TARGETING THE SLAM FAMILY MEMBER, LY-108, PROLONGS ALLOGRAFT SURVIVAL. Qunrui Ye,[1] Christopher C. Fraser,[2] Ran Tao,[1] Liqing Wang,[1] Andrew D. Wells,[1] Andre Veillette,[3] Anthony J. Coyle,[2] Wayne W. Hancock.[1] *[1]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia & University of Pennsylvania, Philadelphia, PA; [2]Inflammation, Millennium Pharmaceuticals, Cambridge, MA; [3]Laboratory of Molecular Oncology, IRCM and University of Montreal, Montreal, QC, Canada.*

Body: Ligation of members of the SLAM (signaling lymphocytic activation molecule) family of receptors expressed on T, B and NK cells promotes intracellular tyrosine phosphorylation via interaction with SH2-containing adaptor protein, SAP (SH2D1A, DSHP), and mutations in SAP occur in patients with X-linked lymphoproliferative disorder. There are 6 known SLAM family members: SLAM (CD150), 2B4 (CD244), CD84, Ly-9, Ly-108 (NTB-A, SF2000) and CD2-like receptor activating cytotoxic cells (CRACC). As a result of our ongoing studies of the activation and function of T cells in vivo during development of allograft rejection, we report the first data on expression and significance of Ly-108 in alloresponses.

Real-time PCR showed lack of Ly-108 mRNA in normal hearts whereas allografts (BALB/c to C57BL/6) showed strong expression of the gene, as did murine thymus, spleen, and CD4+ and CD8+ T cells. Analysis of rejecting cardiac allografts by immunohistology showed localization of both Ly-108 and SAP proteins to infiltrating mononuclear cells. To test the importance of Ly-108 expression on allograft survival, we generated Ly-108-/- mice by homologous recombination. Proliferation of T cells stimulated in vitro with CD3 and CD28 mAbs was comparable for wild-type and Ly-108-/- mice. However, adoptive transfer studies of CFSE-labeled cells across a partial MHC-mismatched (parent to F1) showed decreased proliferation of Ly-108-/- CD4, CD8 and NK cells vs. wild-type cells, especially when recipients were treated with a low dose of rapamycin. Consistent with this, survival of fully MHC-mismatched cardiac allografts in Ly-108-/- recipients was significantly prolonged compared to controls, and use of a subtherapeutic protocol of rapamycin (0.1 mg/kg/day, 14 d), which in wild-type controls led to rejection by 15 d post-Tx, led to mean survival of >100 d in Ly-108-/- recipients ($p<0.001$). Comparison of the latter 2 groups showed a marked suppression of IFN-g, chemokine and chemokine receptor expression in Ly-108-/- recipients.

In summary, using a knockout of a hitherto little studied member of the SLAM family, we report the first data concerning the remarkable therapeutic efficacy of targeting adaptor proteins in vivo. Targeting of such adaptor molecules provides a new approach to immunomodulation which may prove both highly selective and effective in regulating alloresponses and promoting long-term allograft survival.

Abstract# 1400

THE INDUCIBLE NF-KB ACTIVATING KINASE, IKKE, PROMOTES T CELL ACTIVATION AND PROLIFERATION IN VIVO DURING ALLORESPONSES. Tao Wang,[1] Sha-Mei Liao,[2] Tom Maniatis,[3] Anthony J. Coyle,[2] Wayne W. Hancock.[1] *[1]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Inflammation, Millennium Pharmaceuticals, Cambridge, MA; [3]Molecular and Cellular Biology, Harvard University, Cambridge, MA.*

Key TCR-generated signals essential for T cell activation and proliferation include kinase-mediated activation of NF-kB and induction of cytokines and cytokine receptors. The classical NF-kB signalosome involves IKKa, IKKb and IKKg. Targeting of this complex has been dogged by the limited specificity and potency in vivo of various small molecules, and by the ubiquitous distribution of this complex. Recently, an additional IKK, IKKe (IKK-i) was described which is involved in the activation of T cell through CD3 and CD28 costimulation in vitro, though little is yet known as to the functions of this kinase in vivo. We report the first data concerning the role of IKKe in vivo.

Of 18 tissues examined using real-time PCR, IKKe mRNA was expressed primarily in thymus, spleen, lymph nodes and peripheral blood leukocytes. Serially harvested mouse cardiac allografts (BALB/c to B6/129) showed IKKe mRNA expression was highly inducible during allograft rejection, rising from 3 d in conjunction with a T cell influx and peaking at up to 60-fold increase at 6 d, prior to rejection; the basal levels of IKKe were unchanged in isografts. The rise in intragraft IKKe expression correlated with the results of Western blotting and functional assays (immunoprecipitation and IKKe-dependent phosphorylation of NF-kB/p65). IKKe was localized to infiltrating T cells in rejecting murine cardiac allografts and rejecting human renal allografts. By contrast, IKKa, IKKb and IKKg mRNA were constitutively expressed in native hearts, isografts and allografts, and showed only minor changes (<2-fold increase) during allograft rejection. We therefore sought to analyze the importance of IKKe in alloresponses by generating IKKe-/- mice.

Studies using adoptively transferred CFSE-labeled T cells (wild-type vs. IKKe-/- donors, parent to F1 model) showed that alloactivation and proliferation of both CD4 and CD8 T cells was diminished using IKKe-/- vs. control cells. In addition, whereas control mice rejected cardiac allografts by 7 d, survival in IKKe-/- was prolonged to up to 18 d ($p<0.05$), and the benefits of targeting IKKe were enhanced by use of low-dose CsA ($p<0.005$). Analysis at 5 d post-Tx showed that in the absence of IKKe, intragraft expression of the other IKKs was largely unchanged but a marked impairment of IL-2 mRNA production was seen as compared to wild-type controls.

We conclude that IKKe is a new and important target for potential therapy in experimental and clinical allografts.

Abstract# 1401

GENERATION OF POLYCLONAL ALLOSPECIFIC MEMORY BY SORTING ALLOACTIVATED CD25$^+$ T CELLS. Adam W. Bingaman,[1] David B. Leeser,[1] Donna L. Farber.[1] *[1]Department of Surgery, University of Maryland, Baltimore, MD.*

Preface: Memory CD4 T cells specific for alloantigens can play a significant role in allograft rejection and resistance to tolerance induction. However, little is known regarding the mechanism of action of memory cells in transplantation since no system exists to generate a large population of polyclonal allospecific memory cells. Because antigen-specific memory T cells can be generated from effector cells *in vivo*, we hypothesized that alloactivated CD25$^+$ T cells would give rise to allospecific memory T cells in adoptive hosts. **Methods:** Mixed lymphocyte reactions were set up using Balb/c CD4 T cells and B6 APC. After 4 days, cells were sorted into CD25$^+$ and CD25$^-$ subsets and each subset was transferred into RAG2$^{-/-}$ hosts. Splenic memory T cells persisting 4 months post-transfer from recipients of CD25$^+$ (memory^{CD25+}) and CD25$^-$ (memory^{CD25-}) precursors were analyzed for cytokine production, proliferation, and accelerated rejection. *In vitro*, cells were labeled with CFSE, reactivated with donor specific (B6) or third party (B10BR) APC and assayed for IFNγ and divisions by flow cytometry from 12-84 hours (h). For *in vivo* studies, naïve, memory^{CD25+} or memory^{CD25-} CD4 T cells were adoptively transferred into RAG2$^{-/-}$ recipients of B6 or B10BR skin grafts and assayed for graft survival. Naïve CD4 cells were used as controls in all studies. **Results:** Persisting T cells in adoptive hosts were small, CD25$^-$ and CD44hi, consistent with resting memory cells. The response of memory^{CD25+} cells to B6 APC *in*

vitro was remarkable in that 10% produced IFNγ as early as 12h with a maximal response of 40% IFNγ+ at 60h and 32% undergoing cell division by 84h. Response to third-party APC was minimal, demonstrating the donor-specific nature of the recall response. In contrast, IFNγ was produced by few (<2 %) naïve cells at all time points with 6% of cells undergoing division in response to B6 APC, while <0.5% of memory^{CD25-} cells produced IFNγ or divided at any time point. *In vivo*, memory^{CD25+} cells rejected B6 and B10BR grafts with MST 16d and 25d, respectively, whereas naïve CD4 T cells rejected B6 and B10BR skin grafts with MST 21d and 23d, demonstrating accelerated donor-specific rejection. Memory^{CD25-} cells did not reject B6 or B10BR skin grafts for >30d. **Conclusions:** We have developed a novel method of sorting on alloactivated CD25+ T cells to generate polyclonal allospecific memory CD4 T cells. These data establish that alloreactive CD25+ cells are precursors of potent memory cells which can be utilized as a powerful tool to study allospecific memory responses *in vitro* and *in vivo*.

Abstract# 1402

T CELL REPERTOIRE EXPANSION BY B CELLS AND IMMUNOGLOBULIN. Marilia Cascalho,[1,3,5] Cristina Joao,[1] Brenda M. Ogle,[1,2] Jeffrey L. Platt.[1,3,4,5] *[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.*

The classic reports on lymphocyte development hold that the Bursa of Fabricious gives rise to B cells and the thymus to T cells. Since these reports use transplants as a model for T cell responses, they would infer that rejection of grafts – at least of skin and tumor grafts – depends on T cells and hence only on the thymus. Our recent findings however expand this concept. T cell diversity was once thought to depend on the interaction of T cell precursors with thymic epithelial cells. Recent evidence suggests, however, that diversity might arise through interaction of developing T cells with other cells, the identity of which is not known. Here we report that T cell diversity is driven by B cells and by immunoglobulin. T cell receptor Vβ diversity of thymocytes in mice that lack B cells and immunoglobulin is reduced to 6 x 10^2 from wild type values of 1.1 x 10^8; in mice with oligoclonal B cells T cell receptor Vβ diversity of thymocytes is 0.01% of wild type. Adoptive transfer of diverse B cells or administration of polyclonal immunoglobulin increases thymocyte diversity in mice that lack B cells, 8 and 7 fold, respectively, while adoptive transfer of monoclonal B cells or monoclonal immunoglobulin does not. These findings reveal a heretofore unrecognized and vital function of B cells and immunoglobulin for generation of T cell diversity and suggest a potential approach to immune modification for transplantation – that depletion of B cells may constrain T cell responses independent of changes in humoral immune responses.

Abstract# 1403

ICOS AND OX40 MEDIATED COSTIMULATION IS REQUIRED FOR ALLOREACTIVE MEMORY CD8 T CELL ACTIVATION. Wei Gao,[1] Yuan Zhai,[1] Ronald W. Busuttil,[1] Hideo Yagita,[3] Mohammed H. Sayegh,[2] Jerzy W. Kupiec-Weglinski.[1] *[1]The Dumont-UCLA Transplant Center, Surgery, David-Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Laboratory of Immunogenetics and Transplantation, Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [3]Juntendo University, Japan.*

Although T cell costimulation blockade markedly prolongs allograft survival in various animal models, one emerging problem is its failure to control memory T cell-mediated rejection. A number of groups, including our own, have shown that host response to costimulation blockade is very sensitive to its immunological history. Not only allogeneic, but also cross-reactive viral/infectious agent-derived antigens, may prime recipients to abrogate the protective effects of costimulation blockade. Memory CD8+ T cells are considered the primary effector cells that escape the costimulation blockade. We have established a skin-sensitized murine cardiac allograft model to study memory CD8+ T cell-mediated rejection. We have shown that donor skin graft (Balb/c, 40 days prior to heart Tx) primed C57BL/6 recipients to reject subsequent cardiac allografts in an accelerated manner (MST=4.5d vs. 7d in naive mice). CD154 blockade (0.5mg at the time of heart Tx), which uniformly prolongs cardiac allograft survival in naive B6 mice (ca. 50 days), failed to protect cardiac allografts (MST=4.5±2d; n=8) in skin-sensitized hosts. We have also shown that CD8 depletion in conjunction with CD154 blockade synergistically prolonged cardiac allograft survival in sensitized recipients (MST=40±6d; n=6). In this study, we tested the role of three new costimulation pathways, 4-1BB, ICOS, OX40, all of which have been found to be expressed on activated T cells, in the memory alloreactive CD8+ T cell-mediated rejection. A single dose of blocking-antibodies (0.5mg, i.v.) against ligands of each of the three costimulatory molecules at the time of cardiac Tx, resulted in significant prolongation of graft survival in skin-sensitized recipients (anti-4-1BBL MST=15 days; anti-ICOSL MST>25 days; anti-OX40L MST>25 days), which was in sharp contrast to untreated (MST=5 days) or anti-CD154 treated (MST=4 days) controls. Correlated with cardiac allograft survival, alloreactive CD8+ T cell activation was increased in untreated (% of CD44^{high}CD62L^{low} in total CD8 in PBL at day 10 post heart Tx, 5% to 25%) and CD154-treated (26.4%) recipients, but totally suppressed in anti-ICOSL- (3.1%) and anti-OX40L- (2.0%) treated recipients. Interestingly, anti-4-1BBL-treated mice showed a modest increase in activated CD8+ T cells (8.2%). In conclusion, ICOS and OX40 signalling are involved in the activation of memory CD8+ T cells, and their blockade might provide much needed novel therapies to inhibit memory T cell-mediated allograft rejection.

Abstract# 1404

CONTRIBUTION OF CD4 AND CD8 T CELLS TO SKIN AND VASCULARIZED CARDIAC ALLOGRAFT REJECTION IN THE ABSENCE OF CD28. Michael R. Clarkson,[1] Xueli Yuan,[1] Reshma Kewalramani,[1] Toshiro Ito,[1] Hugh Auchincloss, Jr.,[1] Nader Najafian,[1] Mohamed H. Sayegh.[1] *[1]Transplantation Research Center, Brigham and Women's Hospital, Boston, MA.*

T cell costimulation via the CD28-B7 pathway plays an important role in the regulation of alloimmune responses. Blockade of the CD28:B7 pathway with anti-B7 mAbs or CTLA4Ig prevents acute allograft rejection and induces tolerance in several, but not all, murine models of transplantation. In primates, B7 blockade delays allograft rejection but does not induce tolerance, and human trials with CTLA4Ig to spare calcineurin inhibitors are ongoing. The presence of CD28-independent pathways of allograft rejection is confirmed by the findings that CD28 deficient recipients reject both skin and vascularized grafts, albeit the latter with some delay. Understanding the effector mechanisms of CD28-independent alloimmune responses is critical for development of novel tolerogenic strategies involving CD28-B7 blockade to translate to primates and humans. We sought to explore the mechanisms of CD28-independent allograft rejection and specifically test the relative contribution of CD4 versus CD8 T cells in both skin and cardiac transplantation models. C57BL/6 mice deficient in CD28 were crossbred with either CD4 or CD28 deficient mice and F2 offspring were genotyped by PCR to establish founder colonies deficient in both CD28 and CD4 or CD8. We then investigated the role of CD4+CD28- and CD8+CD28--independent rejection using full thickness trunk skin and vascularized cardiac BALB/c allografts. The results are illustrated in the table below (all p values are versus WT recipients):

Skin	Mean Survival Time	p value	Heart	Mean Survival Time	p value
Wild Type	14.2+/-0.8	N/A	Wild Type	7.3+/-0.5	N/A
CD4 KO	13.3+/-1.2	0.2	CD4 KO	96.8+/-31	<0.0001
CD8 KO	12.7+/-1.2	0.07	CD8 KO	12.4+/-3.5	0.015
CD28 KO	15.3+/-0.3	0.08	CD28 KO	17.6+/-3.1	<0.0001
CD4CD28 KO	15+/-0.8	0.19	CD4CD28 KO	>30 (n=4)	<0.0001
CD8CD28 KO	14.5+/-1	0.6	CD8CD28 KO	90+/-67	0.004

In the absence of CD28, neither CD4 nor CD8 T cells alone can mediate acute rejection of vascularized cardiac allografts, while either cell alone can effectively reject skin allografts. These results point to the differential roles of CD28-independent CD4 versus CD8 T cells in contributing to vascularized versus skin allograft rejection, and provide novel tools for studying the role of the new T cell costimulatory pathways in activation of these cells *in vivo*.

Abstract# 1405

INEFFECTIVE ACTIVATION OF GRAFT-SPECIFIC CD8 T CELLS AFTER HEART BUT NOT SKIN TRANSPLANTATION. Alexander Filatenkov,[1] Elizabeth Ingulli.[1] *[1]Center for Immunology, University of Minnesota, Minneapolis, MN.*

INTRODUCTION: T cells are understood to play a critical role in the rejection of various solid organs. Since the mechanisms of rejection of skin and cardiac grafts may be different, we were interested in comparing the activation of graft-specific CD8 T cells after skin or heart transplantation. **METHODS**: To address this, we have used a mouse model system in which we track, in vivo, the behavior of T cell receptor (TCR)-transgenic chicken ovalbumin (OVA)-specific CD8 and CD4 T cells in response to skin grafts mismatched for a single foreign "minor" antigen, OVA. Donor heart graft is obtained from transgenic mice that express OVA under the control of chicken beta-actin promoter on the surface of most cells (Act-mOVA). OT-I RAG deficient (CD8) and/or OT-II RAG deficient (CD4) TCR transgenic T cells were adoptively transferred into wild type congenic B6 recipients. This system allowed us to track graft-specific T cells and evaluate their contribution to graft rejection without risk of homeostatic proliferation as would be observed using lymphopenic recipients. Recipient mice received full thickness skin or heterotopic heart grafts from Act-mOVA B6 mice. **RESULTS**: OT-I cells accelerated skin graft rejection above the endogenous T cell response (mean survival time, MST=21 vs 34 days). Activation of OT-I cells was localaized to the draining lymph nodes. Intersestingly, after heart transplantation, adoptively transferred OT-I cells failed to accelerate graft rejection above the endogenous response (100% heart grafts survived > 40 days in B6 mice that received OT-I cells and in B6 mice that did not receive OT-I cells). Depite the inability to accelerate graft rejection, OT-I cells were not igorant but rather demonstrated evidence of antigen exposure as seen by proliferation (dilution of CFSE), clonal expansion (2-3 fold), and expression of CD25. This activation was systemic; observed in all lymph nodes and spleens. However, OT-I cells did not produce IFN-gamma. This effector defect was overcome upon the co-transfer of OT-II cells. Under those circumstances proliferation, expansion (8-10 fold), CD25 expression, production of IFN-gamma and graft rejection were all enhanced (MST=14 days). **CONCLUSION**: These data suggest that different routes of the antigen administration (systemic circulation vs localized lymphatic drainage) as well as different degree of surgical trauma inflicted during grafting may all play a role in determining the effector pathway of CD8 T cells activation perhaps by affecting the activation a specific dendritic cell subset.

Abstract# 1406

TRANSITION, MAINTENANCE, AND RECALL OF ALLOREACTIVE CD4 AND CD8 MEMORY T CELLS INDEPENDENT OF EACH OTHER AND OF SECONDARY LYMPHOID ORGANS. Jagdeep S. Obhrai,[1] Geetha Chalasani,[1] Lonnette A. Smith-Diggs,[1] Fadi G. Lakkis.[1] *[1]Internal Medicine and Immunobiology, Yale University, New Haven, CT.*

Memory T cells pose a significant risk to transplanted organs because of their survival, migration, and activation advantages over naive T cells. Unknown, however, is whether CD4 and CD8 memory T cells share the same advantages. Here, we compared the ability of antigen-experienced CD4 vs CD8 alloreactive, wildtype (polyclonal) T cells to transition to the memory phenotype and be maintained and recalled independent of each other and of secondary lymphoid organs.

Methods: Wildtype B6 mice were immunized with BALB/c splenocytes to generate either effector or memory T cells. Effector and memory T cells were harvested 4 days or >10 weeks after immunization, respectively, and purified by magnetic separation and high speed sorting (> 98% CD8+CD44high and CD4+CD44high T cells were obtained). 0.5 to 1 million cells of either subpopulation were transferred to splenectomized aly mice which lack secondary lymphoid organs. 12 weeks later, BALB/c skin grafts were placed to test for rejection. Rejection in this model denotes the presence of memory T cells as splenectomized aly mice do not mount primary alloimmune responses.

Results: Either CD4 or CD8 memory T cells alone mediated the rejection of skin allografts even if the transplant procedure was delayed for 12 weeks after cell transfer (Table 1). Similarly, either CD4 or CD8 effector T cells alone transitioned to the memory phenotype and mediated skin transplant rejection 12 weeks later (Table 2). However, more effector CD4 than CD8 T cells were required to generate the same memory response (Table 2). Control mice that received purified naive T cells (CD4+CD44low or CD8+CD44low) did not reject allografts.

Memory maintenance and recall

Population Transferred	Graft Survival (days)
Memory CD4 (0.5 million)	24, 28, 28
Memory CD8 (0.5 million)	32, 38, 40, 45

Transition from Effector to Memory

Population Transferred	Graft Survival (days)
Effector CD4 (0.5 million)	31, 106, 136
Effector CD4 (1 million)	38, 38, 38, 49, 55, 110
Effector CD8 (0.5 million)	38, 38, 45, 45, 49, 55

Conclusions: Alloreactive CD4 and CD8 memory T cells can be generated from effector T cells and can be maintained and recalled independent of each other and of secondary lymphoid organs. This is in contrast to viral immunity where CD8 memory generation and recall is dependent on the presence of CD4 help. Our findings underscore the threat of extralymphoid CD4 and CD8 memory T cells to the longterm survival of transplanted organs.

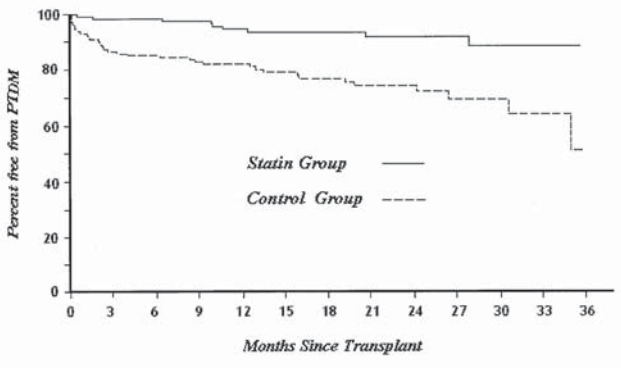
Metabolic Complications and Hypertension After Kidney Transplantation

Abstract# 1407

HMG-CoA REDUCTASE INHIBITORS (STATINS) REDUCE THE INCIDENCE OF POST-RENAL TRANSPLANT DIABETES MELLITUS (PTDM). G. V. Ramesh Prasad,[1,2] S. J. Kim,[1] M. Huang,[2] M. Thenganatt,[2] J. Park,[3] M. M. Nash,[2] J. S. Zaltzman,[1,2] D. C. Cattran,[1,3] S. S. A. Fenton,[1,3] E. H. Cole,[1,3] C. J. Cardella.[1,3] *[1]Medicine, University of Toronto; [2]Renal Transplant Program, St. Michael's Hospital, Toronto; [3]Multi-Organ Transplant Program, University Health Network, Toronto, ON, Canada.*

Background: Statins have anti-inflammatory effects, modify endothelial function, and improve peripheral insulin resistance. We hypothesized that statins influence PTDM development in renal transplant recipients (RTR), a population at high risk. **Methods**: The records of all previously non-diabetic adult single-organ RTR in Toronto between 01/01/99 and 31/12/01 were reviewed with followup to 31/12/02. All RTR receiving a CNI (CsA/Tac), MMF, and prednisone were included. PTDM was diagnosed based on current Canadian Diabetic Association criteria: FBG ≥7.0 mmol/l or 2-h PPG ≥11.0 mmol/l on ≥2 occasions. Statin use prior to PTDM development was recorded alongwith other potential predictors. Multivariate logistic regression analysis and Cox proportional hazards models utilizing PTDM as a time-dependent covariate were separately performed. Kaplan-Meier survival curves were compared by the log-rank test. **Results**: 297 RTR met entry criteria. PTDM incidence was 16% (N=49). PTDM and non-PTDM groups did not differ at baseline. In univariate analysis, statins (p=0.0001), ACE inhibitors (p=0.02), black race (p=0.03), acute rejection (p=0.02), and prednisone dose (p<0.0001) were associated with PTDM. By multivariate logistic regression, statins (p=0.003; RR 0.28[0.12-0.65]), prednisone dose (p<0.0001; RR 2.47[1.78-3.45] per 5mg/d) and acute rejection (p=0.03; RR 2.86[1.11-7.35]) were significant. In the multivariate Cox model, statins (p=0.003, HR 0.32[0.15-0.69]), prednisone dose (p=0.003, HR 1.006[1.002-1.009]), and weight at transplant (p=0.01, HR 1.02[1.004-1.041] per 5kg) were significant. These effects were independent of lipid levels.

Figure 1



Conclusions: Statins may prevent PTDM development in RTR and should be considered in all de-novo RTR to minimize PTDM risk. Prospective evaluation of statin therapy to prevent DM in high risk populations is warranted.

Abstract# 1408

THE USE OF SIROLIMUS AND MYCOPHENOLATE MOFETIL IS ASSOCIATED WITH IMPROVED RENAL FUNCTION, BUT SIMILAR METABOLIC PROFILES TO SIROLIMUS AND TACROLIMUS. Agnes Lo,[1] M. Francesca Egidi,[2] Benjamin Gross,[1] 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: This study examines the renal allograft function and metabolic profiles of sirolimus (SIR) treated renal transplant recipients in combination with either tacrolimus (FK) or mycophenolate mofetil (MMF).

Methods: This is a sub-analysis of a prospective, randomized, single center study. Fifty-nine cadaveric renal transplant recipients were randomized to receive either SIR+FK+Steroids (n=37) or SIR+MMF+Steroids (n=22). Patients with diabetes pretransplant were excluded. Metabolic profiles evaluated were body mass index (BMI), fasting cholesterol (Chol) and triglycerides (TG) levels, incidence of post-transplant diabetes mellitus (PTDM), and metabolic syndrome. All patients had at least 1 year follow-up.

Results: There were no differences in patient demographics between the two groups with 58% male, 78% African-American, and a mean age of 42 ± 12 years. The safety and efficacy data has been previously reported. At 1 year, the serum creatinine was significantly lower in the SIR+MMF group (1.3 ± 0.3 mg/dL) than the SIR+FK group (1.8 ± 0.7 mg/dL), p=0.03. By study design, SIR+MMF group had higher SIR trough levels (12.7±5.9 ng/ml) than SIR+FK group (10.2±4.5 ng/ml), p<0.01. The mean FK trough level was 5.0±2.2 ng/ml during the first year post-transplant. Prednisone daily doses were similar between the two groups. Regardless of regimens, all patients had significant increased in BMI from 26.8 to 28.4 and in Chol (↑21%) and TG (↑ 27%) levels, p<0.01. Lipid lowering therapy was required in 88% of patents. The prevalence of metabolic syndrome was 27% pre-transplant and 1 year incidence of 45%. There were no differences in changes in Chol and TGs and the incidences of PTDM and metabolic syndrome between the groups.

	SIR+FK* (n=37)	SIR+MMF* (n=22)
Cholesterol (pre)**	181±35	168±40
Cholesterol (12 months)	222±77	190±57
Triglycerides (pre)**	162±73	167±119
Triglycerides (12 months)	216±159	248±107
Lipid lowering therapy (pre)**	14%	18%
Lipid lowering therapy (12 months)	89%	86%
Metabolic Syndrome (Pre)**	27%	27%
Metabolic Syndrome (12 months)	51%	36%
PTDM	41%	27%

*p=NS (SIR+FK vs. SIR+MMF), **p=NS (Pre vs. 12 months)

Conclusions: The substitution of FK in a SIR-based triple immunosuppressive regimen with MMF has significant advantage in regards to renal allograft function, but does not alter the metabolic complications of SIR.

Abstract# 1409

USE OF EZETIMBE IN HYPERCHOLESTEROLEMIC KIDNEY TRANSPLANT PATIENTS. Jacob P. John,[1] Joseph Lawen,[1] Bryce A. Kiberd.[2] *[1]Department of Urology, Queen Elizabeth II HSC, Halifax, NS, Canada; [2]Department of Medicine, Queen Elizabeth II HSC, Halifax, NS, Canada.*

Hypercholesterolemia is prevalent in the kidney transplant population. The recommended goal of therapy is an LDL cholesterol <2.6 mmol/L. At our center about half of kidney transplant patients (49%, 220/449) followed in our clinic are on a statin. A significant number of patients (63%, 139/220) on statin therapy are above target (>2.6 mmol/L). Of the 139 statin treated patients above target, 95 (68%) have LDL cholesterols that are between 2.6 and 3.6 mmol/L, the remaining 44 (32%) have an extremely elevated LDL (>3.6 mmol/L). In addition a small proportion of our patients have been intolerant (3) or developed rhabdomyolysis (4) with statin therapy and some are reluctant to start this drug given the bad lay press (9). In order to improve LDL cholesterol control we examined the short-term effect of the oral cholesterol absorption inhibitor, ezetimibe 10 mg daily, in stable kidney transplant recipients.

Ten patients received the drug. Of these 5 were on tacrolimus, 2 on cyclosporine and 3 on sirolimus (no CNI). Patients were 48±13 years old, weight 84± 34 kg, 5/10 were female, 4/10 were diabetic and 8/10 were on a statin (2 were intolerant of statin therapy). The table shows the mean effect of ezetimibe on the fasting lipid profile after 4 weeks of therapy. There were significant improvements in total cholesterol, LDL cholesterol and the cholesterol ratio (TC/HDL). There was no effect on drug levels or serum creatinine. The medication was well tolerated. There was no difference in effect among patients on either CNI or no CNI or whether on a statin or not.

Our results show that the proportion of kidney transplant patients with LDL cholesterols levels above target are substatntial. The use of ezetimibe improves lipid control in this population and is well tolerated. In addition the drug is effective in patients who cannot tolerate statins. Although the effect is limited to about 1 mmol/L reduction in LDL, up to 68% of the patients presently not in target could potentially reach target. How much this translates into reduced CVD events is not known.

Effect of Ezetimibe on Lipids

	Total Chol	LDL	HDL	TG	Ratio	Creatinine
Pre	7.06±1.16	4.22±0.81	1.25±0.59	3.88±1.22	5.86±1.95	154±60
Post	5.78±1.61	3.13±1.11	1.45±0.71	2.63±1.14	4.34±1.37	146±65
% change	-20±13	-30±15	+15±25	-16±36	-27±22	-1±12
p value	0.0029	0.0008	0.13	0.27	0.043	0.95

Abstract# 1410

A PROSPECTIVE, CROSS-OVER TRIAL COMPARING FLOW-MEDIATED BRACHIAL ARTERY VASODILATION IN HYPERCHOLESTEROLEMIC RENAL TRANSPLANT PATIENTS TREATED WITH CYCLOSPORINE A + ATORVASTATIN, TACROLIMUS ALONE AND TACROLIMUS + ATORVASTATIN. K. Martin Wissing,[1] Philippe Unger,[2] Guy Berkenboom,[2] Isabelle Dupont,[3] Nilufer Broeders,[1] Daniel Abramowicz.[1] *[1]Nephrology, Hopital ULB-Erasme, Brussels, Belgium; [2]Cardiology, Hopital ULB-Erasme, Brussels, Belgium; [3]NUTRISUB, Hopital ULB-Erasme, Brussels, Belgium.*

Background: Renal transplant patients frequently have endothelial dysfunction and an impairment of endothelium-dependent arterial vasodilation. The aim of our study was to compare the effects of atorvastatin and conversion to tacrolimus (TRL) on endothelial function of renal transplant patients with hypercholesterolemia.

Methods: Twelve kidney transplant recipients with total cholesterol >240 mg/dl or LDL cholesterol >130 mg/dl and stable graft function under CsA were enrolled. Patients had study visits every three months and treatment was changed 1 week after each study visit. Patients were successively crossed over from CsA alone (baseline) to: 1) CsA plus atorvastatin 10 mg/d (CsA-statin); 2) CsA alone (washout); 3) conversion to TRL (TRL) and 4) TRL plus atorvastatin 10 mg/d (TRL-statin). Endothelial function was assessed by high resolution ultrasound measurement of flow-mediated vasodilation (FMD) during reactive hyperemia following 5 minutes of pneumatic forearm occlusion. FMD was expressed as the percentage increase of brachial artery diameter during reactive hyperemia.

Results: Atorvastatin and conversion to tacrolimus were efficient in reducing LDL-cholesterol. Plasma levels of LDL-cholesterol were 160±41 mg/dl (baseline) ; 89±22 mg/dl (CsA-statin), P<0.0001 vs baseline ; 150±26 mg/dl (washout) ; 129±22 mg/dl (TRL), P=0.01 vs baseline ; 80±21 mg/dl (TRL-statin), P<0.0001 vs TRL, P=NS vs CsA-statin. At baseline renal transplant patients had a FMD of 4.0±1.7% which was significantly lower than FMD in 9 non-transplanted control subjects (6.5±1.0%; P=0.001). Atorvastatin therapy increased FMD to 6.4±4.0% (P<0.05 vs baseline). After cessation of atorvastatin therapy FMD values returned to baseline levels (FMD 4.2±2.3%). Conversion from CsA to TRL slightly but significantly improved FMD (5.1±2.1%, P<0.05 vs baseline). When atorvastatin therapy was added to TRL this had no detectable effect on FMD (5.3±2.4%, P=NS vs TRL).

Conclusions: Our population of CsA-treated renal transplant patients with hypercholesterolemia had an impairment of endothelium-dependent flow-mediated brachial artery vasodilation. Treatment with atorvastatin restored endothelial function close to levels observed in a normal control population. Conversion from CsA to tacrolimus caused a slight improvement of endothelial function.

Abstract# 1411

STEROIDS HAVE NO CALORIES: WEIGHT GAIN POST TRANSPLANTATION IS NOT PREVENTED BY STEROID AVOIDANCE. E. A. Elster,[1] C. Chamberlain,[2] C. Salaita,[2] A. D. Kirk,[1] D. Hale,[1] Roslyn B. Mannon.[1] *[1]Transplantation Branch, NIDDK/NIH/DHHS, Bethesda, MD; [2]Clinical Center, NIH, Bethesda, MD.*

Obesity is a major health care issue in the general, end stage renal disease (ESRD), and renal transplant populations. Previous studies have suggested that chronic corticosteroids increase body weight after transplant. With the recent adoption of steroid-free immunosuppressive strategies, we evaluated the effect of steroid avoidance on body mass index. We examined 101 renal transplant recipients enrolled in NIH clinical transplant trials that received one of three immunosuppressive regimens: 1) steroid-free depletional induction therapy with Alemtuzumab followed by sirolimus or tacrolimus monotherapy; 2) steroid-free high-dose (20mg/kg) rabbit ATG (hRATG) induction followed by sirolimus or tacrolimus monotherapy; and 3) standard triple immunosuppression that included prednisone. Some recipients of hRATG had acute rejections and were converted to chronic prednisone therapy. The results are shown in the table below. Similar to the ESRD population on dialysis, recipients prior to transplant were overweight (BMI 26.0±0.5 kg/m²). There were no significant differences in pretransplant BMI between treatment groups. Despite of the avoidance of steroids, BMI increased post transplant in both depletional steroid-free strategies as well as standard therapy recipients. This increase in BMI represented an average weight gain of 4.6±1.4 kg post transplant. The lowest increase in BMI was seen in the hRATG group, although this was not significantly different than either the Alemtuzumab or standard therapy groups. Additionally, steroid withdrawal at a mean of 100 days post transplant had no impact on weight gain. Interestingly, the addition of chronic steroids following an acute rejection episode in the hRATG group was associated with a significant loss of weight compared to both Alemtuzumab and standard patients (p=0.01). These results demonstrate the overall increase in weight gain following kidney transplantation. Alternative strategies to prevent significant weight gain aside from steroid withdrawal should be considered. Understanding the underlying physiologic milieu that controls weight gain needs to be explored further.

Treatment	PreTx BMI (Mean± SEMkg/m²)	PostTx BMI (Mean±SEM kg/m²)	Change in BMI
Alemtuzumab (N=22)	26.0±1.2	27.9±1.5	1.9±0.6
hRATG (N=12)	26.8±1.4	27.3±1.1	0.5±0.9
Standard (N=30)	25.1±0.8	26.9±0.8	1.7±0.5
Pred Taper (N=19)	25.7±0.9	27.5±1.4	1.8±1.0
hRATG+Pred (N=6)	29.9±3.0	28.6±2.6	-1.3±1.6*

*p=0.01 compared to Standard and Alemtuzumab

Abstract# 1412

PREDICTIVE DIFFERENCES AND MISSCLASSIFICATION BETWEEN CLINIC (Dinamap), AMBULATORY (ABPM), AND HYPERTENSION NURSE (RN) BLOOD PRESSURE MEASUREMENTS IN KIDNEY RECIPIENTS ONE YEAR AFTER TRANSPLANTATION. Stephen C. Textor,[1] Mouhammed AbuAttieh,[1] Adit Mahale,[1] Sandra J. Taler,[1] Driscoll Nancy,[1] Augustine Jo Ellen,[1] Larson S. Timothy,[1] Gloor M. James,[1] Griffin D. Matthew,[1] Schwab R. Thomas,[1] Cosio G. Fernando,[1] Stegall D. Mark.[1] *[1]Transplant Center, Mayo Clinic, Rochester, MN.*

Hypertension after kidney transplantation poses major CV risk and affects long-term allograft outcome. The aims of this study were to compare different BP monitoring methods and to correlate these with renal function after kidney Tx.

Methods: We studied 103 kidney Tx recipients (age 51.5 years, range 20-80 years) (89 living and 14 deceased donor) one year after Tx using clinic oscillometric BP (Dinamap), ambulatory (awake ABPM), and AHA trained RN readings. GFR was measured by iothalamate clearance and resistive index by ultrasound during protocol biopsy. Immunosuppression consisted of tacrolimus (72), sirolimus (23) or cyclosporine (8) along with mycophenolate and steroids. Mean creatinine was 1.55±.05 mg/dL and GFR was 55±2 ml/min/1.73m2. Overall, 87 % were identified as hypertensive, 84% of which were treated with medication (1.7 drugs /patient). BP Control rates ranged between 52% (clinic) and 81% (RN) during Drug RX. Concordance (Conc) was defined as agreement with clinic BP classification as "normotensive" or "hypertensive".[table] Despite BP therapy, Awake ABPM BP levels predicted reduced GFR (R=-0.41, p<.0001) better than clinic BP readings (R=-.24, p=.02). ABPM further predicted renal resistive index, which was highest in tacrolimus treated patients despite GFR similar to sirolimus regimens. Day-night Bp change was reduced compared to normal subjects (Day-night delta SBP: 6±2 vs 14±2 mm Hg, p<.001). Despite similar GFR and clinic BP, deceased donor kidney recipients had higher ABPM (142±4 / 73±2 mm Hg vs 135±1 / 79±1 mm Hg, p< 0.05). These data demonstrate important differences and potential misclassification between BP methods one year after transplantation. Elevated ABPM BP and loss of day-night rhythms independently predict cardiovascular events in hypertensive populations. Our results further indicate that ABPM is most closely associated with reduced GFR and elevated renal resistive index in treated hypertensive Tx recipients. These results will allow more precise characterization and management of BP in the long-term care of renal Tx recipients.

		Normotensive (n=56)	Conc	Hypertensive (n=47)	Conc
Clinic (Dinamap)	SBP	123±2		154±2	
<140/90 mmHg)	DBP	70±1		83±2	
ABPM (Awake:	SBP	131±2*	61%	141±2**	64%**
<135/85 mm Hg)	DBP	76±2*		81±1	
RN (AHA)	SBP	121±2	68%	137±3**	22%**
<140/90 mm Hg)	DBP	69±1		75±2	

Mean±SEM: *p<.05, **p<.01 vs Clinic BP

Abstract# 1413

METABOLIC COMPLICATIONS PRE AND POST RENAL TRANSPLANTATION. Agnes Lo,[1] Barbara Culbreath,[2] M. Francesca Egidi,[3] Ann Cashion,[4] A. Osama Gaber.[2] *[1]Pharmacy, University of Tennessee, Memphis, TN; [2]Surgery, University of Tennessee, Memphis, TN; [3]Nephrology, University of Tennessee, Memphis, TN; [4]Nursing, University of Tennessee, Memphis, TN.*

Introduction: Metabolic syndrome (MS) is gaining recognition as a significant predictor for type 2 diabetes mellitus and cardiovascular diseases. The NCEP-ATPIII expert panel defined MS as the presence of ≥3 of these factors: abdominal obesity, fasting blood glucose (FBG)>110 mg/dL, triglycerides (TG)>150mg/dL, HDL cholesterol (Chol) <50mg/dL in male and <40mg/dL in female, and blood pressure (BP) ≥130/ ≥85mmHg. The purpose of this study is to determine the prevalence and incidence of MS pre and post renal transplantation.

Methods: A retrospective analysis of renal transplants performed between 01/1998 and 12/2000. Patients included had a functioning renal allograft at 1 year and patients with diabetes mellitus pretransplant were excluded. We evaluated changes in systolic and diastolic BP, FBG, fasting Chol and TG levels, body mass index (BMI), and the incidence of MS pre and posttransplant substituting waist circumference with BMI >30 and HDL Chol with total Chol >200mg/dL in our defintion.

Results: The study consisted of 126 patients with a mean age of 43±12 years, 64% male, and 64% African-American. Patients received tacrolimus (76%), cyclosporine (17%), or sirolimus (7%). At 1 year, the serum creatinine was 1.6 ± 0.5 mg/dL. FBG, Chol, TGs and BMI increased significantly posttransplant, while BP improved. The prevalence of MS was 29% pretransplant and increased to 57% at 1 year posttransplant p<0.01. The 1 year incidence of PTDM was 46%.

	Pretransplant	Posttransplant	p
BMI	27.4 ±4.8	29.5±5.2	<0.01
SBP (mmHg)	150 ±22	132±17	<0.01
DBP (mmHg)	86 ±12	81±11	<0.01
Chol (mg/dL)	177±53	208±67	<0.01
TG (mg/dL)	167±88	222±195	<0.01
Fasting glucose	92±19	113±47	<0.01

We also examined achievement of treatment BP, Chol, and TG goals established by the NIH (JNCVII and ATPIII guidelines). Target BP, Chol, and TG goals were not achieved in 82%, 32%, and 48% of patients pretransplant, respectively. Posttransplant, target BP, Chol, and TG goals were not achieved in 54%, 52%, and 74% of patients.

Conclusion: Metabolic complications are common and suboptimally controlled pretransplant. Apart from hypertension, the incidence and severity of these complications increased significantly posttransplant. Treatment of posttransplant patients did not achieve desired goals in a significant proportion of patients. Aggressive medical management should be initiated prior to transplant and continued in the posttransplant follow-up.

Abstract# 1414

AMBULATORY MONITORING OF BLOOD PRESSURE IN A KIDNEY TRANSPLANT POPULATION. Hélène Lord,[1,2] Suzon Collette,[1,2] Raymond Dandavino,[1,2] Michel Morin,[1] Anne Boucher.[1,2] *[1]Département de Médecine, Université de Montréal, Montréal, QC, Canada; [2]Service de Néphrologie, Hôpital Maisonneuve Rosemont, Montréal, QC, Canada.*

INTRODUCTION: High blood pressure (HBP) in kidney transplant patients (KTP) contributes to chronic allograft dysfunction (CRF) and cardiovascular morbidity. The prevalence is 60-80%. Nocturnal dip (ND) influences progression to CRF and cardiovascular morbidity. ND is absent in 25-78% of KTP. We evaluated the repercussion of white coat on BP and the correlation between BP and various parameters (graft function, antihypertensive Rx, magnesium (Mg), parathormone (PTH), ND).

PATIENTS AND METHODS: Prospective study, still enrolling. Inclusion criteria: KTP with HBP or upper limit BP. Ambulatory monitoring of BP (AMBP) every 30 minutes (day) and 60 minutes (night). 82 patients: 37 women, 45 men; 79 cadaveric grafts; mean time since transplantation 76.5 months; age 54.1 ± 12.7 yrs; BMI 27 ± 4.7; creatinine 137 ± 44.8 µmol/L; creatinine clearance (Ccl) 51.8 ± 13 ml/min; LDL 2.7 ± 0.7; Hb 130.3 ± 13.6; Mg 0.72 ± 0.1; PTH 7.5 ± 7. Prior to KT: 87% HBP, 71% dyslipidemic, 21% CAD, 17%DM(diabetes mellitus). Medication used: 98.5% corticosteroids, 46.3% CsA, 43.9% FK, 8.5% sirolimus, 67.1% MMF, 13.4% azathioprine. Antihypertensive Rx: 32% 1 Rx, 32% 2 Rx, 16% 3 Rx; 40% CCB, 38% ACEI, 33% BB, 27% diuretics, 17% ARB, 4% alpha blockers.

RESULTS: We compared the BP at the clinic before and during AMBP: systolic 149 vs 139 mmHg (p<0.0005), diastolic 87 vs 84 mmHg (p<0.04). We compared the BP at the clinic after the AMBP: systolic 145 vs 139 mmHg (p<0.003), diastolic 84 vs 84 mmHg (p=NS). There was no correlation between AMBP and creatinine, Ccl, Mg, PTH and class of Rx. ND was absent in 42% KTP; there was no correlation between ND and mean AMBP, DB, Ccl, time since KT, CsA and FK. 33% of KTP had adjustment in antihypertensive Rx after AMBP, mostly by addition of ARB (38%), followed by ACEI or CCB (19%).

DISCUSSION AND CONCLUSION: AMBP eliminates white coat effect on BP, especially systolic BP. AMBP showed that 33% of KTP with HBP at the clinic are well controlled at home while 11% of KTP with adequate control at the clinic are hypertensive. We conclude that BP at the clinic is not a good indicator of everyday BP; AMBP should be added to our investigation tools. Our Rx don't treat the ND in most of the KTP. Further results are pending regarding LVH and specific therapeutic intervention on ND.

Abstract# 1415

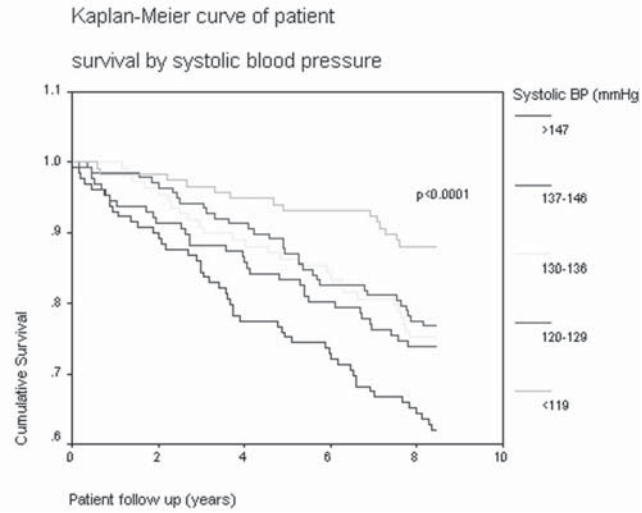
HYPERTENSION AND SURVIVAL FOLLOWING RENAL TRANSPLANTATION-POTENTIAL CARDIOVASCULAR PROTECTION WITH BETA BLOCKERS. Patrick B. Mark,[1] Viliami Tutone,[2] Graeme Stewart,[2] C. C. Tan,[2] R. S. C. Rodger,[2] Colin Geddes,[2] Alan G. Jardine.[1] *[1]Medicine and Cardiovascular Sciences, Western Infirmary, Glasgow, Scotland, United Kingdom; [2]Nephroplogy, Western Infirmary, Glasgow, Scotland, United Kingdom.*

Background Hypertension is common following renal transplantation and adversely affects graft and patient survival. However strategies for antihypertensive drug therapy and target blood pressure have not been clearly defined.

Methods We undertook a prospective study of 622(57.2% male; mean age 45.2 +/-13.0) patients with functioning grafts from December 1994-June 2003. Blood pressure (BP) was determined from mean of three clinic readings and drug therapy recorded. We analyzed patient and graft survival after102 months follow up to assess the influence of hypertension and drug therapy.

Results There were 158(2.9% per annum) deaths and 115(2.2% per annum) graft failures. Univariate analysis identified age (p<0.001, hazard ratio (HR) 1.066), creatinine (p=0.03, HR 1.002), diabetes (p<0.001, HR 3.989), pulse pressure (p<0.05, HR 1.013) as predictors of patient survival with creatinine (p<0.001, 1.012), number of antihypertensives (p<0.01, HR 1.413) and pulse pressure (p<0.05, HR 1.018) as predictors of graft survival. Mortality increased with increased systolic blood pressure. Patient survival was reduced with increasing number of antihypertensives (p<0.05) as was graft survival (p<0.05). Reduced patient and graft survival was seen in patients prescribed calcium channel antagonists (p<0.01). There was no increased patient mortality in those patients on beta blockers or ACE inhibitors although the number of patients on ACE inhibitors was small (11.3%)

Conclusion Hypertension is a risk factor for reduced patient and graft survival. This risk remains despite use of antihypertensives. Calcium channel antagonists are associated with greater patient mortality than beta blockers. Beta blockers may have a cardio protective effect and therefore reduce patient mortality.



OUTCOMES AND COMPLICATIONS AFTER CARDIAC TRANSPLANTATION

Abstract# 1416

MORTALITY FOLLOWING CARDIAC TRANSPLANTATION FOR STATUS 1 PATIENTS WITH AND WITHOUT MECHANICAL VENTRICULAR ASSIST DEVICES: AN ANALYSIS OF THE UNITED NETWORK FOR ORGAN SHARING THORACIC REGISTRY. Rajan Krishnamani,[1] Kamal R. Khabbaz,[1] Wenjun Li,[1] Richard D. Patten,[1] Marvin A. Konstam,[1] David DeNofrio.[1] *[1]Division of Cardiology and Cardiothoracic Surgery, Tufts-New England Medical Center, Boston, MA.*

Purpose

Among United Network for Organ Sharing (UNOS) Status 1 patients, we sought to compare the post-transplant mortality of patients not supported with a ventricular assist device (VAD) to those bridged to cardiac transplantation with an extracorporeal VAD or intracorporeal VAD.

Methods

A retrospective analysis was conducted using Organ Procurement and Transplantation Network data as of 5/31/2002 from the UNOS Thoracic Registry. The analysis was restricted to UNOS Status 1 first-time single organ cardiac transplant recipients between 1/1/1995 to 12/31/2001. The one and five-year mortality were compared among patients supported with an extracorporeal VAD (Abiomed, Thoratec)[Group 1], an intracorporeal VAD (HeartMate, Novacor)[Group2], or no VAD support [Group 3]. Mortality rates were estimated using the Kaplan-Meier method. Risk ratios were estimated using Cox proportional hazard regression models. For risk factors that exhibited non-proportional hazard over time, hazards were modeled either as a function of time or by stratification.

Results

Of the 10,523 patients transplanted as UNOS Status 1, 876 were supported with an extracorporeal VAD and 750 with an intracorporeal VAD. The one-year mortality was 21.2%, 17% and 14.7% in Groups 1, 2 and 3, respectively. The cumulative mortality at 5 years was 35.7%, 33.8% and 28.3% in Group 1, 2 and 3, respectively (p<0.00001). The hazard ratio for patients with intracorporeal VAD support compared to UNOS Status 1 patients without VAD support was 1.32 (95% CI: 1.09-1.59, p<0.001). The adverse effect of extracorporeal VAD use was nonproportional over time (p<0.001), with a hazard ratio of 1.47 (95% CI: 1.28-1.70, p<0.001) at the first month which decreased gradually to a hazard ratio of 1.16 (95% CI: 0.98-1.38, p=0.09) 12 months following transplantation.

Conclusion

Thus, compared to UNOS Status 1 patients not supported with a VAD, those bridged with an extracorporeal or intracorporeal VAD demonstrate a higher short and long term mortality following cardiac transplantation.

Abstract# 1417

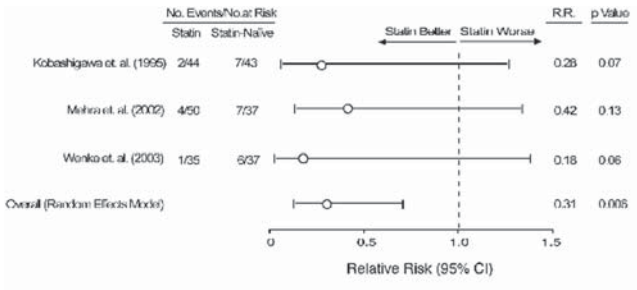
STATINS AND SURVIVAL IN DE NOVO CARDIAC TRANSPLANTATION: A META-ANALYSIS. Nirav Y. Raval,[1] Mandeep R. Mehra.[2] *[1]Tulane University Health Sciences Center, New Orleans, LA; [2]Cardiomyopathy and Heart Transplantation Center, Ochsner Clinic Foundation, New Orleans, LA.*

Background: The use of HMG Co-A reductase inhibitors (statins) after cardiac transplantation has been suggested to decrease severe rejection and improve survival. Individual investigations leading to this suggestion are randomized (but not placebo controlled), include small numbers (and thus underpowered) and enroll heterogeneous patients (including retransplant recipients). The purpose of this pooled analysis was to quantify the benefit of statins on survival in de novo cardiac transplant recipients.

Methods: Medline (1966-2003) was queried using keywords statin, HMG Co-A reductase inhibitors, cardiac, transplantation, transplant, cholesterol, atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin. In addition, we searched cited literature and previously published systematic reviews. Summary outcome data were extracted from final trial reports. Of 36 articles retrieved, 3 randomized controlled studies met our population inclusion criteria (>18 years, de-novo heart transplant, statin therapy within 3 months of transplant, at least 1 year follow-up of statin treated and controls). Pooled data were meta-analyzed using Mantel-Haenszel testing in a random effects model that included tests for heterogeneity.

Results: The 3 pooled studies included 246 patients (statin n=129, no-statin n=117) and 27 events (11%). Although individual studies trended to favor statin therapy for mortality at 1 year, the 95% CI's crossed unity. Pooled analysis demonstrated a significant reduction in mortality with statin use (RR 0.31 (95% CI 0.13-0.7, p=0.006) without significant heterogeneity (p=0.7) among studies. (Figure 1). Two of the 3 studies reported allograft rejections with hemodynamic compromize (HDC). Pooled analysis demonstrated a significant benefit on this end-point (RR 0.22 (95% CI 0.08-0.63) p=0.004).

Conclusion: This meta-analysis has convincingly demonstrated that statin therapy decreases rejection with HDC and improves 1 year survival in heart transplantation.



Abstract# 1418

THE RELEVANCE OF HLA ANTIBODY AND A DONOR SPECIFIC FLOW CYTOMETRY CROSSMATCH TO REJECTION AND GRAFT SURVIVAL IN CARDIAC ALLOGRAFT RECIPIENTS. Peter Przybylowski,[2] Branislav Radovancevic,[1] O. H. Frazier,[1] F. Smart,[1] Noriel Acordia,[2] Paula Juarez,[1] L. A. Nemeth,[1] Barry D. Kahan,[2] Ronald H. Kerman.[2] *[1]Texas Heart Institute, Houston, TX; [2]Surgery, University of Texas Medical School-Houston, Houston, TX.*

At our transplant center cardiac allograft recipients undergo transplantation (Tx) following negative (=) IgG anti-human globulin (AHG) crossmatch (XM). Recent data from renal Tx studies suggested that recipients with pre-Tx sera containing Flow PRA identified IgG HLA antibodies (Ab) and displaying positive (+) IgG donor specific flow cytometry XMs (FCXM) experienced a high rejection frequency and graft loss. Therefore, we retrospectively evaluated the pre-Tx sera of 127 cardiac allograft recipients for the presence of HLA Abs and a (+) or (=) IgG donor-specific FCXM. The results were correlated to post-Tx rejection and patient survival. Recipients with little or no (< 5%) IgG HLA Ab experienced significantly fewer rejections (10%, 9/86 vs 56%, 23/41, p<0.001) and better graft survival (92%, 65/71 vs 78%, 32/41, p<0.01) than recipients with ≥5% (39 ± 19%) IgG HLA Ab. More importantly, those recipients with pre-Tx IgG HLA Abs (4 class I only, 3 class II only and 3 class I and II) and a (+) IgG donor specific FCXM experienced a 90% (9/10) rejection frequency and only had a 70% (7/10) one year graft survival compared with an 11% (7/64) rejection frequency and a 92% (59/64) survival for recipients with no HLA Ab and a (=) FCXM, p<0.01 for rejection and survival respectively. Recipients presenting with no HLA Ab and a (+) IgM FCXM experienced an 8% (1/13) rejection frequency and a 92% (12/13) survival. These data suggest that the pre-Tx presence of Flow PRA detected IgG HLA Ab in recipient sera correlates with a significant risk for rejection. Recipients presenting pre-Tx with both IgG HLA Abs and a (+) IgG donor specific FCXM are at risk for both rejection and graft loss. The presence of IgM may be beneficial to graft outcome. Having pre-Tx IgG HLA Ab information may influence clinicians to desensitize patients prior to transplantation and/or to choose different immunosuppressive regimens for those sensitized vs nonsensitized recipients.

Abstract# 1419

RISK FACTORS FOR ACUTE REJECTION AND VASCULOPATHY AMONG 634 PATIENTS TREATED WITH EVEROLIMUS OR AZATHIOPRINE. R. C. Starling,[1] J. Kobashigawa,[2] H. Eisen,[3] J. Jaffe,[4] Y. Li.[4] *[1]Cleveland Clinic Foundation, Cleveland, OH; [2]UCLA, Los Angeles, CA; [3]Temple University, Philadelphia, PA; [4]Novartis Pharmaceuticals, E. Hanover, NJ.*

The goal of this study is to determine the effect of multiple pre- and post-transplant risk and protective factors on the incidence of acute cardiac allograft rejection ISHLT grade 3A (AR) among 634 patients participating in a multi-center double-blind comparator-controlled trial, randomized to either everolimus (EV) or azathioprine (AZA). Univariate logistic regression modeling of effect on AR was performed for risk factors: PRA, donor age, recipient age, recipient coronary artery disease (CD), body mass index (BMI), cold ischemia time (IT), use of LVAD, and hypertension. Of these, the use of LVAD was significantly associated with AR (p=0.002, odds ratio [OR]= 3.2, 95% CI 11.5-6.7). We also determine whether the above risk factors plus AR, mean CsA trough, mean steroid dose, creatinine level>200umol/mL, triglyceride 4.5 mmol/L, cholesterol 6.2 mmol/L, CMV, statin, ACE inhibitor, or calcium channel blockers (CCB) influenced the subsequent occurrence of IVUS-defined cardiac allograft vasculopathy (CAV) at one year (y), an endpoint studied because of its value as a surrogate for survival and MACE at 5 y after transplant. CAV was defined as an increase in maximal intimal thickness of 0.5 mm or more in any segment studied at both baseline and 1 y. Among the 211 patients with baseline and 1 y IVUS, there were too few patients with CMV or without statin use to determine whether these factors affected CAV. AR, and choice of statin were not risk factors for the occurrence of CAV. Of the risk factors analyzed donor age >50 y ((p=0.005, OR=3.6, CI=1.5-9.0) was associated with increased risk of CAV as was recipient CD (p=0.04, OR=1.83, CI=1.02-3.25) Conclusion: Metabolic risk factors did not influence AR or CAV at 1 y. In this mixed population, LVAD use emerged as a significant risk factor for AR, but AR itself did not influence the occurrence of CAV during the same interval. Patients receiving hearts from older donors, or whose reason for transplant is related to a diagnosis of CD may need their regimen tailored to address CAV.

Abstract# 1420

ANTI-DONOR IMMUNITY IN CARDIAC ALLOGRAFT RECIPIENTS WITH AND WITHOUT TRANSPLANT VASCULOPATHY (TV). Emilio Poggio,[1] Meagan Roddy,[1] Michael Clemente,[1] Jocelyn Riley,[1] Randall Starling,[1] Patrick McCarthy,[1] James Young,[1] Barbara Gus,[1] Mohamad Yamani,[1] Peter S. Heeger.[1] *[1]Immunology and The Heart Transplant Center, The Cleveland Clinic Foundation, Cleveland, OH.*

Chronic graft dysfunction with transplant vasculopathy (TV) represents a dominant cause of late heart transplant failure. The development of TV may involve cellular and humoral immunity as well as nonimmune factors. To assess the role of anti-donor immunity in the development of human TV we evaluated peripheral immune responses in 22 human cardiac allograft recipients with angiographic evidence of TV and 27 controls with normal angiograms. Recipient peripheral blood lymphocytes (PBLs) were tested for immune reactivity to indirectly presented peptides derived from donor HLA molecules by IFNg ELISPOT. Serum anti-HLA class II alloantibodies were assessed by flow cytometry screening beads, and positive sera were further tested to determine specificity. There was no significant difference in immunosuppression regimens between the TV+ and TV- groups (predominantly a calcineurin inhibitor, an anti-proliferative agent and steroids). The number of HLA mismatches did not differ between groups (5.0 vs 5.2, p=NS) and there was no difference in the frequency of CMV positivity between groups. The TV+ group had longer follow-up (median 115 months) than the controls (median 54 months). Within the TV+ group, 6/22 (27%) had detectable responses to donor but not recipient HLA peptides and 9/22 (41%) had anti-donor HLA class II antibodies. Only 1 patient exhibited evidence of both cellular and humoral immunity. 8 patients had no detectable anti-donor immunity despite angiographic evidence for TV. In contrast, only 4/27 (15%) TV- controls exhibited anti-donor peptide immunity and 1/27 (4%) had anti-donor class II alloantibodies in the serum. The overall incidence of anti-donor immunity in the TV+ group (63%) was statistically greater than in the TV- group (22%, p < 0.05). This held true for those patients in each group followed for the same time period of > 5 years. Our results provide strong correlative evidence that anti-donor immunity participates in the development of TV in human heart graft recipients but further demonstrates the heterogeneity of the induced responses. Comprehensive approaches to assessing both cellular and humoral immunity will be required to identify those individuals at highest risk for this disease. Ultimately, a noninvasive ability to identify (and perhaps predict) those individuals with persistent anti-donor immunity should permit individualization of therapy aimed at prolonging graft survival.

Abstract# 1421

FURTHER ANALYSIS OF THE INTRAVASCULAR ULTRASOUND DATA FROM THE RANDOMIZED MYCOPHENOLATE MOFETIL (MMF) TRIAL IN HEART TRANSPLANT RECIPIENTS. Jon A. Kobashigawa,[1] Jonathan Tobis,[1] Robert M. Mentzer,[2] Hannah A. Valantine,[3] Robert C. Bourge,[4] Frank W. Smart,[5] Leslie W. Miller,[6] Robert D. Gordon.[7] *[1]University of California at Los Angeles, Los Angeles, CA; [2]University of Kentucky, Lexington, KY; [3]Stanford University, Stanford, CA; [4]University of Alabama at Birmingham, Birmingham, AL; [5]Texas Heart Institute, Houston, TX; [6]University of Minnesota, Minneapolis, MN; [7]Roche Pharmaceuticals, Basel, Switzerland.*

First year intravascular ultrasound (IVUS) measurements, including the change from baseline to 1-year maximal intimal thickness (MIT) greater than 0.5mm, have been recently reported to be a surrogate marker for long-term outcome after heart transplantation. This IVUS measurement most likely represents a heightened immune response of the recipient to the donor heart, which can lead to allograft vasculopathy and subsequent poor outcome. First year IVUS data can be analyzed by site-to-site analysis (recent reports) or by morphometric analysis (average of 10 sites, without matching sites which was done in the MMF Trial) in the same coronary artery from baseline to 1-year. Since intimal thickness is heterogeneous with most sites having little or no intimal thickening, morphometric analysis will not be sensitive to detect changes at any one particular site as it averages data from multiple (usually 10) sites. In this regard, morphometric analysis of the first year MMF IVUS data may not have accurately depicted the impact of MMF on first year MIT in the study patients. MMF has been reported to decrease intimal thickening in animal transplant models. The MMF trial involved 650 heart transplant patients from 28 centers to receive MMF (3000 mg/day) or azathioprine (1.5-3.0 mg/kg/day), in addition to cyclosporine and corticosteroids. Baseline and 1-year IVUS were performed in 196 patients (102 MMF and 94 azathioprine patients) with no differences in IVUS results by morphometric analysis found between the study groups. Further analysis of the IVUS data using site-to-site analysis from baseline to 1-year will be performed at the Core IVUS Laboratory. The number of patients with change in MIT greater than 0.5mm from baseline to 1-year at any one site will be determined for MMF-treated patients compared to azathioprine-treated patients. Results of this analysis will be presented at the ATC Scientific Sessions 2004.

Abstract# 1422

MULTI-CENTER INTRAVASCULAR ULTRASOUND VALIDATION STUDY AMONG HEART TRANSPLANT RECIPIENTS: OUTCOMES AFTER 5 YEARS. Jon A. Kobashigawa,[1] Jonathan M. Tobis,[1] Randall C. Starling,[2] Murat E. Tuzcu,[2] Mandeep R. Mehra,[3] Alan Yeung,[4] Hannah Valantine,[4] Andrew Smith,[5] Hitoshi Anzai,[1] Brandy T. Oeser,[1] Kamal Abeywickrama,[6] Jane Murphy,[6] Nathalie Cretin.[6] *[1]University of California at Los Angeles, Los Angeles, CA; [2]Cleveland Clinic, Cleveland, OH; [3]Oschner Clinic, New Orleans, LA; [4]Stanford University, Stanford, CA; [5]Emory University, Atlanta, GA; [6]Novartis Pharma, Basel, Switzerland.*

Background: Cardiac allograft vasculopathy (CAV) is a major impediment to long-term graft survival. Intravascular Ultrasound (IVUS) is an invasive procedure that is more sensitive than coronary angiography and detects intimal thickening (early CAV) in the coronary arteries of the donor heart. There have been single center studies suggesting that the first year IVUS results might predict or be a surrogate marker for long-term outcome however this has not been established in a large multicenter study. Methods: First- year IVUS results and subsequent five-year clinical data follow-up were reviewed in 125 cardiac transplant recipients from 5 institutions skilled in performing IVUS in heart transplant patients. IVUS tapes (at baseline and 1-year follow-up) were re-analyzed at a core IVUS laboratory. The greatest change in maximal intimal thickness (MIT) from baseline to year 1 was recorded for matched sites in the LAD coronary artery. Patients were divided into two groups: Those with an increase of 0.5 mm in the MIT in any matched site and those without this increase. Analysis of the results is ongoing and will correlate these two IVUS groups with 5-year patient outcomes (death/retransplant, non-fatal major adverse cardiac events, cardiac function, morbidity factors, and subsequent development of angiographic/autopsy confirmed cardiac allograft vasculopathy). The results will serve to validate first year IVUS data as a surrogate marker for poor outcome after heart transplantation. Data has been collected and the complete analysis will be available at the time of the ATC Scientific Sessions.

Abstract# 1423

DECREASED SURVIVAL IN HEART TRANSPLANT PATIENTS REQUIRING CHRONIC DIALYSIS: A CANADIAN EXPERIENCE. M. Cantarovich,[1] F. Ivis,[2] K. Badovinac.[2] *[1]Medicine, McGill University Health Center, Montreal, QC, Canada; [2]Canadian Organ Replacement Register, Canadian Institute for Health Information, Toronto, ON, Canada.*

End-stage renal disease (ESRD) requiring chronic dialysis constitutes a serious complication in 2%-10% of heart transplant (HTx) pts. Purpose: To describe the prevalence of ESRD and the survival of HTx pts requiring chronic dialysis in Canada. Methods: We studied 2,555 pts who received HTx between Jan 1, 1981 and Dec 31, 2001. Data was extracted from the Canadian Organ Replacement Register, managed by the Canadian Institute for Health Information. An attempt was made to match each HTx pt on dialysis with 2 dialysis controls (n=180) without a HTx (No HTx-Dialysis group). Results: During the follow-up period, 94 pts (3.7%) developed ESRD requiring chronic dialysis (HTx-Dialysis group) and 2,461 pts did not develop ESRD (HTx-No dialysis group). There was no difference on pts' age at HTx between the HTx-Dialysis group and the HTx-No Dialysis group (48±11 yrs vs. 45.5±16.5 yrs). There was no difference in the primary disease. The mean time elapsed between HTx and the initiation of chronic dialysis was 87±49 months (median 86.5). Pt's years after HTx in the HTx-Dialysis group was 9.7±4.3 and in the HTx-No Dialysis group was 5.6±4.7 (P<0.0001)

	Survival post-heart Tx						
Survival	0-1 yr	1-3 yrs	3-6 yrs	6-9 yrs	9-12 yrs	12-15 yrs	>15 yrs
HTx-Dialysis group	100%	99%	97%	85%	67%	52%	31%*
HTx-No Dialysis group	100%	83%	78%	71%	64%	56%	49%

*P=0.009

Mean time elapsed between the initiation of chronic dialysis and death was 21±22.5 months (median 11.5) in HTx pts. Causes of death in the HTx-Dialysis group and in HTx-No Dialysis controls included: cardiac (40% vs. 19%, P=0.0017), vascular (4% vs. 7%, P=NS), infection (12% vs 8%, P=NS), social (10% vs. 2%, P=0.003), other (22% vs. 25.5%, P=NS) and not reported (12% vs. 39%, P=NS).

	Survival after the initiation of dialysis					
Survival	0-1 yr	1-2 yrs	2-3 yrs	3-4 yrs	4-5 yrs	>5 yrs
HTx-Dialysis group	100%	72%	58%	49%	29%	22%*
No HTx-Dialysis group	100%	88%	72%	61%	47%	38%

*P=0.009

When compared to the HTx-Dialysis group, the causes of death in the No HTx-Dialysis group included: cardiac (41%, P=NS), vascular (7%, P=NS), infection (12%, P=NS), social (13%, P=NS), other (18%, P=NS) and not reported (9%, P=NS). Conclusion: The incidence of ESRD after HTx in Canada is low. HTx pts requiring chronic dialysis lived longer vs. HTx pts not experiencing ESRD. Survival post-dialysis initiation was lower for HTx pts vs. dialysis controls without a HTx. Cardiac and social causes are the main etiologies of death. Future studies should focus on renal protective strategies to reduce the risk of ESRD after HTx.

Abstract# 1424

SEROPOSITIVITY FOR CYTOMEGALOVIRUS PREDISPOSES TO ALLOGRAFT VASCULOPATHY IN HEART TRANSPLANT RECIPIENTS REGARDLESS OF GANCICLOVIR-PROPHYLAXYS. Ombretta Biadi, Luciano Potena, Cecile Holweg, Helen I. Luikart, Sharon A. Hunt, Hannah A. Valantine. *Cardiovascular Medicine, Stanford University, Stanford, CA.*

Background Cytomegalovirus (CMV) infection is a risk factor for cardiac allograft vasculopathy (CAV) in heart transplant (HT) recipients. However it is unclear whether baseline sensitization of the immune system to CMV, as expressed by positive serology for CMV, may represent a risk factor for CAV, beyond viral cytotoxicity. In this study we hypothesized that donor or recipient positive CMV serology might be a risk factor for CAV, regardless of signs of acute CMV infection, and despite anti-CMV prophylaxis.
Methods Consecutive patients who underwent HT between 1994 and 2002 at our Center (n=331) were reviewed. All HT recipients who survived at least one year and underwent at least one coronary angiography were included in the study. Since 1994, all HT recipients seropositive for CMV (R+) undergo a prophylaxis regimen consisting of 4 weeks of intravenous ganciclovir, 5 mg/Kg/bid (14 days) and daily until Day 28 post-transplant. Seronegative recipients (R-) receiving the graft from seropositive donor (D+) additionally received CMVIgG until the 4th postoperative month. Immunosuppression consisted of induction with OKT3 or daclizumab, and a 3-drug regimen of CSA, prednisone and MMF.
Results Of the patients reviewed, 155 fulfilled inclusion criteria, and demographics were characterized by age 46±17years; 117(75%) men; and 45(29%) pre-transplant coronary artery disease. CMV serostatus was R+/D+: 55(35%); R+/D-: 32(21%); R-/D+: 45(29%), and R-/D-: 23(15%) No baseline differences were recorded among groups, except that R+ were older than R- (50±15 vs. 42±17y, P<0.01). 21(14%) patients experienced clinical CMV disease, confirmed by a positive CMV-PCR. After a follow-up of 7 years, the estimated incidence of CAV was 61±13% in R+/D+, 70±22% in R+/D-, 19±7% in R-/D+ and 20±12% in R-/D-, P=0.05. This trend was confirmed by multivariate analysis which showed that seropositive recipients had the highest risk of developing CAV, independent of recipient demographics, donor serology and CMV disease (OR[95%CI] = 2.22 [1.03 to 4.71]; P=0.04).
Conclusions Recipient CMV seropositivity is an independent risk factor for CAV, suggesting that CMV sensitization even in the absence of acute disease may play a role in CAV pathogenesis. However, the effect of sub-clinical viral replication on CAV requires further investigation.

Positive Crossmatch Kidney Transplantation and Sub-Clinical Rejection

Abstract# 1425

RENAL TRANSPLANTATION USING LIVING DONORS WITH A POSITIVE CROSSMATCH: CAN WE PREDICT EFFICACY OF DESENSITIZATION WITH IVIG BY AN "IN VITRO" TEST? Maria Cristina Ribeiro Castro,[1] Nicolas Panajotopoulos,[2] Liliam M. P. Araujo,[1] Helcio Rodrigues,[2] Renata Maciel,[1] Carla Ronda,[2] Carlos Sérgio Viggiani,[2] Willian Nahas,[1] Elias David-Neto,[1] Luiz Estevam Ianhez,[1] Jorge Kalil Filho,[2] Denis Glotz.[2,3] *[1]Renal Transplant Unit, University of São Paulo, São Paulo, SP, Brazil; [2]Laboratory of Immunology (Heart Institute), University of São Paulo, São Paulo, SP, Brazil; [3]Renal Transplant Unit, Hôpital European George Pompidou, Paris, France.*

High-dose IVIg (intravenous immunoglobulins) administration has proven its efficacy in reducing anti-HLA antibodies titers in most patients. However, success varies from 50 to 87% of treated patients, and, due to the cost of treatment, it is of importance to devise an "in vitro" test which could predict the clinical response of the patients. It has been proposed that inhibition of the cytotoxic CDC crossmatch by a fixed dose of IVIg could predict the subsequent clinical response to IVIg.
We initiated a protocol of desensitization of patients with a positive crossmatch against their potential living donor. Five patients have been so far treated with 3 monthly courses of 2 g/Kg of IVIg (ENDOBULIN, Baxter), with crossmatch negativation in 4 cases (the fifth case is still under treatment). In all five cases, adjunction of IVIg (25 mg/ml) had no effect on the crossmatch test (Table I), while administration of IVIg to the patient has been successful in all cases.

"In vitro" inhibition of cytotoxic CDC crossmatch by IVIg

Patients	Serum untreated	Serum + IVIG (v/v)	Serum + Medium (v/v)
MRCB	Pos	Pos	Pos
ZRS	Pos	Pos. (1/16)	Pos. (1/32)
GMC	Pos	Pos. (1/4)	Pos. (1/4)
HCR	Pos	Pos. (1/16)	Pos. (1/16)
MCC	Pos	Pos. (1/64)	Pos. (1/64)

Table I

Patients received their transplant under a regimen of THYMOGLOBULIN, CELLCEPT, PROGRAF and steroids, with additional courses of IVIg on days -1/0, 20/21 and 42/43 post-transplant. One steroid sensitive rejection (Banff 1a) was seen and one graft was lost to vascular rejection.
We conclude that, at the moment, there is no assay that should preclude IVIg treatment of sensitized recipients, particularly those with a potential living donor.

Abstract# 1426

IMPROVED RESULTS WITH SELECTIVE USE OF SPLENECTOMY AND ANTI-CD 20 FOR POSITIVE CROSSMATCH TRANSPLANTS. Christopher E. Simpkins,[1] Andrea A. Zachary,[2] Matthew Cooper,[1] Daniel S. Warren,[1] Lloyd E. Ratner,[1] Robert A. Montgomery.[1] *[1]Surgery; [2]Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD.*

Background: 30% of the kidney transplant waiting list is sensitized to allo-HLA molecules. These patients endure disproportionately long waiting times and inferior graft survival. Many of these patients have a live donor who has been excluded due to a positive crossmatch (+XM).

Methods: A total of 58 +XM transplants were performed during the study period (2/98-11/03) utilizing our plasmapheresis/low dose CMVIg (PP/CMVIg) protocol. The first 41 patients (Group 1) were preconditioned with a one-size-fits-all protocol utilizing PP/CMVIg and standard immunosuppression. Results from this group are compared to the most recent 17 patients (Group 2) in which the therapeutic plan was individualized to match the level of risk of graft loss.

Results:. Graft survival for Group 1 and 2 are 86% and 100%, respectively. Five patients in Group 2 were determined to be at high risk for graft loss (wide breadth of HLA reactivity, previous transplants and early graft losses, multiple repeat mismatches or donor reactive antibodies, rising or rebounding DSA titers) and their preconditioning was enhanced with splenectomy and/or anti-CD20. The rate of overall graft loss was 14.6% in Group 1 and 0% in Group 2 ($p < 0.05$). Graft loss due to severe antibody-mediated rejection occurred in 4 patients (9.7%) in Group 1 and 0 patients in Group 2. Mean current creatinine clearance is 59.1 ± 38.0 and 70.6 ± 17.6 mL/min for patients in Group 1 and Group 2, respectively (p=NS). Mean follow-up times for Group 1 and Group 2 are 21.3 ± 17.7 and 2.3 ± 1.1 months, respectively.

Conclusions: Lower rates of graft loss can be achieved in + XM transplants when the treatment plan is crafted to match the immunologic risk of the recipient.

Abstract# 1427

HIGHLY SUCCESSFUL LIVING DONOR KIDNEY TRANSPLANTATION AFTER CONVERSION TO NEGATIVE OF A PREVIOUSLY POSITIVE FLOW-CYTOMETRY CROSS-MATCH BY PRE-OPERTIVE PLASMAPHERESIS. Howard N. Sankary,[1] James Thielke,[2] Philip DeChristopher,[3] Kerri Kraft,[2] Diego Bogetti,[1] Jose Oberholzer,[1] Giuliano Testa,[1] Enrico Benedetti.[1] *[1]Transplant Surgery; [2]Pharmacy Practice; [3]Department of Pathology, University of Illinois at Chicago, Chicago, IL.*

BACKGROUND: A positive cross-match between donor and recipient has been traditionally considered an absolute contraindication to kidney transplantation. In an effort to further expand our ability to utilize living donors for kidney transplantation, we have implemented a strategy to prevent immune mediated graft loss when the recipient has a positive cross-match against a potential living donor.

METHODS: Between 7/01 and 6/03, 15 highly sensitized candidates to kidney transplantation, with cross-match positive potential living donors, were enrolled in this study. The average age was 34 years; 10 were African-American, 1 Caucasian and 4 Hispanic. One patient had a prior transplant. All patients received plasmapheresis QOD starting 1 week before the scheduled transplant. In 5 patients a single dose of Rituximab was given at the beginning of the treatment. If the cross-match by flow-cytometry converted to negative after the initial 3 treatments, the transplant was performed as scheduled; otherwise the protocol was aborted. Post-operatively, the recipients continued to receive QOD plasmapheresis for the initial week. Immunosuppression therapy consisted in induction with Thymoglobulin x 5 days along with combination of tacrolimus, mycophenolate and steroids. Average follow-up was 1 year.

RESULTS: Four patients failed to convert with pre transplant immunomodulation, and were not transplanted. The other 11 underwent uncomplicated kidney transplant. One-year patient and graft survival was 100%. All the recipients continue to do well to date. There were 3 early episodes of antibody mediated rejection successfully treated with plasmapheresis, and 2 episodes of cellular rejection treated with bolus steroids. The average serum creatinine at 6 months was 1.4 mg/dl. There were two episodes of systemic cytomegalovirus infection successfully treated with gancyclovir; in both cases the donor was CMV positive and the recipient CMV negative. No other significant infectious complications occurred.

CONCLUSIONS: Pre transplant immunomodulation with plasmapheresis ± rituximab can effectively lower antibody titer and convert to negative a previously positive flow-cytometry cross-match in over 70% of the cases. Kidney transplantation can be thereafter performed with high success and acceptable rate of rejection and infection complications on a standard immunosuppressive protocol.

Abstract# 1428

MODULATION OF ANTI-CLASS I, ANTI-CLASS II AND ANTI-ENDOTHELIAL CELL ANTIBODIES BY INTRAVENOUS GAMMAGLOBULIN (IVIG) IMPROVES TRANSPLANT RATES AND REDUCES ALLOGRAFT REJECTION IN HIGHLY-SENSITIZED PATIENTS. Mieko Toyoda,[1] Anna Petrosyan,[1] Lara Hilo,[1] Andy Pao,[1] Gail Rodich,[1] Matthew McIntosh,[2] Stanley C. Jordan.[1] *[1]Transplant Immunology Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA; [2]The EMMMES Corporation, Rockville, MD.*

Backgound: IVIG treatment (Rx) reduces crossmatch (CMX) positivity and increases rates of (Tx) in highly sensitized patients (HS pts). Here, we quantified anti-HLA class I and class II Ab levels in HS pts by ELISA and correlated them with Tx rates after IVIG-Rx and risk of AR. We also measured , anti-endothelial cell Ab (AECA), anti-CMV, total IgG and IgM. **Methods**: Pre-Rx serum samples obtained from 80 highly sensitized ESRD patients entered in the NIH IGO2 study (42 IVIG/38 placebo, Mean PRAs >80%) were submitted for ELISA. Anti-class I, class II and anti-CMV ELISA by kits, AECA and IgG/IgM ELISAs developed in our lab.**Results:** The Pre-Rx levels of anti-class I (43,404±102,943units), anti-class II (51,796±136,784 units) and AECA (16±11units) were elevated in 94, 76 and 31% of patients compared to normal (<300, <300 and <5.5, respectively). Anti-CMV levels were also elevated in 77% of CMV sero-positive pts (141±177 vs. <36IU/ml). Total IgG and IgM levels were similar to normals. Levels of anti-class I and II Abs correlated (c.c. 0.849). PRA-G correlated with anti-class I (cc:0.312), but to a lesser degree with anti-class II (cc:0.223). Pre-Rx levels of anti-class I (18,256±28,331 vs. 65,379±139,082, p=0.054), class II (5,499±7,470 vs. 74,051±16,6391, p=0.023), PRA-G/M (61±28 vs. 73±26, p=0.028), PRA-G (60±28 vs. 76±27, p=0.01) in pts who received transplants were significantly lower than those who did not. Of 29 Tx pts, 5 lost their grafts.The pre-Rx AECA levels in these pts were higher than those with functioning graft (17.9±16.1 vs. 5.7±6.8, p=0.062). 10 of 29 Tx pts developed AR. Their pre-Rx anti-HLA class I, class II and AECA levels were higher than those without AR (class I: 35,458±42,468 vs. 9,655±11,996, p=0.054; class II: 8,981±8,531 vs. 3,759±6,440, p=0.065; AECA: 11.9±14.4 vs. 6.3±7.2, p=0.13). PRA-G/M and PRA-G did not discern those who developed AR. **Conclusions**: 1) HS pts show elevations of AECA and anti-CMV in addition to anti-class I and II Abs, suggesting a possible polyclonal activation of B-cells. 2) Pts with low anti-class I, class II and AECA levels pre-Rx have high rates of Tx, 3) Pts with higher anti-class I, class II and AECA levels pre-Rx have higher risks for AR and graft loss, 4) Modulation of anti-class I, class II, AECA and PRA by IVIG improves transplantation and reduces AR, 5) PRA G/M and PRA-G did not predict AR.

Abstract# 1429

INTRAVENOUS IMMUNGLOBULIN (IVIG) AND THYMOGLOBULIN INDUCTION TREATMENT DOWNREGULATE PREFORMED DONOR-SPECIFIC ANTI-HLA ANTIBODIES (DSA), BUT DO NOT PREVENT THE DEVELOPMENT OF DE NOVO DSA. Enver Akalin,[1,2] Marilena Fotino,[3] Rex Friedlander,[3] Barbara Murphy,[1,2] Scott Ames,[2] Vinita Sehgal,[1,2] Lisa Daly,[2] Jonathan S. Bromberg.[2] *[1]Renal Division; [2]Recanati/ Miller Transplantation Institute, Mount Sinai Medical School; [3]Rogosin Immunogenetics Laboratory, New York, NY.*

An increasing number of recent studies have implicated a role for anti-donor HLA antibodies in the pathogenesis of early and late graft failure. We recently reported the effects of IVIG and Thymoglobulin induction treatment in B cell complement-dependent-cytotoxicity (CDC), and T and/or B cell flow-cytometry (FC) cross-match positive kidney transplant (KT) recipients (Transplantation, in press). In this study, we investigated the effects of IVIG and Thymoglobulin induction treatment on the production of donor specific antibodies (DSA) in 10 KT recipients (9 female, 1 male, 8 Caucasian or Hispanic, 2 African-American, mean age 45± 13) with positive B cell CDC and FC cross-matches. Five also had a positive T cell FC cross-match. Only one allograft was from a cadaveric donor. There were 6 first, 3 second, and 1 fourth transplant. Mean peak PRA±SD was 40±30. Six patients had pre-transplant preformed DSAs by CDC and/or Flow Beads (Luminex). Two patients had anti-HLA class I (anti-A2, B57), 3 had anti-class II (2 anti-DR4, 1 anti-DR13), and 1 had anti-A26, B39 and DR7 (Table). Each recipient received IVIG 100 mg/kg for 3 days and Thymoglobulin 1.5 mg/kg for 5 days after transplantation and maintenance triple immunosuppression with emulsion cyclosporin, mycophenolate mofetil (9 patients) or rapamycin (1 patient), and prednisone taper. DSAs were repeated at 6-12 months post-transplantation by Flow Beads. Three patients lost pre-transplant class II DSAs and 2 lost class I DSAs. One patient each with anti-class I and anti-class II DSA antibodies retained their DSA. However, 4 patients developed de novo DSAs to A29, DR 11, DR17, and DR18. One patient with pretransplant anti-A2 had acute humoral rejection and responded to treatment with plasmapheresis, IVIG, and Rituximab. Two patients with de novo DSAs lost their allograft at 15 and 18 months after transplantation due to polyoma nephritis and thrombotic microangiopathy. Remaining 2 patients with de novo DSAs have stable renal function.These results indicate that IVIG and Thymoglobulin induction treatment might downregulate DSAs, but do not prevent the development de novo DSAs. Clinical significance of de novo DSAs requires further follow-up.

Patient#	Pre-Tx DSA	Post-Tx DSA
1	DR4	0
2	0	0
3	DR4	0
4	A2	A2, DR11
5	0	0
6	A26, B39, DR7	B39
7	0	A29
8	DR13	DR13, DR18
9	0	DR17
10	B57	0

Abstract# 1430

SAFETY AND ADVERSE EVENT PROFILES OF INTRAVENOUS GAMMAGLOBULIN (IVIG) PRODUCTS USED FOR IMMUNOMODULATION: A SINGLE CENTER EXPERIENCE. Ashley A. Vo,[1] Marina Lukovsky,[1] Mieko Toyoda,[1] Dechu Puliyanda,[1] Vinh Cam,[1] Suphamai Bunnapradist,[1] Stanley C. Jordan.[1] *[1]Kidney Transplant Program & Transplant Immunology Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA.*

Introduction: High-dose IVIG is used in management of highly-sensitized patients. With increased use, reports of significant complications including acute renal failure (ARF), thromboembolic events and aseptic meningitis have emerged. Here we describe our experience with IVIG related complications. Methods: 3 products were used from 1997-2003: Gamimune-N 10% (n=76), Polygam (n=105) and Carimmune (n=98). Infusion-related side effects (headache, aseptic meningitis, stroke, myocardial infarction (AMI), acute renal failure, rigors, chills, etc.) were monitored. Viral PCRs (CMV, EBV, Parvo B-19 and Polyoma) were also performed on 55 patients receiving IVIG for desensitization. Results: Headache was the most common complaint (52%) in patients receiving Gamimune-N and this was related to infusion rate. No patient receiving Gamimune-N experienced AMI or ARF. Subsequent infusions were well tolerated when infusion rate decreased and pre-medications given. Between 4/01 and 2/02, 5 patients (4.7%) in the Polygam group experienced AMI. A Naranjo algorithm was done to assess causality and yielded moderate probability. This was reported to FDA Medwatch. Eight patients (8.2%) in the Carimmune group developed ARF 1-21 days after IVIG infusion. 5/ 8 had impaired renal function prior to IVIG infusion, all recovered. Reduction of osmolality reduced this complication. Viral PCR data showed only 1/ 55 receiving IVIG for desensitization experienced viral infection post-transplant (CMV/Polyoma); 4 had CMV viremia. Patients receiving IVIG at 2gm/kg on hemodialysis experienced fewer AE than those receiving IVIG off HD (>400 infusions). Conclusions: 1). IVIG products differ in osmolality, pH , sugar and sodium content, this results in specific side effects. 2). Polygam (sodium excipient) resulted in no ARF, but an increase in MI. The Carimmune product at 9% (sucrose excipient) resulted in an increase in ARF, but no AMI. Gamimune-N (isosmolar) was associated with more HA, but no patients developed AMI or ARF. 3). IVIG products tend to prevent viral infections post-transplant. 4). Administration of IVIG on HD appears to be safe and effective with minimal AE. This is likely due to removal of the excipient by the dialyzer and heparinization of the patient. 5). All IVIG products can be administered safely if side effect profiles are recognized with concomitant product modification and pre-medication given.

Abstract# 1431

INCIDENCE OF SUBCLINICAL REJECTION IN PROTOCOL BIOPSIES PROCURED FROM RENAL TRANSPLANT RECIPIENTS ON TACROLIMUS-BASED IMMUNOSUPPRESSIVE REGIMEN. Mark L. Lipman,[1] Paula Lavery,[1] Yanping Shi,[1] Yingnian Shen,[1] Ziba Aalamian,[1] Rolf Loertscher.[1] *[1]Division of Transplantation, McGill University, Montreal, QC, Canada.*

Subclinical rejection has been defined as the presence of histological rejection in the absence of graft dysfunction, and may contribute to the development of chronic allograft nephropathy and poorer graft outcomes. The incidence of subclinical rejection has been reported to be as high as 30% on cyclosporin-based regimens. Here, we report the incidence of subclinical rejection in recipients on a tacrolimus-based regimen.

53 adult renal transplant recipients from our center were enrolled in a study wherein protocol biopsies were procured at 1,2,3,6, and 12 months. These were in addition to any clinically indicated biopsies that were performed for assessment of graft dysfunction. Donor source was as follows; 41 cadaver, 6 living-related, 6 living-unrelated. Initial immunosuppressive therapy included; ATG induction followed by Tacrolimus, MMF, Pred in 43 recipients, Sirolimus/Tacrolimus/Pred in 7 recipients and various alternative combination therapies in the remaining 3 recipients.

Of the non-protocol biopsies performed to assess graft dysfunction there were 3 confirmed episodes of acute clinical rejection. Of the 130 protocol biopsies performed, 4 biopsies met the criteria for acute rejection (3.1%) and 18 (13.8%) exhibited features of borderline rejection using Banff 97 criteria.

Importantly, a histological diagnosis consistent with polyoma viral infection (confirmed by urine cytology and PCR) was found in 5 protocol biopsies and in one instance where a non-protocol biopsy was performed to investigate graft dysfunction. In addition, there were 6 episodes of CMV viremia/disease and 3 cases of HZV.

This study demonstrates an exceptionally low incidence of both clinical and subclinical rejection in our study population wherein the majority of recipients received antibody induction (83%), followed by a Tacrolimus-based maintenance regimen (94%). However, this is at the expense of a significant incidence of viral complications.

These results underscore the need for balance between prevention of rejection and promotion of viral replication.

Abstract# 1432

A NOVEL DRUG TOXICITY PATHOLOGY SCORE IN PROTOCOL BIOPSIES CAN PREDICT CLINICAL GRAFT AND PATIENT OUTCOMES IN PEDIATRIC RENAL TRANSPLANTATION. Suja Nagarajan,[1] Sheryl Shah,[1] Megan Troxell,[2] Minnie Sarwal,[1] Neeraja Kambham.[2] *[1]Pediatric Nephrology, Stanford University, CA; [2]Pathology, Stanford University, CA.*

Purpose: Standardized pathology scoring exists for both acute and chronic rejection, but there is an unmet need for assessing the severity of drug nephrotoxicity (DT) in an objective manner and evaluating its clinicopathological significance in pediatric renal transplantation (Txp) patients. By studying serial protocol renal biopsies, we aim to establish a DT score that would predict the clinical outcome of the graft based on creatinine clearance (CrCl) and hypertension (HTN).

Methods: Our study aims to conduct a retrospective analysis of 140 consecutive protocol kidney biopsies (done at 3, 6, 12 and 24 months post Txp) from 50 patients with functioning renal allografts and on a steroid-free immunosuppression protocol. At present 70 biopsies have been reviewed in a blinded fashion by the pathologist. The severity of DT was arbitrarily scored in a semiquantitative fashion based on % glomerulosclerosis, tubular atrophy, interstitial fibrosis, isometric tubular vacuolization and arteriolar medial hyaline (0- none; 1-<25%; 2-26-50%; 3->50%). Linear correlation analysis with Pearson's correlation coefficient r was conducted between DT scores and CrCl and HTN. The non DT biopsies were scored for chronicity based on percentage glomerulosclerosis, mesangial matrix increase, tubular atrophy, interstitial fibrosis and arterial intimal fibrosis.

Results: A diagnosis of DT was made in 25 of 70 biopsies; 5 were classified as acute rejection, and 40 as chronic allograft nephropathy. The average DT score was 3.6±1.9 and found to increase over time (delta DT scores 3 to 24 month post Txp : 2). The 3 month DT score correlated significantly with 24 month CrCl (r=-0.996, p=0.05) but there was no significant correlation with HTN (r=0.11). There was no significant correlation with calcineurin inhibitor drug levels (r = 0.29) either. Average DT scores were similar in acute rejection and non rejection biopsies. The average chronicity score was 3.6±2.4, and these scores did not correlate significantly with graft outcome.

Conclusion: A semiquantitative DT scoring system can be predictive of 2 year graft function. DT score can be an useful tool for early titration of calcineurin inhibitor drugs, to minimize accruing nephrotoxicity and preserve graft function. Inclusion of this DT score in standard biopsy pathology analyses may be considered after further studies to determine long term predictive value.

Abstract# 1433

LOW MANNOSE-BINDING LECTIN LEVELS ARE ASSOCIATED WITH IMPROVED OUTCOME IN CADAVER RENAL TRANSPLANTATION. Stefan P. Berger,[1] Anja Roos,[1] Marko J. K. Mallat,[1] Johan W. de Fijter,[1] Mohamed R. Daha.[1] *[1]Department of Nephrology, Leiden University Medical Centre, Leiden, Netherlands.*

Background: The complement system has been shown to be an important component of the immune response in various models of organ transplantation and ischemia/reperfusion damage. The mannose-binding lectin (MBL) pathway of complement is activated by pattern recognition and leads to cleavage of the complement components C4 and C2. Genetic MBL variants with low MBL serum levels are frequent and are associated with recurrent infection. We hypothesized that low MBL levels may be associated with less damage in the setting of ischemia/reperfusion and rejection. As a consequence this may lead to less delayed graft function and improved graft survival in low MBL individuals as compared to patients with normal MBL levels.

Methods: 266 pretransplant serum samples collected from consecutive cadaver kidney transplantation recipients between january 1990 and december 1994 at our institution were analysed for MBL concentration by ELISA. Subsequently the cohort was analysed for the occurrence of delayed graft function and rejection. All transplantations with a functioning graft three months after transplantation were included in the analysis for graft survival.

Results: 36 % of the graft recipients had a MBL level below 400 ng/ml. There was no significant difference in the incidence of delayed graft function in recipients with a low MBL level (< 400 ng/ml) compared to those with a normal MBL level (≥400 ng/ml) (37.1 vs. 34.9%). Similarly there was no difference in the occurrence of acute rejection between both groups. However, average death censored graft survival was significantly longer in those recipients with a low MBL level (12.2 vs. 10.9 years, p=0.02). Multivariate analysis including traditional risk factors for graft loss showed a hazard ratio of 2.7 (CI 1.2 - 6.3) for death censored graft loss if the pretransplant MBL level was above 400 ng/ml. If MBL levels were analysed as a continuous variable the hazard ratio for graft loss was 1.034 (CI 1.008-1.061) for every 100 ng/ml increase in the MBL level.

Conclusion: In our cohort higher MBL levels are associated with poorer graft survival. Our data point to MBL as a progression factor for chronic graft loss in post mortal kidney transplantation. In the setting of inflammation triggered by ischemia/reperfusion or rejection high circulating MBL levels may lead to a potentiation of the inflammatory response and thereby increase tissue damage. Measurement of pretransplant MBL levels may give additional information for risk stratification prior to kidney transplantation.

Abstract# 1434

INTERNATIONAL MULTICENTER TRIAL OF ISLET TRANSPLANTATION USING THE EDMONTON PROTOCOL IN PATIENTS WITH TYPE 1 DIABETES. James Shapiro,[1] Camillo Ricordi,[3] Bernhard Hering,[2] Barbara DiMercurio,[4] Robert Lindblad,[5] Enrico Cagliero,[6] Mathias Brendel,[7] Paul Robertson,[8] Thierry Berney,[9] Antonio Secchi,[10] Daniel Brennan,[11] Eleanor Ramos,[12] Lisa Viviano,[12] Edmond Ryan,[1] Nicole Close,[5] Jonathan Lakey.[1] *[1]University of Alberta, Edmonton, AB, Canada; [2]University of Minnesota, Minnesota, MN; [3]University of Miami, Miami, FL; [4]National Institutes of Health, Bethesda, MD; [5]The EMMES Corporation, Rockville, MD; [6]Massachusetts General Hospital, Boston, MA; [7]Justus-Liebig University, Giessen, Germany; [8]Pacific Northwest Research Institute, Seattle, WA; [9]University of Geneva, Geneva, Switzerland; [10]University of Milan, Milan, Italy; [11]Washington University, St Louis, MO; [12]Immune Tolerance Network, San Francisco, CA.*

The Immune Tolerance Network international multicenter trial using the Edmonton Protocol was designed to explore the safety and efficacy of islet-alone transplantation in patients with Type 1 diabetes. Stringent enrollment criteria included metabolic lability, recurrent hypoglycemia or progressive complications of diabetes. Only optimal patients were selected based on strict weight limitation (£70kg) and adequate renal reserve (CC >80ml/min/1.73m). The trial involved nine sites in Canada, the US and Europe, with a target enrollment of 36 patients. The trial is ongoing and we anticipate all transplants to be completed in the next several months. All 36 participants have been enrolled and have received at least one islet transplant. Clinical follow-up will continue for three years post final transplant with the primary endpoint assessed at the one-year time-point. Updated information regarding the number of transplants, insulin independence, glucose control, complications related to transplantation and overall safety will be available for presentation.

Conclusion: The preliminary findings from this trial support the safety and efficacy of islet-alone transplantation, and indicate that the Edmonton Protocol may be successfully replicated across centers internationally. Standardization of islet processing between centers remains a major challenge, but it should not delay the development of successful clinical protocols in the field of islet transplantation.

Abstract# 1435

GRAFT SURVIVAL ANALYSIS IN 20 TYPE 1 DIABETIC ISLET ALLOGRAFT RECIPIENTS. Bernhard J. Hering,[1] Raja Kandaswamy,[1] Jamen Parkey,[1] Kathryn Duderstadt,[1] Jeffrey D. Ansite,[1] Hui-Jian Zhang,[1] Masahiko Nakano,[1] Ippei Matsumoto,[1] Toshiya Sawada,[1] Baolin Liu,[1] Andrea Bauer,[1] Lukas Guenther,[1] Robin Jevne,[1] Angelika Gruessner,[1] Jeffrey A. Bluestone,[2] David E. R. Sutherland.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN; [2]Diabetes Center, UCSF, San Francisco, CA.*

Background: Islet transplants are an emerging treatment option for type 1 diabetes. Long-term outcomes and results in single-donor recipients will have important implications for the transition of islet transplants from clinical investigation to practice of medicine.

Methods: We analyzed results of 24 consecutive islet allotransplants in 20 type 1 diabetic recipients followed for 59 to 1194 (685 ± 311) days. These recipients participated in 4 pilot clinical trials. Induction immunosuppression was with hOKT3g1 (Ala-Ala), anti-thymocyte globulin, or daclizumab; maintenance immunosuppression was with sirolimus plus tacrolimus, sirolimus plus mycophenolate mofetil, or everolimus plus cyclosporine. Peritransplant etanercept was administered in 10 recipients to promote islet engraftment.

Results: Kaplan-Meier analysis of the entire cohort showed a 1-yr insulin-independent graft functional survival rate of 85%. Only 1 graft was lost in the 2nd or 3rd yr. Recipient survival was100%. Serious, unexpected, and protocol-related adverse events were not experienced; in particular, procedural complications such as portal vein thrombosis or bleeding and immunosuppressive complications such as opportunistic infections or malignancies were not observed. Severe adverse advents included transient neutropenia in 7/20, transient anemia in 1/20, transient LFT elevations in 3/20, and acute cholecystitis in 1/20 recipients. A subgroup analysis of single-donor islet transplant recipients showed that 16 of 18 of these recipients achieved insulin independence. To date, 11 single-donor recipients have maintained insulin independence for more than 1 yr. Their average body weight was 62 ± 5 kg; islet dose was 8.3 ± 2.7 KIE/kg; beta cell dose was 6.0 ± 3.6 x10^6/kg; days to insulin independence was 57 ± 28 days; 1-yr HbA1c was 5.5 ± 0.5%; 2-yr HbA1c (n=7) was 5.1 ± 0.6%; 3-yr HbA1c (n=3) was 5.5 ± 0.3%; OGTT-2hr glucose was 136 ± 32 mg/dl, and acute C-peptide response to arginine was 1.1 ± 0.3 ng/ml.

Conclusion: These preliminary results indicate that outcomes in islet recipients can now match outcomes previously only consistently attainable in pancreas transplant recipients. Thus, increased support for transitioning islet transplants into clinical care should become a priority for the transplant and diabetes community.

Abstract# 1436

COMPARISON OF WHOLE ORGAN PANCREAS AND ISOLATED ISLET TRANSPLANTATION FOR TYPE 1 DIABETES. Adam M. Frank,[1] Shaoping Deng,[1] Thavachenthan Thambi-Pillai,[1] Ergun Velededeoglu,[1] Niraj M. Desai,[1] Xiaolun Huang,[1] Moh Moh Lian,[1] Muhammad M. Mohiuddin,[1] Yong Suk Bae,[1] Gerald R. Stephenson,[1] Peter L. Abt,[1] Marty T. Sellers,[1] Clyde F. Barker,[1] Ali Naji,[1] James F. Markmann.[1] *[1]Surgery, Division Transplantation, Hospital of the University of Pennsylvania, Philadelphia, PA.*

Objectives: Beta cell replacement is the only therapy that can fully normalize glucose homeostasis in Type 1 Diabetes. The recent success of isolated islet transplants(IIT) using the Edmonton Protocol has led some to believe that it should replace whole-organ pancreas transplants (WPT). Direct comparison of these therapies has not been reported.

Methods: We compared our ongoing series of IIT performed 9/01-11/03(n=12 patients, 22 infusions, 9 Islet Transplant Alone, 3 Islet After Kidney) with consecutive WPT performed concurrently (n=23 patients, 20 Simultaneous Cadaveric Pancreas Kidney Transplants(SPK), 3 Cadaveric Pancreas after Kidney). Only pancreata declined for WPT (because of steatosis or age >50) were utilized for IIT. Compared with WPT, IIT donors were older (25.3vs.49.3 yrs), and obese (23.4kg/m² vs. 34.3kg/m²).

Results: The average number of islet-equivalents (IEQ)/infusion was 551,100 (range 293,600-881,700; or 8,440IEQ/kg-recipient) and the total infused/patient 15,475IEQ/kg. WPT and IIT recipients were similar in age(42 years) and BMI(24 kg/m²). WPT cold ischemia time averaged 13.25hrs; from cross-clamp to infusion of IIT was 15.25hrs (5.75hrs of recovery/storage and 9.5hrs of processing). Hospital LOS was markedly longer for WPT(12.6 versus 2.0days). Of 23 WPT's, 22 are still functioning (mean follow-up 383 days). The one failure was due to intractable perigraft infection and was explanted at one month. The kidney graft in this recipient as well as all SPK recipients retain excellent function (mean serum Creatinine 1.24mg/dl range 0.8 to 2.1 mg/dl). Of the12 IIT recipients (mean follow-up 393 days), 10 became insulin independent (1 withdrew), 4 remain totally insulin independent (up to 2.2 years) and 2 require only minimal insulin (<5units/day); three grafts failed (8, 9, and 17 mo.). One patient is awaiting a second dose.

Conclusions: IIT was performed with older, obese donors and was associated with less morbidity and shorter LOS. However, more donors/recipient were needed to achieve insulin independence. Sustained IIT function was inferior to WPT. Fortunately, older and obese donors can be utilized successfully for IIT without decreasing utilization of young lean donors most suitable for WPT. At present, IIT and WPT should be considered complementary therapies in the treatment of type 1 diabetes.

Abstract# 1437

PROSPECTIVE COMPARISON OF CLINICAL OUTCOMES WITH FRESH VS CULTURED ISLETS AT A SINGLE INSTITUTION. James Shapiro,[1] Edmond Ryan,[1] Deborah McGhee-Wilson,[1] Peter Senior,[1] Breay Paty,[1] Parastoo Dinyari,[1] Tara McCready,[1] Huey Chong,[1] Norman Kneteman,[1] David Bigam,[1] Jonathan Lakey.[1] *[1]Clinical Islet Transplant Program, University of Alberta, Edmonton, AB, Canada.*

Practical and safety considerations in clinical islet transplantation have recently led to the increased use of islet storage in culture rather than immediate implantation. Culture allows patients to live remotely from the isolation center, and often improves the purity of the islet preparation, thereby reducing the packed cell volume, which in turn lowers the risk of portal vein thrombosis. Successful, prolonged culture has been made possible by the availability of insulin-transferrin-selenium (ITS) supplemented CMRL media.

Of a total of 59 patients transplanted at a single institution to date, 45 have received completed islet infusions. Of these, 20 patients (32 procedures) received **cultured** islets (mean age 43±10; weight 69±12kg; pre-transplant insulin requirement 0.7±0.2 U/kg/d; total islet mass 11,955±4,052 IE/kg) and 25 patients (63 procedures) received **freshly prepared** islets (mean age 43±11; weight 68±9kg; pre-transplant insulin requirement 0.6±0.2 U/kg/d; total islet mass 12,990±3,131 IE/kg). The mean culture time was 19.2±14.5 hours (range 6.5 to 60 hours). Prolonged islet culture generally reduces the post-purification tissue volume by 2 to 5cc. The volume of islet preparation transplanted was 3.9±1.7cc in the cultured vs 4.2±1.9 in the fresh group.

Islet culture did not adversely impact graft survival as determined by attainment (90% cultured vs 95% fresh) or maintenance (75% cultured vs 76% fresh) of insulin independence within the first year. In terms of C-peptide secretion (>0.5ng/ml), 93% cultured and 95% fresh of grafts were functioning at one year, and 93% cultured and 85% fresh continued to function at two years. HbA1C was normalized in both patient groups (6.0% fresh vs 6.2% culture at one year; 6.3% fresh vs 5.9% culture at two years). In conclusion, a prospective comparison of one and two year outcomes with fresh and cultured islets demonstrates similar islet graft function rates. Islet culture additionally confers the benefits of increased practicality and safety. Therefore islet culture is beneficial in clinical islet transplantation.

Abstract# 1438

INSULIN INDEPENDENCE FOLLOWING TRANSPLANTATION OF CULTURED HUMAN ISLETS IN PATIENTS WITH TYPE 1 DIABETES: THE MIAMI EXPERIENCE. Rodolfo Alejandro,[1] Jacqueline V. Ferreira,[1] Tatiana Froud,[1] David A. Baidal,[1] Muhammad Hafiz,[1] Norma S. Kenyon,[1] Camillo Ricordi.[1] *[1]University of Miami - Diabetes Research Institute, Miami, FL.*

Introduction: Several clinical trials in islet transplantation are currently in progress utilizing fresh human islet allografts, including a multi-center trial. We proposed to assess the efficacy of in-vitro culture of human islets using an immunosuppression protocol similar to the one in Edmonton.

Methods & Results: 15 consecutive patients (7 males, 8 females) with type 1 DM received human islet allografts isolated using automated method with minor modifications and cultured in Miami defined media and administered via ultrasound guided percutaneous transhepatic intraportal infusion. Immunosuppression consisted of daclizumab induction and sirolimus/tacrolimus maintenance. 14 patients became insulin independent (13 with 2 islet infusions and 1 with 1); 1 patient was awaiting a second infusion but was discontinued from immunosuppression and will not be discussed below. First transplant (Tx) averaged 6919± 1938 IEQ/kg (n=14; range 4592-12013) and second 7230± 2031 IEQ/kg (n=13; range 3577-10306), with total of 13633± 3010 IEQ/kg (n=14; range 6250-18403). Insulin requirements pre Tx were 0.48± 0.1 U/kg/day (n=13; range 0.29-0.58). Currently 9 patients are insulin independent with stable islet allograft function and glycemic control 888, 887, 864*, 822*, 782*, 770, 675, 628* and 419 days post first islet infusion (*=3rd islet infusion). 9 out of 14 patients have had deterioration of metabolic control requiring the reintroduction of insulin (13± 7 units) on post Tx day 395± 112 (range 208-513). 5 patients received a 3rd infusion and 2 are being considered for a supplemental infusion. 2 additional patients with deterioration of metabolic control were withdrawn from the trial (1 was lost to follow up and 1 discontinued immunosuppression due to intercurrent illness). There was no correlation between recurrence of autoimmunity and partial loss of graft function, but there was correlation with elevation of cytotoxic lymphocyte genes expression especially granzyme B.

Conclusions: Human islet allografts cultured in Miami defined media resulted in insulin independence in 14 patients with normalization of glycemic control under steroid free immunosuppression regimen. However, there seems to be a partial loss of islet function or islet mass as a function of time similar to what is seen with fresh islets. The mechanism(s) of this loss need(s) to be clarified. The introduction of supplemental infusions when this occurs may result again in insulin independence.

Abstract# 1439

CO-TRANSPLANTATION OF PORCINE ISLETS WITH SERTOLI CELLS IN TYPE 1 DIABETIC PATIENTS PRODUCES ATYPICAL HUMORAL IMMUNE RESPONSES AS ASSED BY ELICITED ANTI-PIG ANTIBODIES. R. A. Valdes-Gonzalez,[1] L. M. Dorantes,[1] G. N. Garibay,[1] E. Bracho,[1] L. Teran,[1] L. Silva,[1] P. Valencia,[1] L. Copeman,[2] D. J. G. White.[2] *[1]Surgery, Endocrinology and Pathology, Hopital Infantil de Mexico, Mexico City, Mexico; [2]Robarts Research, University of Western Ontario, London, ON, Canada.*

Protocol 12 patients with type 1 diabetes were transplanted with neonatal pig islets and sertoli cells (35-100/islet) derived from a SPF herd of large white pigs. The cells were inserted into neovascularised collagen tubes created by implanting a closed stainless steel mesh containing an inner Teflon stent subcutaneously 8 weeks prior to transplantation. No immunosuppression was given. 11 patients received a second transplant 6 months after the first procedure. 6 of these recipients have a significant reduction in the requirement for exogenous insulin at more than 2 years post transplant with one patient being insulin independent for more than one year. Patients were monitored for lytic antibodies to porcine RBC and anti-GAL IgM and IgG antibodies. IgG isotypes were measured by FACS analysis using a pig kidney cell line as targets. **Results** All patients demonstrated an increase in anti-pig antibodies in response to the first transplant. Mean GAL IgM responses rose from a pre-transplant value of 1:90 to 1:180 (1 $\log_2$). This declined to pretransplant levels (1:90) by 6 months. These anti-GAL IgM responses were significantly ($P<0.01$) less than those previously reported for islet transplants alone (in immunosuppressed patients) where the titre increased at one month from 1: 85 to 1: 980. Anti-GAL IgG responses in the two patient populations were comparable at one month. In this study mean IgG titres increased from 1:150 to 1:3200. By 6 months post transplant these had declined to a mean titre of 1:750. IgM Responses at one month to a second transplant were similar to those seen after the first transplant. Thus anti-GAL IgM mean titres increased from 1:90 to 1:190 (1.1 $\log_2$). IgG titres increased from a pre-transplant level of 1:750 to 1:2500. Thus these anti-GAL IgG responses to a second transplant (1.7 $\log_2$) were significantly less than those induced by the first transplant (4.4 $\log_2$ $P=0.03$). Anti-pig haemolytic antibodies mimicked the results seen with anti-GAL IgM **Conclusion** Patients transplanted with a combination of porcine islets and Sertoli cells produce atypical humoral immune responses to their graft. Anti GAL IgM responses are significantly reduced, as is the secondary response by Anti-GAL IgG.

Abstract# 1440

SUCCESFSUL HUMAN ISLET TRANSPLANTATION FOLLOWING DISCONTINUOUS GRADIENT RESCUE PURIFICARTION. Hirohito Ichii,[1] Antonello Pileggi,[1] R. Damaris Molano,[1] Aisha Khan,[1] Ismail Al-Abdullah,[1] Yoshikazu Kuroda,[2] Luca Inverardi,[1] 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. More than 5,000 islet equivalents (IEQ)/kg recipient body weight are generally required as a pre-transplant product release criteria in clinical trials of human islet transplantation. Despite improvements in the isolation methods, it is difficult to produce consistent islet yields that meet the minimal requirements for transplantation. The relative inefficiency of the islet purification process may not allow us to obtain enough islets for transplantation even when pre-purification counts are adequate. In this study, we determined the effect of an additional purification step, termed Rescue Purification (RP).

Method. Twenty pancreata were processed by using a standard protocol of Liberase perfusion, mechanical dissociation with the automated method and continuous gradient purification. In all cases the less pure islet fractions and exocrine pellets were subjected to an additional RP step using discontinuous Eurocollins-Ficoll gradients (density: 1.132, 1.108, 1.096, 1.037). The islets obtained from the RP were combined and transplanted into recipients with type I diabetes.

Results. The average IEQ from continuous and rescue purification were 344,572±138,036 IEQ and 140,888±113,589 IEQ respectively. The rescue purification yielded a 27.2%±18.3% contribution to the total IEQ. There were no significant differences in the quality of these islets, as assessed by viability and insulin secretion *in vitro* and *in vivo* following transplantation in diabetic nude mice. In 10 of 20 preparations (50%), the IEQ obtained from continuous gradients yielded less than 5,000 IEQ/kg in the absence of the RP. However the total IEQ/kg were higher than the minimal requirement of 5,000 IEQ/kg. In these cases, the transplant could not have been done unless the RP step was performed. All of the islet preparations were successfully transplanted and resulted in insulin independence and sustained C-peptide production, with normalization of HbA1C levels.

Conclusions. An additional RP step may be of assistance to maximize the number of islet preparations that can be successfully used in transplantation protocols.

Abstract# 1441

INSULIN RESISTANCE AND PROCESSING, APOPTOTIC AND IMMUNOLOGICAL PROCESS IN THE COURSE OF ISLET GRAFT FAILURE IN ISLET TRANSPLANTED PATIENTS. Paolo Fiorina,[1] Alberto M. Davalli,[1] Federico Bertuzzi,[1] Paola Maffi,[1] Chiara Gremizzi,[1] Marta Bruno Ventre,[1] Lucilla Monti,[1] Ezio Bonifacio,[1] Emanuele Bosi,[1] Antonio Secchi.[1] [1]*Medicine, San Raffaele Scientific Institute, Milan, Italy.*

Background: Our aim was to study peripheral markers of insulin resistance, insulin secretion, autoimmunity and apoptotic/immunological processes in islet transplanted patients who experienced an early failure of the transplanted islet or not.

Patients and Methods: 37 islet transplanted patients were enrolled, they were splitted in long term (*Long*= 17 pts with long-term islet fx) or short term functioning islet (*Short*,=20 pts, with lost of 50% of C-peptide within 6 months). In all the patients we analyze at baseline and twice during the follow-up (at best islet function moment and at failure moment) markers of: (i) Islet function (exogenous insulin requirment, C-peptide, glucose and insulin levels); (ii) Insulin resistance (HOMA IR); (iii) Insulin processing (Pro-Insulin/C-peptide ratio); (iv) Islet overworking (activin and amylin levels); (v) Apoptotic process (sFas and sFasL); (vi) Immunological process (sIL2r); (vi) Autoimmunity (Ab anti-GAD, anti-IA2, anti-insulin).

Results: *Long*, but not *Short*, group showed a persistent C-peptide secretion up to 36 months (*Long*=1.7±0.4 vs. *Short*=0.6±0.2 ng/ml, p<0.05), with higher exogenous insulin requirment (p=0.04 and p=0.01 at 12 and 36 months respectively). An early and persistent increase of HOMA was evident in *Short* group (HOMA at full function point: *Short*= 7.5±2.3 vs. *Long*=3.5±0.5, p<0.01). A higher ratio Pro-Insulin/C-peptide ratio was evident in the *Short* group particularly at the time of failure (*Short*=32.4±11.9 vs. *Long*=7.0±1.6, p<0.05) as well as sFasL. SIL-r is high during all the follow-up in *Long* group, particularly at the failure moment (*Long*=4901±1033 vs. *Short*=2754±490 pg/ml, p<0.05). Increased islet working, as suggested by high activin levels, was evident in *Short* group. Finally, at 6 months a rise in Ab anti-GAD and IA2 (baseline: *Short*=1.07±0.62 vs. *Long*=2.52±1.21 AU; 6 months: *Short*=15.45±13.94 vs. *Long*=2.37±1.30 AU) were evident in *Short* group.

Discussion: Patients who experienced an early worsening of islet function showed higher insulin resistance, altered pro-insulin processing and islet overworking, higher levels of sFasL lower levels of sIl2-r, suggesting a reduced tolerance process associated with recurrence of autoimmunity.

Abstract# 1442

AN INITIAL EXPERIENCE WITH LAPAROSCOPIC(LAP) DONOR DISTAL PANCREATECTOMY FOR LIVING DONOR PANCREAS TRANSPLANTATION. Miguel Tan,[1] Raja Kandaswamy,[1] Rainer W. Gruessner.[1] [1]*Department of Surgery, University of Minnesota, Minneapolis, MN.*

Introduction: Although the pancreas was the first extrarenal organ to be used from living donors, this resource has been underutilized because of the potential morbidity of an open distal pancreatectomy in an otherwise healthy donor. With the advent of lap donor nephrectomy, the lap approach has become the procedure of choice for kidney donation because of reduced hospital stay and more rapid convalescence. It has been shown to be equivalent to the open procedure in terms of donor safety and quality of allograft. Consequently, we performed the first lap donor distal pancreatectomy in 1999 and present our initial experience with this procedure.

Methods: Between March 1999 and August 2003 we performed 5 hand assisted lap donor pancreatectomies. All donors underwent standardized metabolic workup. The mean donor age was 48.4 yrs +/- 8.7 with a BMI of 23.7 kg/m^2. The average length of surgery for pancreas donors alone (PTA) was 4.5hrs +/-0.13; for pancreas/kidney donors (SPK), 7.9 hrs +/-0.38. Mean blood loss was 330 mL +/-228. Mean recipient age was 36.6 yrs +/-9.4. OR time for PTA recipients was 8.4 hrs +/-2; for SPK recipients, 9.6 hrs +/-3.4.

Results: Donor and recipient survival was 100% at up to 3-yrs follow-up. None of the donors required blood products perioperatively. There were no episodes of pancreatitis, leaks, or pseudocysts. One splenectomy was performed at the time of the original surgery for a non-viable spleen. The average length of hospital stay was 8 days +/-2. All donors are back to their pre-operative state of health and back to work. None of the donors have required oral antidiabetic medications or insulin. In the recipients, pancreatic and renal allograft function is 100% at 3-yrs. There were 2 instances of graft pancreatitis, 3 intra-abdominal infections, and 2 anastomotic leaks. There were 3 rejection episodes in one patient.

Discussion: We conclude that (1) lap donor distal pancreatectomy is a safe procedure with excellent donor outcome; (2). hand assisted (vs. fully) lap distal pancreatectomy is preferable, because of the added margin of safety due to increased tactile feedback and ease of pancreatic dissection; (3) the procedure can be accomplished with a single 6-cm periumbilical incision and two 10-mm ports, resulting in excellent cosmesis and high donor satisfaction; (4) at 3-yr follow-up, graft and recipient survival are 100%.

Abstract# 1443

OVEREXPRESSION OF HUMAN COPPER/ZINC-SUPEROXIDE DISMUTASE (SOD1) SUPPRESSES ISCHEMIA REPERFUSION INJURY AND SUBSEQUENT DEVELOPMENT OF GRAFT CORONARY ARTERY DISEASE IN MURINE CARDIAC GRAFTS. Masashi Tanaka,[1] Feny Gunawan,[1] Raya D. Terry,[1] Golnaz K. Mokhtari,[1] Anthony D. Caffarelli,[1] David T. Cooke,[1] Theo Kofidis,[1] Robert C. Robbins.[1] [1]*Department of Cardiothoracic Surgery, Stanford University, Stanford, CA.*

Ischemia reperfusion (I/R) injury is an important risk factor for the development of graft coronary artery disease (GCAD). We hypothesized that overexpression of SOD1 in donor hearts would suppress I/R injury and reduce GCAD. **Methods:** In one series, donor hearts of C57BL/6 (H-2^b) transgenic mice overexpressing human SOD1 (Tg) or C57BL/6 wild-type (WT) mice were heterotopically transplanted into C57BL/6 recipients (n = 6 each group). Grafts were procured after 4 hours of reperfusion, and parameters of I/R injury including superoxide generation by the spin-trapping method; TNF-α and MCP-1 cytokine levels by ELISA; myeloperoxidase (MPO) activity by MPO activity assay; and cardiomyocyte apoptosis by TUNEL, caspase-3, -8, and -9 activities were measured. Graft injury was assessed by measuring serum levels of CPK-MB. In a second series, transgenic and WT hearts were transplanted into C-H-2^{bm12}KhEg (H-2^{bm12}) mice (n = 7 each group). Cardiac graft beating (scale of 0 to 4) was assessed by abdominal palpation on days 14, 28, 42, and 56. After 56 days, MCP-1, ICAM-1, and VCAM-1 levels were measured by ELISA and GCAD was assessed by computerized morphometry. **Results:** In the first series, production of superoxide, TNF-α, and MCP-1 was significantly reduced in the Tg group in comparison to such production in the WT group. MPO activity was significantly decreased in the Tg group, indicating decreased neutrophil infiltration and activation. Cardiomyocyte apoptosis and caspase-3 and -9 activities were significantly decreased in the Tg group; caspase-8 activity was unchanged. Serum CPK-MB level was significantly decreased in the Tg group. In the second series, graft beating scores were significantly better in the Tg group at POD 28 ($p = 0.01$), 42 ($p < 0.001$), and 56 ($p < 0.001$). The production of MCP-1, ICAM-1 and VCAM-1 were significantly decreased in the Tg group at 56 days. The SOD1 transgenic donor group showed significantly less luminal narrowing (SOD1 vs WT: 18 ± 12 vs 45 ± 17%, $p < 0.01$), intima/media ratio (SOD1 vs WT: 0.2 ± 0.1 vs 0.7 ± 0.3, $p < 0.01$), and % of diseased vessels (SOD1 vs. WT: 45 ± 27 vs. 78 ± 24 %, $p = 0.04$) at 56 days. **Conclusions:** Overexpression of SOD1 attenuates I/R injury and subsequent development of GCAD. Gene therapy approaches that target SOD1 may be useful in clinical cardiac transplantation.

Abstract# 1444

OVEREXPRESSION OF HUMAN BCL-2 BY ADENOVIRAL GENE TRANSFER IN DONOR CORONARY ARTERY SMOOTH MUSCLE CELLS REDUCES DONOR GRAFT APOPTOSIS AND LEADS TO AN EARLY TH2 CYTOKINE PROFILE IN RAT CARDIAC ALLOGRAFTS. David Tom Cooke,[1] Jeremy I. Pearl,[1] Masashi Tanaka,[1] E. Grant Hoyt,[1] Robert C. Robbins.[1] [1]*Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA.*

Purpose: Overexpression of the human anti-apoptotic protein Bcl-2 by adenovirus mediated gene transfer in donor rat hearts decreases acute graft oxidative stress, as well as chronic graft coronary artery disease (GCAD). Other studies have suggested that TH2 cytokines, such as IL-4 and IL-10 may assist graft survival in solid organ transplantation, and TH1 cytokines, such as IL-2 may inhibit graft survival. We tested the hypothesis that human Bcl-2 overexpression in donor rat coronary arteries by adenovirus gene transfer would promote an early protective TH2 cytokine profile after allotransplantation.

Methods: PVG (RT1c) hearts were infused by antegrade aortic injection with $1.0x10^9$pfu of either AdvBcl-2 (N=5) or AdvNull (N=5) in 300µl of PBS, heterotopically transplanted into the abdomen of syngeneic PVG recipients, and then reperfused for 4 days. Grafts were excised and retransplanted into allogeneic ACI (RT1a) recipients treated with one dose of oral cyclosporine (7.5mg/kg). Hearts were procured on postoperative day one. Donor heart tissue was evaluated by histology and protein extracts by ELISA and activated caspase-3 and -9 assays.

Results: Human Bcl-2 was overexpressed in AdvBcl-2 treated hearts compared to AdvNull treated controls on postoperative day one (234.4±86.2 pg/mg vs. 44.2±7.9, p<0.05). Human Bcl-2 localized to the medial smooth muscle cells of large coronary arteries as well as the perivascular area, and patchy areas of adjacent myocardium. AdvBcl-2 treated hearts exhibited diminished apoptosis compared to controls, characterized by reduced activated caspase-3 (4.2±3.5 µM/mg vs. 60.7±12.3, p<0.01) and caspase-9 activity (2.8±2.8 µM/mg vs. 52.7±35.1, p<0.05). In addition, donor AdvBcl-2 hearts had reduced intragraft expression of ICAM-1. Moreover, AdvBcl-2 hearts expressed significantly high intragraft levels of IL-4 compared to control hearts (5836.2±951.2 pg/mg vs. 2734.4±332.7, p<0.05), increased IL-10, and reduced IL-2 (3961.5±538.8 pg/mg vs. 6212.6±479.8, p<0.05).

Conclusion: Adenoviral gene transfer of human Bcl-2 in smooth muscle cells of donor coronary arteries reduces donor graft post-transplant apoptosis, and leads to an early intragraft TH2 cytokine profile in rat allografts, suggesting a role for gene therapy in human heart transplantation in the future.

Abstract# 1445

OVEREXPRESSION OF Bcl-2 REDUCES GRAFT CORONARY ARTERY DISEASE BY SUPPRESSING ISCHEMIA REPERFUSION INJURY AND IMMUNE RESPONSE DURING ACUTE AND CHRONIC PHASE IN MURINE CARDIAC ALLOGRAFTS. Masashi Tanaka,[1] Susumu Nakae,[2] Raya D. Terry,[1] Theo Kofidis,[1] Robert C. Robbins.[1] *[1]Department of Cardiothoracic Surgery; [2]Department of Pathology, Stanford University, Stanford, CA.*

Ischemia reperfusion (I/R) injury and acute rejection are risk factors for the development of graft coronary artery disease (GCAD). We hypothesized that Bcl-2 overexpression in donor hearts would reduce GCAD by suppressing I/R injury and immune response during acute and chronic phase. **Methods:** Hearts of FVB ($H\text{-}2^q$) transgenic mice overexpressing human Bcl-2 (Tg) and FVB wild-type mice (WT) were heterotopically transplanted into C57BL/6 ($H\text{-}2^b$) mice. In the first series, parameters of I/R injury, including TNF-α and MCP-1 levels by ELISA, cardiomyocyte (CM) apoptosis by TUNEL, caspase-3, -8, and -9 activities were measured after 2 h (n = 5 each). In a second series, immune response during acute rejection was assessed at 8 days (n = 6 each). Intragraft T cell subsets ($CD4^+$ and $CD8^+$), B cells ($B220^+$), macrophages ($Mac1^+$), and dendritic cells ($CD11c^+$) were quantified by immunostaining. IFN-γ, MCP-1, IP-10, MIG, ICAM-1, VCAM-1 levels by ELISA, and CM apoptosis by TUNEL and caspase-3 activity were measured. In a third series, host spleen cell response to the allograft was assessed by flow cytometry (FACS) and mixed lymphocyte reaction (MLR) at 1, 3, 5, and 7 days (n = 3 each). In a fourth series, IFN-γ, MCP-1, IP-10, MIG, ICAM-1, and VCAM-1 levels by ELISA, CM apoptosis, and GCAD indices were assessed at 30 days (n = 8 each). The recipients in this series received cyclosporine A. **Results:** At 2 h, TNF-α and MCP-1 levels, CM apoptosis, and caspase-3 and -9 activities were significantly decreased in the Tg group, but not caspase-8 activity. At 8 days, $CD8^+$, $CD11c^+$, and $Mac1^+$ cell infiltration were significantly decreased in Tg group but $CD4^+$ and $B220^+$ were not different. IFN-γ, MCP-1, IP-10, MIG, ICAM-1, VCAM-1 levels and CM apoptosis were reduced in Tg group. FACS and MLR did not show a difference in host spleen cell response ($CD4^+$ and $CD8^+$ cell count, proliferation profile, and TNF-α and IFN-γ production). At 30 days, IFN-γ, MCP-1, IP-10, MIG, ICAM-1, VCAM-1 levels and CM apoptosis were reduced in Tg group. The Tg group showed decreased luminal narrowing (17 ± 11 vs. $46 \pm 12\%$, $p < 0.01$), intima/media ratio (0.2 ± 0.1 vs. 0.6 ± 0.2, $p < 0.01$), percent of diseased vessels (42 ± 23 vs. $98 \pm 7\%$, $p < 0.01$). **Conclusions:** Bcl-2 overexpression in donor heart reduces I/R injury and the immune response during acute and chronic phase without affecting host spleen cell response. This reduction is correlated with a decrease in GCAD.

Abstract# 1446

IMMUNOSUPPRESSANT PROPERTIES OF α-TOCOPHERYL (PEG-1000) SUCCINATE THAT SYNERGIZES WITH LOW-DOSE CYCLOSPORINE IN CARDIAC ALLOGRAFT REJECTION. Galen M. Pieper,[1] Thanh K. Nguyen,[1] Vani Nilakantan,[1] Christopher P. Johnson,[1] Allan M. Roza,[1] Mark B. Adams,[1] Christopher C. Felix,[2] Ashwani K. Khanna.[3] *[1]Surgery (Transplant); [2]Biophysics; [3]Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Purpose: To evaluate the immunosuppressant activities of the water-soluble vitamin E derivative, α-tocopheryl (PEG-1000) succinate (TPGS, 80 IU/100g daily until rejection) vs. low-dose cyclosporine (CsA, 2.5 mg/kg for days 0-7) alone and in combination on cardiac allotransplantation. *Methods:* Heterotopic cardiac transplantation was performed in Wistar-Furth to Lewis rat strains. ISHLT rejections scores and graft survival time were determined. Graft function was measured by *in situ* sonomicrometry. DNA binding of NF-κB and AP-1 and expression of inducible nitric oxide synthase (iNOS) were determined by gel shift assay and Western blots, respectively. Direct effects on TPGS were evaluated in mitogen-stimulated lymphocyte cultures. *Results*: Graft survival in untreated allografts (6.8 ± 0.1 days) was prolonged by TPGS (10.8 ± 1.2 days, $P<0.05$) equipotent to low-dose CsA (12.0 ± 0.3 days, $P<0.05$). Combination therapy caused a synergistic action on graft survival (31.0 ± 3.7 days, $P<0.001$). Histological rejection scores were improved by monotherapy and combination therapy (untreated: 4.9 ± 0.3; TPGS: 3.3 ± 0.5, $P<0.01$; CsA: 2.6 ± 0.3, $P<0.01$; TPGS + CsA: 2.0 ± 0.3, $P<0.001$). Short-axis fractional shortening was improved by TPGS alone ($P<0.05$) while increases in systolic and diastolic length were prevented by TPGS or CsA alone or both in combination. Downstream protein targets of nitric oxide (e.g. nitrosylation of myocardial heme protein) was significantly inhibited by TPGS alone and abolished by CsA alone or in combination with TPGS. Western analysis revealed that iNOS protein was downregulated by nearly 50% by TPGS alone equipotent to low-dose CsA but apparently occurred via a NF-κB and AP-1 independent pathway. Combination therapy further decreased iNOS expression compared to either agent alone. In addition to decreased cell infiltration *in vivo*, we found that TPGS markedly inhibited mitogen-stimulated lymphocyte proliferation *in vitro* (68, 85 and 95% inhibition at 1, 10 and 20 μM TPGS, respectively, $P<0.01$ each vs. untreated). *Conclusions:* The vitamin E analog, TPGS, has significant effects *in vitro* and *in vivo* to limit cell proliferation and activation. This might explain the equipotent action of TPGS vs. low-dose CsA to prolong graft survival and limit histological rejection and explain its action to synergize with CsA on prolonging graft survival.

Abstract# 1447

INDUCED HEME OXYGENASE-1 PLUS DONOR SPECIFIC TRANSFUSION YIELDS ANTIGEN SPECIFIC TOLERANCE MEDIATED BY REGULATORY $CD4^+CD25^+$ T CELLS. Kenichiro Yamashita,[1] James McDaid,[1] Robert Öllinger,[1] Hongjun Wang,[1] Eva Csizmadia,[1] R. Neal Smith,[2] Miguel P. Soares,[1] Fritz H. Bach.[1] *[1]Immunobiology Reserch Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [2]Pathology, Masachusetts General Hospital, Harvard Medical School, Boston, MA.*

Aim: Up-regulation of heme oxygenase-1 (HO-1), a stress inducible 'protective' gene, prolongs allograft survival in rodents. We studied the combined effects of HO-1 induction and donor-specific transfusion (DST) in allo-transplantation (Tx).

Methods: MHC incompatible DBA/2 ($H\text{-}2^d$) cardiac grafts were transplanted into B6AF1 ($H\text{-}2^{k/d,b}$) mice. Tx recipients received DST ($20x10^6$ of DBA2 splenocytes) either 7 days before (D-7) or immediately after Tx (D0). HO-1 was induced or inhibited by using cobalt- or zinc-protoporphyrin (CoPP or ZnPP, 5 mg/kg/day), respectively. Protoporphyrins were given i.p. to recipient for 15 days starting one day before DST without further treatments.

Results: Untreated control mice rejected allografts with a median survival time (MST) of 11.5 days (n=6). DST (D0) alone did not prolong survival (MST: 9.5 days, n=6). In contrast, HO-1 induction plus DST (D0) significantly prolonged graft survival (MST: >100 days, n=8) with 87.5% of grafts surviving over 100 days; however, ZnPP treatment did not (MST: 8 days, n=7). DST (D-7) prolonged graft survival (MST: > 60 days, n=8) with 50% of grafts survived more than 100 days. HO-1 induction plus DST (D-7) led to a long-term acceptance in all grafts (MST: >100 days, n=7), while ZnPP treatment plus DST resulted in a prompt graft rejection (MST: 13 days, n=9). CoPP plus DST treated recipients carrying long-term surviving hearts accepted second heart grafts from the donor (n=6) but not third party (FVB, $H\text{-}2^q$) strain (n=6) showing that those recipients were tolerant to the donor antigens specifically. Sub-lethally irradiated naïve B6AF1 recipients were reconstituted by adoptive transfer with leukocytes ($50x10^6$ lymph node and spleen cells) of tolerant animals. DBA/2 hearts survived for >100 days (n=3) in such animals. Depletion of $CD4^+CD25^+$ T cells from the adoptively transferred leukocytes abolished the "tolerizing" effect (MST: 11 days, n=4). Animals receiving naïve B6AF1 leukocytes promptly rejected DBA2 hearts (MST: 12.5 days, n=6).

Conclusion: Induction of HO-1 plus DST can lead to donor-specific tolerance to cardiac allografts based on the action of regulatory $CD4^+CD25^+$ T cells. HO-1 induction may be worth considering in some strategies to achieve tolerance.

Abstract# 1448

PROTEIN KINASE C EPSILON DEFICIENCY IN KIDNEY TRANSPLANTS IMPROVES ALLOGENIC TRANSPLANT SURVIVAL. Song Rong,[1] Joon-Keun Park,[1] When Zen,[1] Anke Schwarz,[1] Michael Leitges,[2] Hermann Haller,[1] Faikah Gueler.[1] *[1]Nephrology, Medical School Hannover, Hannover, Germany; [2]Experimental Endocrinology, Max Planck Institute, Hannnover, Germany.*

Protein Kinase C (PKC) contributes to vascular reactivity in response to hypoxia and is involved in different signaling pathways which are relevant for ischemia reperfusion injury and transplant rejection. The isoform PKC epsilon regulates various physiological functions including the activation of inflammatory and immunological reactions. The role of PKC isoenzymes in acute kidney transplant rejection has not been studied so far.

Allogenic kidney transplantation was performed after removal of the right native kidney. Donor kidneys from 12-16 weeks old PKC epsilon knock out (-/-) donor mice (mixed background: C57Bl6/SV129) were transplanted into BalbC recipients. The contralateral kidney was removed at day 4. Donor kidneys from littermates (C57Bl6/SV129) served as wild type (WT) controls. Animals were sacrificed 24 hours and 6, 14, 21 and 28 days after transplantation. Plasma creatinine was measured spectrophotometrically. Kidneys were examined by western blot analysis and by immunohistology for infiltrating cells, adhesion molecules, i-NOS expression and apoptosis.

PKC epsilon -/- mice displayed normal renal function and histology. Renal function and pathology deteriorated rapidly in rejecting kidneys from WT but not from PKC epsilon -/- donors. Recipients from WT donors died within 7-10 days after transplantation, whereas recipients from PKC epsilon -/- mice survived 4 weeks until the end of the planned observation period. Recipients from PKC epsilon -/- mice had only 2 fold creatinine elevation 6 days after transplantation and kidney function remained almost stable within the next 4 weeks. WT recipients suffered from severe loss of kidney function with 5 fold creatinine elevation 6 days after transplantation. The number of infiltrating monocytes and macrophages in recipients from PKC epsilon -/- donors was dramatically reduced compared to WT donors. PKC epsilon deficient transplants showed almost no apoptosis 24 hours after transplantation, whereas WT transplants had severe apoptosis in all renal compartments, this difference in apoptosis remained even at d6 after transplantation.

Our results indicate that PKC epsilon deficiency in renal allografts protects from severe acute rejection and early loss of renal function. This effect is most likely attributed to reduced apoptosis early after transplantation.

Abstract# 1449

ROLE OF INTERFERON-GAMMA IN THE REDUCTION OF HUMAN ARTERIAL INJURY IN THE SCID HUMAN TRANSPLANT MODEL VIA SYNERGY OF SIROLIMUS & CYCLOSPORINE. Tai Yi,[1] Lisa Geiselhart,[1] George Tellides,[1] Jordan Pober,[2] Marc Lorber.[1] *[1]Surgery, Yale University School of Medicine, New Haven, CT; [2]Pathology & Immunobiology, Yale Univ. School of Med., New Haven, CT.*

Interspecies differences in immune responses have made correlation of results from animal models with the human transplant experience difficult to establish. We have developed a relevant model for studying human alloimmune arterial injury consisting of a SCID mouse reconstituted with allogeneic human PBMC and bearing human artery. Previous results demonstrated reproducible arterial injury with lymphocytic infiltration, endothelial destruction & intimal expansion by smooth muscle cells. The purpose of this work was to determine whether the Sirolimus (SIR), alone or in combination with cyclosporine (CsA) affects arterial injury in this model. CB17 SCID/beige mice were transplanted with size matched human arteries and 7 days later, reconstituted with 3x10^8 PBMC. Mice (n=4) given only vehicle were compared to mice given CsA (5 mg/kg/d), SIR (0.1mg/kg/d), or CsA plus SIR. CsA was given at the time of PBMC, but due to reconstitution concerns, SIR was given 7 days later. The combination of CsA & SIR resulted in more reduction of myointimal expansion than separate use (P<0.01). This reduction was not caused by decreased human CD3+ cells in mouse blood (P>0.05) or human artery (infiltration) (P>0.05). Under these conditions, there was no statistical myointimal expansion difference between SIR treated & control (P>0.05), perhaps due to low dose (higher doses impacted reconstitution) or the timing of SIR administration (7 days after PBMC introduction). To study the mechanism by which CsA & SIR synergistically inhibit arterial injury, we determined that both CsA & SIR inhibit IFN gamma. Our results revealed that IFN gamma (0.2ug, 3X/week) given to human PBMC reconstituted, artery bearing SCID mice resulted in ENHANCED myointimal expansion. Under these conditions, CsA & SIR were not as effective at preventing myointimal expansion. Further studies with IFN gamma treated mice (0.2ug, 3X/week) in the absence of PBMC indicated that IFN gamma alone induced myointimal expansion. When CsA & SIR were given to these artery bearing SCID mice given IFN gamma, myointimal expansion was NOT reduced. While CsA & SIR synergistically ameliorate PBMC induced myointimal expansion in this model, they cannot prevent myointimal expansion induced by IFN gamma alone. Taken together, these data help to delineate the mechanism by which CsA & SIR ameliorate human arterial injury, and the the importance of reduction of gamma IFN as part of this mechanism.

Abstract# 1450

THERAPEUTIC EFFICACY OF TARGETING ANGIOGENESIS IN ALLOIMMUNITY. Masayuki Sho,[1] Naoya Ikeda,[1] Yukiyasu Kuzumoto,[1] Satoru Akashi,[1] Yoshikazu Tsurui,[1] Hisanori Kashizuka,[1] Takashi Mizuno,[1] Hiromichi Kanehiro,[1] Marlies Reinders,[3] Daniel J. Hicklin,[2] Yoshiyuki Nakajima,[1] David M. Briscoe.[3] *[1]First Department of Surgery, Nara Medical University, Nara, Japan; [2]ImClone Systems Inc, New York, NY; [3]Children's Hospital, Harvard Medical School, Boston, MA.*

Angiogenesis is a characteristic component of chronic vascular processes including atherosclerosis and graft vascular disease (GVD). However, little is known of the mediators or the function of angiogenesis in the development of GVD. In this study, we used a MHC class II disparate cardiac transplant model in which B6.C-H2bm12 hearts transplanted into C57BL/6 are monitored for progressive GVD by histological analysis at 6 weeks posttransplant. Recipients were treated with the angiogenesis inhibitor endostatin/Fc (20mg/kg three times a week), either in the initiation phase (0-3 weeks) or in the progression phase (4-6 weeks). Remarkably we found that early treatment failed but that delayed treatment significantly inhibited the development of GVD (n=8, P=0.026 vs. control). Moreover, there was less fibrosis and less cellular infiltration of grafts in the delayed treatment group. VEGF is a major angiogenesis factor known to be predictive of GVD in humans. Moreover, VEGF is a chemoattractant and thus may accelerate GVD via monocyte recruitment into lesions. To evaluate this possibility, we treated animals with a blocking anti-VEGF anti-serum on alternate days; and hearts were harvested 28 days after transplantation for immunohistochemical analysis. We found that control-treated animals developed persistent infiltrates but we observed only a mild infiltrate, reduced numbers of monocyte/macrophages and minimal vasculitis in grafts from anti-VEGF treated mice (n=6). Since early monocyte recruitment is a predictor for disease progression, these data suggest that the early expression of VEGF may be of critical importance for subsequent monocyte recruitment and the late angiogenesis response. VEGF mediates its effects via 2 high affinity receptors flt-1 and flk-1. In an initial attempt to evaluate which VEGFR is involved VEGF-mediated leukocyte trafficking, we treated fully mismatched BALB/c recipients of C57BL/6 hearts with anti-flk-1 and anti-flt-1 monoclonal antibodies and found that they significantly prolonged graft survival (P=0.004). Together, these findings provide important new insights into the significance of angiogenesis in chronic rejection and the potential that angiogenesis inhibitors can therapeutically interrupt the progression of GVD. Moreover, we suggest that VEGF-VEGFR interactions may be of importance in early processes leading to GVD.

Abstract# 1451

INTRAGRAFT EXPRESSION OF SDF-1 IS A NOVEL PREDICTIVE MARKER OF POOR GRAFT OUTCOMES FOLLOWING ACUTE RENAL TRANSPLANT REJECTION. Sheryl Shah,[1] Elaine Mansfield,[1] Neeraja Kambham,[1] Szu-Chuan Hsieh,[1] Rosa Liu,[1] Minnie Sarwal.[1] *[1]Pediatrics and Pathology, Stanford University, Stanford, CA.*

Purpose: To develop risk stratification of acute renal transplant rejection (AR) episodes, by identification of a molecular marker predictive of graft outcomes following AR.

Methods: Cox regression analysis was conducted between 25 AR biopsies with good (n=12) and poor (n=13) outcomes (incomplete functional recovery and/or graft loss) across a differentiated subset of 1340 cDNAs, identified from analysis of a 30,000 cDNA microarray. A chemokine, stromal cell derived growth factor (SDF-1) correlated with poor AR outcomes. Real-time PCR for SDF-1 and immunostaining for SDF-1β, CD20 and CD45RO was conducted on core biopsies; SDF-1β staining was also performed on tissue microarrays (TMAs) of normal tissues and transplant nephrectomies. PCR-RFLP genotyping of the SDF-1 3' G/A polymorphism was performed on all patients.

Results: Reduced intra-graft SDF-1 mRNA was confirmed by PCR in patients with poor AR outcomes (73.08 ± 76.95 vs. 8.02 ± 11.2; p=0.018 for graft loss and 75.72 ± 77.39 vs. 22.53 ± 48.3; p=0.11 for graft function recovery). On TMA analysis, the 92 amino acid protein, SDF-1β, was ubiquitously expressed in lymph node, spleen, heart and liver. In normal kidney, SDF-1β localized to podocytes, arterial endothelium, tubular epithelial cells, but not mesangial or glomerular endothelial cells. SDF-1β staining intensity correlated with CD45RO and B cell density by CD20 staining in biopsies with graft loss. Although variable, core biopsies and TMA analysis revealed that AR had increased SDF-1β positive lymphocytes; in areas of fibrosis, interstitial SDF-1β was higher. Chronic rejection had reduced SDF-1β staining within arterial media. All AR patients, regardless of graft loss status, were G/G homozygotes indicating that expression differences of SDF-1 are not due to genetic differences at the SDF-1 locus.

Conclusions: Reduced intra-graft SDF-1 mRNA may be used as a novel prognostic marker of graft outcome after AR. Further studies are needed to unravel the relationship between SDF-1 translation and transcription within the graft. B cell production, survival and APC function may be regulated by intra-graft SDF-1, which may result in resistance to conventional AR therapy and poor outcomes. Reduced vascular SDF-1β may have reduced ischemic neovascularization, resulting in greater tissue chronicity after injury. Manipulation of SDF-1 intra-graft expression may be valuable for graft rescue after AR.

Abstract# 1452

GENE TRANSFER OF HUMAN BCL-2 IN DONOR RAT LUNG ALLOGRAFTS LIMITS ISCHEMIA/REPERFUSION MEDIATED INFLAMMATORY RESPONSE AND APOPTOSIS AND IMPROVES POST-TRANSPLANT LUNG FUNCTION. David Tom Cooke,[1] Jeremy I. Pearl,[1] Masashi Tanaka,[1] Justin Cole,[1] E. Grant Hoyt,[1] Robert C. Robbins.[1] *[1]Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA.*

Purpose: Ischemia/reperfusion (IR) injury is a major cause of graft dysfunction in 30% of lung transplant recipients. IR injury in lung transplantation generates pro-inflammatory cytokines such as IL-1β, and results in donor respiratory epithelial apoptosis. We hypothesized that intratracheal administration of a non-replicating adenovirus, overexpressing the human anti-apoptotic protein Bcl-2, will inhibit IR injury to rat donor lung allografts.

Methods: $1.0x10^9$pfu of either AdvBcl-2 (n=7) or AdvNull (n=6) in PBS or PBS alone (n=8) were administered intratracheally to PVG (RT1c) rats. The left lungs were procured after 24hrs of *in-vivo* incubation. Donor lungs were orthotopically transplanted after 1hr (Group I) or 24hrs (Group II, AdvBcl-2 vs. AdvNull) of cold ischemia into allogeneic ACI (RT1a) recipients, and then reperfused for 2hrs. Peak inspiratory pressures (PIPs) were measured in all grafts, and donor pulmonary vein paO_2 was measured in Group II lungs. Allografts were excised and evaluated by histology, and graft protein extracts analyzed by ELISA, and activated caspase-3, -9 and -8 assays.

Results: Human Bcl-2 protein was overexpressed in AdvBcl-2 treated lungs compared to AdvNull and PBS alone treated lungs at the time of transplantation (p<0.05), and localized to the alveolar epithelium. The human Bcl-2 overexpression in AdvBcl-2 lungs was identical in groups I and II. Human Bcl-2 expression was restricted to donor lung grafts, and no human Bcl-2 could be detected in heart, kidney and liver tissue or serum from donor animals. In Group I, AdvBcl-2 treated lungs had diminished PIPs (p<0.01) and intragraft IL-1β cytokine (p<0.05), compared to AdvNull and PBS alone treated controls. Also, AdvBcl-2 treated lungs had reduced caspase-3, -9 and surprisingly caspase-8 activity (p<0.05), as well as ISOL TUNEL positive nuclei (1.7%±0.7% vs. 4.7%±1.1%, and 6.1%±0.4%, p<0.05). In Group II animals, AdvBcl-2 lungs had higher donor pulmonary vein paO_2(p<0.05) compared to AdvNull lungs, and maintained significant decreases in intragraft IL-1β production, caspase-3 activity, and ISOL TUNEL positive nuclei (3.6%±0.8% vs.19.3%±3.6%).

Conclusions: Intratracheal gene transfer of human Bcl-2 ameliorates ischemia/reperfusion injury, and preserves post-transplant lung function, and may one day be utilized as a gene therapy protocol in human lung transplantation.

Abstract# 1453

ISCHEMIA-REPERFUSION (I-R) INDUCES GLOMERULAR AND TUBULAR ACTIVATION OF PRO-INFLAMMATORY AND ANTI-APOPTOTIC PATHWAYS: DIFFERENTIAL MODULATION OF RAPAMYCIN (RM). A. Loverre,[1] C. Capobianco,[1] P. Ditonno,[2] M. Battaglia,[2] S. Palazzo,[2] A. Crovace,[3] G. Stallone,[1] B. Infante,[1] A. Schena,[1] S. Di Paolo,[1] F. P. Selvaggi,[2] E. Ranieri,[1] L. Gesualdo,[4] F. P. Schena,[1] G. Grandaliano.[1] *[1]Div of Nephrology, Italy; [2]Div of Urology, Italy; [3]Section of Veterinary Surgery, DETO, Univ. of Bari, Italy; [4]Chair of Nephrology, Univ. of Foggia, Italy.*

I-R injury in transplanted kidney, a key pathogenic event of delayed graft function (DGF), is characterized by tubular cell apoptosis and interstitial inflammation. Among the signaling pathways that regulate cell survival and inflammation, Akt-mTOR-S6k and NIK-NFkB may play a crucial role in the I-R-induced injury. The clinical relevance in transplantation of Akt pathway, is due its to specific inhibition by RM. The aim of this study was to evaluate Akt and NFkB axis activation in a pig model of I-R and in pts with DGF receiving or not R and the role of coagulation priming in this setting. I-R was induced in 6 pigs clamping the renal artery for 15 min. Biopsies were performed before, 15 min after I and 15, 30, 45, 60 min after reperfusion. In addition, we evaluated 12 pts with DGF and biopsy-proven acute tubular injury with no evidence of acute rejection treated with (6) or without RM (6). The activation of Akt, S6k and NIK was evaluated by confocal microscopy using antibodies recognizing the phosphorylated (p) and active form of the enzymes. In pig kidneys, we observed, by immunofluorescence, a significant interstitial deposition of fibrin upon reperfusion. Before I-R, pAkt was observed in podocytes and proximal tubular cells (PTC). In I-R pAkt increased in PTC, interstitial and mesangial areas in a time-dependent manner with a clear nuclear translocation. The same pattern of activation was observed for S6k. pNIK was detected before I-R only in mesangial cells. Following I-R, pNIK increased both at the glomerular and tubular levels with a peak at 30 min. This pattern of activation was confirmed in pts not receiving RM, while in pts treated with RM, we observed a significant reduction of S6k but not NIK activation. Interestingly, in cultured PTC thrombin induced a striking increase in pS6k and pNIK. RM inhibited thrombin-induced S6k but not NIK activation.

Our data suggest that: 1. Priming of coagulation may play a pathogenic role in I-R injury. 2. Akt axis may represents an anti-apoptotic mechanism and its inhibition may explain the prolonged DGF observed in pts receiving RM. 3.The striking NIK activation observed both in I-R and in pts with and without RM-treatment, may be a powerful pro-inflammatory and RM-insensitive signal, potentially leading to progressive graft injury.

Abstract# 1454

CASPASE-3 AND -8 BLOCKADE REDUCES ISCHEMIA/ REPERFUSION DIRECTED APOPTOSIS AND INFLAMMATORY CHEMOKINE AND CYTOKINE PRODUCTION IN DONOR RAT LUNG ALLOGRAFTS. David Tom Cooke,[1] E. Grant Hoyt,[1] Robert C. Robbins.[1] *[1]Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA.*

Purpose: Ischemia/reperfusion (IR) injury in lung transplantation contributes to morbidity and mortality, and may increase the incidence of acute rejection and bronchiolitis obliterans. IR injury causes apoptosis of donor lung epithelium and produces pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-12 and chemokines such as IL-8. Caspases are proteinases that direct apoptotic cascades. We have shown that blockade of Caspase-3 and -8 with a cell permeable caspase inhibitor can ameliorate IR injury in rat cardiac allotransplants. In this study we investigated the hypothesis that blockade of caspase-3 and -8 reduces IR injury in rat donor lung allografts.

Methods: Donor PVG (RT1c) and allogeneic ACI (RT1a) recipient rats were pretreated with either 500µg of the reversible caspase-3 and -8 inhibitor DEVD-CHO in 300µl of PBS/10% DMSO *i.p.* (N=4) or vehicle alone (N=4) 2 hrs before orthotopic transplantation. At the time of donor lung procurement, animals pretreated with caspase inhibitor received intratracheal administration of 250µg of DEVD-CHO in 300µl of PBS/.25% DMSO, compared to vehicle alone in the control animals. The left lungs from donor animals were flushed antegrade with 250µg of DEVD-CHO in 25cc of low potassium dextrose and glucose perfusate (LPDG) or LPDG alone in control animals. After procurement, donor lungs underwent 1hr of cold ischemia; followed by 2hrs of post-transplant reperfusion. Allografts were then excised and graft protein extracts were evaluated by activated caspase-3 and -8 assays and ELISA.

Results: Compared to vehicle treated controls, DEVD-CHO treatment significantly diminished donor lung apoptosis, as measured by reduced activated caspase-3 activity (58.7±25.1 µM/mg vs.168.0±53.2) and caspase-8 activity (52.5±20.8 µM/mg vs. 129.6±5.8). The DEVD-CHO treated group had significantly less IL-1β levels (1360.0±98.5 pg/mg vs. 1761.6±39.3) and reduced TNF-α (909.9±37.6 pg/mg vs. 1062.5±59.1) and IL-12 levels (2.9±0.3 pg/mg vs. 3.6±0.1) compared to the control group. In addition, the DEVD-CHO treated donor lungs had diminished intragraft levels of the chemokine GRO/CINC-1 (IL-8) compared to vehicle only controls (424.4±44.1 pg/mg vs. 605.2±161.4).

Conclusions: Preoperative treatment of donor and recipient, in addition to intratracheal and perfusate treatment of the donor lungs at the time of transplantation with a caspase inhibitor reduces IR injury. Preoperative caspase inhibition may serve as an invaluable regimen in human lung transplantation.

Abstract# 1455

CASPASE 8 SMALL INTERFERING RNA DECREASES ISCHEMIA-REPERFUSION INJURY TO THE LIVER IN MICE. Mario Vilatoba,[1] Guadalupe Bilbao,[1] Christopher Eckstein,[1] Anthony Thompson,[1] Devin E. Eckhoff,[1] Juan L. Contreras.[1] *[1]Surgery, University of Alabama at Birmingham, Birmingham, AL.*

Ischemia/reperfusion-injury (I/R-I) of the liver remains a significant problem during liver surgery and transplantation. I/R-I is associated with liver apoptosis (Apo), which is mediated by death receptors (DR) such as Fas, TNF-α, and TNF-related apoptosis-inducing ligand. Caspase 8 is presumed to be the apex of the DR-mediated Apo pathway. Synthetic small interfering RNAs (siRNAs) 21-23 nt in length, specifically suppress gene expression by RNA interference. Therefore, we evaluated the therapeutic efficacy of caspase 8 siRNA in a murine model of liver I/R-I. **Methods**: C57BL/6 mice were used. 45 or 70% of the liver mass was clamped for 90 minutes. *In vivo* delivery of siRNA was performed via portal vein by high-volume injection (0.2-0.5 nmol of siRNA in 1 mL containing 10% Lipiodol) 60 minutes before I/R-I. As a control, some animals received non-sense siRNA (siRNA-scrambled). **Results:** Liver uptake of siRNA was analyzed in transgenic mice that express β-gal (C57BL/6J-TgN(MTn-LacZ)204Bri), after administration of siRNA-LacZ. 3-4-fold decrease in β-gal activity was accomplished at 0.5 nmol. No significant change in β-gal activity was demonstrated in mice receiving non-sense siRNA. Immunohistochemical studies found that 70% of liver cells took up siRNA. Lower serum AST, assessed 6 hours after injury was found in animals treated with siRNA (AST=308±102 IU/L), compared with siRNA-scrambled (810±122) or saline (802±79, n=8, $P<0.05$). Caspase 8 activity was higher in saline (0.138±0.010 O.D. at 405) and siRNA-scrambled mice (0.131±0.009) *versus* siRNA-treated animals (0.058±0.014, $P<0.05$). Liver Apo assessed by ELISA was higher in controls (saline=Enrichment Factor=8.3±1.8; siRNA-scrambled=8.6±1.2) *versus* siRNA (3.8±0.9, $P<0.001$). 100% of the control mice died within 5 days. In contrast, 40% of the siRNA animals survived indefinitely. **Conclusions**: Small interfering RNA targeted to Caspase 8 provide significant protection against ischemia-reperfusion injury to the liver. This approach could be therapeutic in liver transplantation and other conditions associated with ischemia-reperfusion injury to the liver.

Abstract# 1456

LYSOSOMAL RELEASE OF CATHEPSIN B POTENTIATIES LIVER DAMAGE OF STEATOTIC LIVERS AFTER COLD ISCHEMIA AND WARM REPERFUSION INJURY. E. Baskin-Bey,[1] A. Canbay,[1] S. Bronk,[1] N. Werneburg,[1] S. Nyberg,[1] G. Gores.[1] *[1]Mayo Clinic College of Medicine, Mayo Foundation, Rochester, MN.*

Cold ischemia/ warm reperfusion (CI/WR) liver injury of steatotic livers prohibits their use in liver transplantation. The cellular mechanisms resulting in liver injury of steatotic livers after CI/WR still remains obscure. Recently, a lysosomal pathway of cell death in fatty liver disease was described involving the lysosomal protease, cathepsin B. However the role of this pathway in CI/WR injury of steatotic livers remains unknown. Thus, our **AIM** was to ascertain if inactivation of cathepsin B attenuates liver injury of steatotic livers after CI/WR. **METHODS:** Wild type ($catB^{+/+}$) and cathepsin B-deficient (knockout) ($catB^{-/-}$) mice were fed the methionine choline deficient (MCD) diet for 2 weeks to induce hepatic steatosis. Mouse livers were stored in the University of Wisconsin (U/W) solution for 24 hrs at 4° C and reperfused for 1 hr at 37° C in vitro. Lysosomal permeabilization was determined by cellular distribution of cathepsin B and D by immunohistochemistry. Liver injury was assessed by histology and transmission electron microscopy (EM). Hepatocyte apoptosis was quantitated using the TUNEL assay, and immunohistochemistry for activated caspase 3/7. **RESULTS:** After two weeks on the MCD diet, both wild type and cat B knock out animals equally developed hepatic steatosis. Cathepsin D cellular localization was punctate in both $catB^{+/+}$ and $catB^{-/-}$ animals fed standard or MCD diets under basal conditions consistent with its lysosomal localization. However, following CI/WR, cathepsin D immunohistochemistry was diffuse in wild type animals fed the MCD diet consistent with lysosomal permeazation. In contrast, cathepsin D remained punctate in MCD diet fed $catB^{-/-}$ animals following CI/WR suggesting cat B is necessary for the lysosomal permeabilization. Histology and EM showed increased microscopic liver damage in $catB^{+/+}$ vs. $catB^{-/-}$ after MCD plus CI/WR. In particular, mitochondrial were condensed in $catB^{+/+}$ but normal appearing in $catB^{-/-}$ MCD fed animals following CI/WR. TUNEL positive hepatocytes were 6.3-fold greater ($p<0.002$) in $catB^{+/+}$ vs. $catB^{-/-}$ with MCD plus CI/WR. Similarly, caspase 3/7 activity was 9.5-fold greater ($p<0.002$) in $catB^{+/+}$ vs. $catB^{-/-}$ with MCD plus CI/WR. In **CONCLUSION** these findings suggest for the first time that the lysosomal pathway of apoptosis occurs in steatotic livers after CI/WR. These observations suggest cathepsin B inactivation or inhibition maybe a therapeutic strategy for improving the function of steatotic livers for transplantation.

Abstract# 1457

DONOR PRE-TREATMENT WITH ATORVASTATIN UP-REGULATES HEME-OXYGENASE 1 IN RENAL TISSUE AND PREVENTS COLD PRESERVATION INJURY IN ISOGENEIC TRANSPLANTATION IN RATS. Uwe Goettmann,[1] Paul Brinkkoetter,[1] Christian Karle,[1] Benito Yard,[1] Fokko J. van der Woude,[1] Claude Braun.[1] *[1]Vth Department of Medicine, University Hospital Mannheim, Mannheim, Germany.*

Pleiotropic effects of HMG-CoA-reductase inhibitors (statins), e. g. anti-inflammatory and immunmodulatory effects, are well recognized. Recent studies have shown that statins decrease I/R-injury in rats. The aim of the present study was to evaluate the effect of atorvastatin on cold preservation injury in isogeneic renal transplantation in rats. Male Lewis donor rats were pre-treated with atorvastatin (ATOR: 50mg/kg/d, n=7) or vehicle (VEH, n=7) daily by oral gavage 2 days prior to and on the day of explantation. After explantation one kidney was snap frozen in liquid nitrogen for determination of heme-oxygenase-1 (HO-1) expression by light-cycler PCR. The second kidney was stored for 24h at +4° in UW solution and then transplanted in a bilaterally nephrectomized male Lewis rat. Serum creatinine was measured on days 0, 1, 3, and 5 after transplantation. After 5 days grafts were harvested for determination of monocyte infiltration and MHC II expression. Pre-treatment with atorvastatin improved renal function, as measured by lower serum creatinine on days 1 and 3 after transplantation (day 1: ATOR 2.04 mg/dl vs. VEH 3.10 mg/dl, p<0.0001; day 3: ATOR 1.26 mg/dl vs. VEH 3.47 mg/dl, p=0.009; t-test). Atorvastatin markedly reduced monocyte infiltration 5 days after transplantation (ATOR 9.48 ± 1.36 vs. VEH 19.77 ± 1.36 pos. cells/high power field, p<0.0001), whereas there was no difference in MHC II positive cells. In donor kidneys pre-treated for 2 days with atorvastatin, a significant up-regulation of the protective enzyme HO-1 was observed compared to animals pre-treated with vehicle. Our data demonstrate for the first time, that donor pre-treatment with atorvastatin improves renal function and decreases renal inflammation after prolonged cold storage and isogeneic renal transplantation in rats. The positive effect may be related to the up-regulation of the protective enzyme HO-1 in kidney tissue by atorvastatin. This observation is of high clinical significance, since it could offer a new strategy to prevent transplantation-associated injury.

Abstract# 1458

LECTIN-LIKE OXIDIZED LDL RECEPTOR (LOX-1) MEDIATED PATHWAY AND VASCULAR OXIDATIVE INJURY IN RAT RENAL TRANSPLANTS WITH OLDER AGE. Duska Dragun, Jan H. Braesen, Melina Nieminen-Kelhae, Denise Markmann, Wolfgang Schneider, Hans H. Neumayer, Klemens Budde, Friedrich C. Luft. *Nephrology and Transplantation, Charite University Hospitals, Berlin, Germany.*

Increased age is associated with inferior survival of renal transplants by mechanisms that are unclear. Oxidants participate in normal ageing and age-related diseases like atherosclerosis. We reasoned that same mechanisms may be involved in acute transplant vasculopathy. We investigated how mediators of oxidative stress may increase susceptibility to vascular injury in aged transplants. We employed low-responder allogeneic F344-to-Lew rat functional model of renal transplantation using non-immunosuppressed young (donors and recipients 12 weeks of age) versus old (donors and recipients 52 weeks of age). Grafts were transplanted after 2-hours cold preservation in UW solution and harvested 7 and 10 days later. Additional group of old animals receiving continous 1.5 mg/kg CyA immunosuppression was included to the study design. Renal allograft pathology was scored according to Banff criteria. We studied intragraft expression and distribution of VCAM-1, lectin like oxidized LDL-receptor (LOX-1), hypochlorous acid/hypochlorite ($HOCl/OCl^-$) modified LDL, and infiltration with ED-1+ macrophages and CD8+ lymphocytes. Intragraft in situ superoxide anion production was determined with dihydroethidium (DHE) assay on cryosections. Compared to young transplants old transplants demonstrated higher vascular lesion score and functional impairment. The degree of VCAM-1 expression and infiltration with ED-1+ monocytes/macrophages and CD8+ lymphocytes directly correlated with the transplant age. VCAM-1 and LOX-1 staining were localized predominantly on the endothelium of arterial vessels, shifting the distribution to vascular smooth muscle layer strongly dependent on age and the grade of vascular injury. LOX-1 staining colocalized with HOCl generated oxidized epitopes in the media of injured arteries. In corresponding areas we measured increased in situ superoxide anion production. Immunosuppression with CyA had no protective effect on features of vascular injury and LOX-1 expression. Induction of LOX-1 related oxidation pathways and increased susceptibility to oxidative stress may play an important role in promoting vascular injury in old renal transplants.

Abstract# 1459

EFFECT OF A NOVEL INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR IN PREVENTION OF RENAL ISCHEMIA/ REPERFUSION INJURY IN VERVET MONKEYS. Shijie Qi,[1] Dasheng Xu,[1] Anlun Ma,[1] Xiaochun Zhang,[1] Yang Wang,[1] Huijuan Gao,[1] Noboru Chida,[2] Yuji Sudo,[2] Kouichi Tamura,[2] Pierre Daloze,[1] Huifang Chen.[1] *[1]CHUM, Notre-Dame Hospital, University of Montreal, Montreal, QC, Canada; [2]Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan.*

Background. Ischemia/reperfusion (I/R) injury can be involved in almost all tissues and organs during pathosis. Over the past decade, an enormous number of studies have focused on the role of nitric oxide (NO). Cytotoxic nitric oxide (NO) is produced during ischemia/reperfusion (I/R) injury by expression of inducible NO synthase (iNOS). Therefore, continuous iNOS inhibition might prevent early graft dysfunction. FR260330 is a potent and selective inhibitor of iNOS activity by preventing iNOS monomers from dimerization. In this study, the effect of FR260330 in prevention of renal I/R injury was evaluated in the model of one kidney ischemia in Vervet monkeys.

Methods. A total of 18 male Vervet monkey were randomly assigned to two groups (n=9). Transient (60 min) left renal ischemia was produced with simultaneous contralateral nephrectomy in FR260330 20 mg/kg/day treated and control groups. Renal function and other biochemistry determinations, and FR260330 concentrations were tested until day 15 after I/R injury.

Results. All the monkeys were survival after 60 min I/R injury until sacrifice on day 15 at study termination. Serum creatinine in untreated group increased significantly to compare with FR260330 treated group on day 2, 3, 4, and 7 (p<0.05, ANOVA). Plasma concentration of FR260330 after oral administration showed that Cmas is 3.251 ± 2.526 mg/ml, and Tmax is 4 hrs.

Conclusion. A selective inhibitor of nitric oxide synthase, FR260330 effectively prevents renal I/R injury in Vervet monkeys. Potential applications of FR260330 in prevention of delayed graft function patients and other I/R injury pathosis are thus suggested.

Abstract# 1460

ATORVASTATIN (Ator) DECREASES LOSS OF HUMAN PANCREATIC ISLET (PI) MASS RELATED TO INSTANT BLOOD-MEDIATED INFLAMMATORY REACTION (IBMIR) BY DOWN-REGULATION OF TISSUE FACTOR (TF) AND MACROPHAGE CHEMOATTRACTANT PROTEIN (MCP-1) EXPRESSION. Juan L. Contreras,[1] Cheryl A. Smyth,[1] Christopher Eckstein,[1] Carlton Young,[1] Guadalupe Bilbao,[1] Anthony Thompson,[1] Devin Eckhoff.[1] *[1]Surgery, UAB, Birmingham, AL.*

PI constitutively express TF and MCP-1. After exposure to blood, PI trigger a detrimental clotting reaction referred as IBMIR, effect associated with significant reduction in islet mass early after transplantation. TF as an initiator and MCP-1 as a chemoattractant have been demonstrated to play a critical role in this process. We hypothesized that statins, which have been demonstrated to decrease the activation of the coagulation/inflammation systems, may down-regulate TF and MCP-1 expression in isolated human PI. **Methods**: Immediately after isolation, groups of human hand-picked PI were incubated with Ator (10mmol/L) or vehicle for 3 days (n=5). TF and MCP-1 were determined from islet homogenates on day 1 by ELISA. To evaluate the effects of Ator on IBMIR, 5000 IEQ were perfused with ABO compatible human blood *in vitro* using loops of heparin-coated PVS (flow ~45 mL/min, 37 °C, 60 minutes). Plasma pro-insulin (hPI), β-tromboglobulin (TG) and thrombin-antithrombin (TAT) complexes were quantified by ELISA. **Results**: Ator decreased spontaneous PI loss during culture (day 3, 91.2±2.4% viable islet cells) compared with vehicle (82.1±3.1, P<0.05). A dose-dependent decrease in TF and MCP-1 was demonstrated after Ator treatment (10mmol/L, TF=12.3±6.4 pg/IEQ, MCP-1 3.8±2.1 pg/IEQ) compared with controls (TF=38.3±12.4, MCP-1 9.8±4.1, P<0.05). *In vitro*, Ator significantly reduced platelet aggregation (platelet counts in the loops: PI media=289±21x10^9/L, PI=12.3±1.1x10^9/L, PI + Ator=160.1±2.1x10^9/L, n=6, P<0.001), platelet activation (TG: media=366±110 IU/mL; PI=891±156; PI+Ator=590±89, P<0.05), activation of coagulation (TAT: media=5.1±1.1 mg/L; PI=367±152; PI+Ator=122±88, P<0.05), and complement activation (C3: media=57.1±4 mg/dL; PI=16.3±7; PI+Ator33.8±8.9= P<0.05). In parallel with the activation of coagulation, PI destruction was higher in controls (proinsulin= 4998±324 pmol/L) compared with Atorv-treated PI (2656±556, P<0.05). Immunohistochemical analysis after PI perfusion demonstrated reduced platelets, PMNs, monocytes and fibrin surrounded the PI treated with Ator compared with controls. **Conclusion**: Atorvastatin significantly reduced human islet expression of TF and MCP-1. This effect was associated with reduced loss of islet mass after exposure to blood. The early use of statins may improve the results in islet transplantation.

CHEMOKINES AND ADHESION MOLECULES

Abstract# 1461

ROLE OF CXCR2 IN RENAL ISCHEMIA/REPERFUSION INJURY. Motoo Araki,[1] Masayoshi Miura,[1] Hiromi Kumon,[2] John Belperio,[3] Robert Strieter,[3] Andrew Novick,[1] Robert Fairchild.[1] *[1]Urology, Glickman Urological Institution, Cleveland Clinic Foundation, Cleveland, OH; [2]Urology, Okayama University Medical School, Okayama, Okayama, Japan; [3]Critical Care Medicine, UCLA School of Medicine, Los Angeles, CA.*

Introduction: Ischemia/reperfusion injury has a critical impact on the outcome of organs transplanted for treatment of end-stage heart, lung and kidney disease. The factors directing cell infiltration into parenchymal tissues during this injury remain unclear. Chemokines are cytokines having chemoattractant properties for leukocytes expressing the specific receptors. Previous studies from this laboratory in a mouse model have shown rapid induction of neutrophil chemoattractant chemokine expression and parenchymal neutrophil infiltration shortly following reperfusion of ischemic kidneys. The goal of the current study was to investigate the induction and role of the neutrophil chemokine receptor, CXCR2 in this model of renal ischemia/reperfusion injury.

Methods: BALB/c wild-type and CXCR2$^{-/-}$ mice were anesthetized and the renal hilum was clamped for 45 min while the temperature was maintained at 32°C. At various times post-reperfusion, ischemic and control kidneys were removed, whole cell RNA was prepared and tested for expression of chemokine receptor genes by ribonuclease protection assay. Sections were prepared from kidneys and stained for the chemokines KC, MIP-2 ,and neutrophils by immunohistochemistry. Serum creatinine was measured each day post-reperfusion. In some experiments mice were depleted of neutrophils by treatment with anti-Ly6G mAb and/or were treated with goat anti-CXCR2 antiserum. Controls were treated with normal rat IgG or normal goat serum.

Results: CXCR2 was the most highly expressed chemokine receptor in ischemic kidneys with expression peaking 18 h after reperfusion. The ligands for CXCR2, KC and MIP-2, were clearly present in the tubular epithelial cells 9 h after reperfusion by immunohistochemistry. Renal dysfunction was abrogated in CXCR2$^{-/-}$ mice as well as in wild-type animals depleted of neutrophils before reperfusion. TUNEL assay showed the decreased cell damage compared to the wild type. Treatment of wild-type mice with anti-CXCR2 antibodies at the time of reperfusion resulted in extremely high serum creatinine levels 24-48 h after reperfusion followed by 100% loss in viability. This renal failure was dependent on neutrophils as it was not observed in mice depleted of neutrophils before renal ischemia and reperfusion.

Conclusions: CXCR2 plays an important role in kidney ischemia/reperfusion injury. However, the use of CXCR2 antagonists to attenuate neutrophil infiltration and the injury following reperfusion should be carefully considered.

Abstract# 1462

EXPLOITING TISSUE-SPECIFIC HOMING TO THERAPEUTIC EFFECT: TARGETING THE TECK/CCR9 PATHWAY PROLONGS SMALL BOWEL ALLOGRAFT SURVIVAL. Ran Tao,[1] Qunrui Ye,[1] Rongxiang Han,[1] Paul E. Love,[2] Wayne W. Hancock.[1] *[1]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA; [2]Lab of Mammalian Genes & Development, National Institute Child Health and Human Development, National Institutes of Health, Bethesda, MD.*

Analysis of the contributions of inflammatory chemokine/chemokine receptor pathways to the rejection of vascularized allografts in rodent models has led to the identification of key effector molecules (e.g. CXCR3, CCR5), and their targeting is now being tested in non-human primate allograft recipients. By contrast, little attention has been given to homeostatic or tissue-specific chemokine pathways and alloresponses. The chemokine TECK (CCL25) is expressed exclusively in the thymus and small intestine, and mediates gut-specific homing of CCR9+ T cells. We now report how manipulating the TECK/CCR9 pathway can impair effector T cell infiltration and depress the robust rejection of small bowel transplants.

We first studied CCR9 expression in peripheral T cells in naive mice. CCR9 was expressed by <5% of mesenteric and peritoneal lymph node and 15-20% of splenic CD4 and CD8 T cells. CCR9-/- mice showed negligible defects in T cell development in the thymus, only minor changes in the small bowel IEL populations, and had normal numbers of CD4+ CD25+ T-reg cells. Analysis of fully MHC-mismatched small bowel transplants (BALB/c to B6/129) using real-time PCR showed peak up-regulation of TECK (500-fold) and CCR9 (12-fold) mRNA expression at day 3; upregulation was not observed in syngeneic small bowel transplants. Increased CCR9 mRNA expression was also found in recipient spleen and mesenteric lymph nodes, consistent with the induction of CCR9 expression by recipient T cells encountering donor antigen-presenting cells within secondary lymphoid tissues. Immunohistology showed markedly increased accumulation of CCR9+ T cells within serially harvested small bowel allografts, as compared to isografts or recipient small bowel. Lastly, and critically, wild-type B6/129 mice promptly rejected BALB/c SBT by 6-8 days, whereas CCR9-/- mice displayed prolongation of SBT survival for up to 15 days ($p<0.0005$).

These studies indicate that besides its role in the normal homing of T cells to the small intestine, the TECK/CCR9 pathway promotes the recruitment of effector T cells to small bowel transplants. Our ongoing studies are directed towards further understanding how manipulating this pathway, alone or in conjunction with immunosuppression or costimulation blockade, may help to prevent acute rejection of small bowel allografts, and eventually promote a better outcome in clinical small bowel transplantation.

Abstract# 1463

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) INDUCES THE EXPRESSION OF THE CHEMOKINE IP-10 THROUGH THE PI-3 KINASE SIGNALING PATHWAY IN ENDOTHELIAL CELLS. Gwenola Boulday,[1] Marlies E. J. Reinders,[1] Soumitro Pal,[1] David M. Briscoe.[1] *[1]Transplantation Research Center, Children's Hospital, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Local intragraft expression of IFNγ-inducible protein-10 (IP-10) is associated with ischemia-reperfusion injury and acute rejection. IP-10 dependent interactions are known to mediate the local infiltration of T cells into allografts. VEGF is expressed in the immediate post transplant period and in rejection, and induces the expression of the chemokine IP-10. However, little is known of the mechanism(s) or signals involved in the VEGF or cytokine-dependent regulation of IP-10. Here, we have analyzed VEGF and IFNγ inducible intracellular signal(s) that regulate IP-10 expression in endothelial cells (EC). EC were transiently transfected with a full length IP-10 promoter-luciferase construct and were cultured in the presence of VEGF or IFNγ alone, or in combination. We found that VEGF alone resulted in weak IP-10 promoter activation, as compared to IFNγ. In contrast, the VEGF+IFNγ combination resulted in a synergistic increase in IP-10 transactivation. VEGF mediates its primary functions via interactions with the VEGF-receptors Flt-1 and KDR. IP-10-promoter transfected ECs were next stimulated with the selective Flt-1 agonist PlGF (instead of VEGF), but it failed to induce IP-10, either alone or in combination with IFNγ. These data suggest that KDR is the functional receptor mediating VEGF-inducible IP-10. Post KDR intracellular signaling pathway(s) are reported to involve the PI-3K molecule. We next treated transfected EC with Wortmannin (a PI-3K inhibitor) or co-transfected them with the IP-10-luciferase construct and a PI-3K dominant negative mutant. Our findings were that both PI-3K antagonists inhibited the ability of IFNγ, VEGF or combinations to induce IP-10 transcriptional activation. Thus, PI-3K is central to cytokine-mediated expression of IP-10. To further assess the associated kinases and pathway(s), we used an active form of PDK1 (a critical signaling component of the PI-3K pathway) in co-transfection assays and found that it also led to the IP-10 promoter activation. Together these findings establish for the first time a role for the PI-3K pathway in IP-10 expression and suggest that PI-3K/PDK1 could represent novel targets to modulate IP-10-dependent T cell trafficking post transplantation.

Abstract# 1464

ORGAN SPECIFIC DIFFERENCES IN THE FUNCTION OF CHEMOKINES DURING ALLOGRAFT REJECTION. Zdenka Haskova,[1] Atsushi Izawa,[1] Stuart W. Robertson,[1] Jocelyn Corradi,[1] David M. Briscoe.[1] *[1]Transplantation Research Center, Children's Hospital & Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Monocyte chemoattractant protein-1 (MCP-1) and the CXC chemokine receptor 3 (CXCR3) are two of a family of chemokines and chemokine receptors that are proposed to play a critical role in leukocyte trafficking into allografts and in effector cell activation. CXCR3 is the receptor for the chemokines IP-10, I-TAC and Mig that are produced mainly by activated Th1 and NK cells. The MCP-1 chemokine is produced mainly by monocytes and endothelial cells, and binds to its hematopoietic cell receptor CC chemokine receptor 2. Both CXCR3 and MCP-1 are expressed in association with rejection and promote the migration of T cells into inflamed tissues. We used fully MHC mismatched BALB/c - C57BL/6 tail to trunk skin or cardiac transplant models to determine the relative role(s) of donor or recipient MCP-1 and CXCR3. Compared with wild-type control mice (median survival time (MST)=9d, n=8) CXCR3-/- or MCP-1-/- recipients did not show delayed rejection of fully mismatched skin allografts. To our surprise, when CXCR3-/- and MCP-1-/- mice were used as graft donors, all skin allografts survived significantly longer than their wild-type controls (MST=15d, 12d vs. 9d; n=10, 10 and 8 resp., $p<0.0001$). This finding is in contrast to results following cardiac transplantation in which CXCR3-/- cardiac allografts do not survive significantly longer, and our preliminary observation that the lack of MCP-1 expression in cardiac allografts does not affect leukocyte trafficking or acute rejection (MST=8d vs.9d, n=6 and 5 resp). Also, we found that wild-type cardiac allografts in CXCR3-/- or MCP-1-/- recipients had prolonged survival ($p<0.005$). We hypothesized that the reason for the different role of CXCR3 and MCP-1 in skin and cardiac allograft rejection is due to vascularization. To address this possibility, we developed a model in which fully mismatched skin grafts were allowed to heal in SCID recipients. Four weeks later SCIDs received recipient wild-type spleen cells, and subsequently rejected the vascularized skin grafts by day 16 (n=8). Adoptive transfer of CXCR3-/- or MCP-1-/- spleen cells in this vascularized skin model did not result in a significant prolongation of survival. We conclude that donor expression of CXCR3 and MCP-1 in the heart and skin have different functions in rejection. Our data imply that there are organ specific differences in the usage of chemokine pathways that mediate selective leukocyte trafficking into allografts.

Abstract# 1465

BLOCKADE OF FIBRONECTIN-α4β1 ADHESIVE INTERACTION SPECIFICALLY DOWN-REGULATES METALLOPROTEINASE-9 AND PROLONGS RECIPIENT SURVIVAL IN A 24 HOUR MODEL OF COLD HEPATIC I/R INJURY. Carolina Moore,[1] Xiu-Da Shen,[1] Michael Witkosky,[1] Feng Gao,[1] Jerzi W. Kupiec-Weglinski,[1] Ana J. Coito.[1] *[1]Department of Surgery, The David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Ischemia/reperfusion (I/R) injury is often the cause of primary failure in orthotopic liver transplants (OLT). Expression of fibronectin (FN) by sinusoidal endothelium is an early event after liver injury. We hypothesized that FN-leukocyte interactions are critical in liver I/R injury. This study evaluated the efficacy of CS1 peptide mediated blockade of FN-α4β1 interactions in a 24h cold I/R model. **Methods and Results:** CS1 peptides (500 μg/rat) were administered through the portal vein of Sprague-Dawley (SD) rat livers before and after the 24h cold storage. SD recipients received a 3d-course of CS1 peptides (1mg/rat, i.v.). CS1 peptide therapy significantly increased the 14d OLT survival rate as compared with controls (100% vs 50%, n=6rats/gr, p<0.007). Moreover, it profoundly improved liver function, as assessed by sGOT levels (1251 ± 195 vs. 3733 ± 455, p<0.01). CS1 treated OLTs at day 1 showed good preservation of lobular architecture with no evidence of congestion or necrosis, contrasting with severe necrosis and sinusoidal congestion in controls (score: 1.0 ± 0.5 vs 3.5±0.2, p<0.01). COX-2 (0.8±0.1 vs. 2.1±0.6, p<0.05) and iNOS (non-detected vs. 1.4±0.4), important mediators of inflammation, were significantly depressed in CS1 treated livers. Leukocyte migration requires adhesion and focal matrix degradation events. Interestingly, CS1 therapy had no effect on MMP-2 (gelatinase A) production (1.6±0.1 vs. 1.8±0.4, p<0.3); however, it profoundly decreased MMP-9 (gelatinase B) expression (1.4±1 vs. 4.5±1, p<0.02). T cells and monocyte/macrophages were the primary sources of MMP-9 in injured control livers. To evaluate whether FN-α4β1interactions specifically regulate MMP-9 expression, RAW 264.7 macrophage-like cells, which express α4β1, were cultured in FN coated plates. Indeed, MMP-9 mRNA expression increased by 2.5-3 fold in cells cultured on FN, as compared with cells cultured on polylysine. However, cells cultured in FN plates pre-treated with anti-FN Ab or cells incubated with an anti-α4 Ab, before being plated on FN, showed significantly reduced MMP-9 levels. MMP-2 was virtually absent in cultured macrophages. **Conclusion:** CS1 peptide therapy significantly decreased iNOS and COX-2 production, specifically down-regulated MMP-9 expression, and improved liver function/ OLT recipient survival. This novel study demonstrates the potential utility of targeting FN-α4β1 interactions for improving outcomes in OLT.

Abstract# 1466

THE INTERACTION BETWEEN VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND CHEMOKINE EXPRESSION AND FUNCTION IN ACUTE ALLOGRAFT REJECTION. Atsushi Izawa,[1] Stuart W. Robertson,[1] Zdenka Haskova,[1] Masayuki Sho,[1] Mohamed H. Sayegh,[1] David M. Briscoe.[1] *[1]Transplantation Research Center, Children's Hospital & Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Recent studies suggest that chemokine and chemokine receptor interactions: interferon-inducible protein-10 (IP-10)/CXCR3 or monocyte chemoattractant protein-1 (MCP-1)/CCR2 play key roles in allograft rejection. VEGF, a known regulator of MCP-1 and IP-10 is expressed and is functional for leukocyte recruitment into allografts. In this study, we evaluated the interactions between VEGF-mediated trafficking of leukocytes and rejection, and MCP-1 or CXCR3 function *in vivo*. Fully MHC-mismatched hearts from BALB/c mice were transplanted into C57BL/6 wild type (WT), MCP-1, or CXCR3 gene knockout ($^{-/-}$) recipients; and were treated with either neutralizing VEGF anti-serum or normal rabbit serum i.p. on days -1, 0, 2, 4, 6, 9, 12, 15. Our findings were that anti-VEGF inhibited rejection and prolonged survival from 9 days (n=5) to as much as 14 days (n=5, p<0.005) in WT recipients. Anti-VEGF also prolonged survival in MCP-1$^{-/-}$ recipients (median survival time (MST) 15 days, n=2, -> 21 days, n=4) and in CXCR3$^{-/-}$ recipients (MST 15 days, n=3, -> 19 days, n=6). Histological examination of the grafts confirmed massive infiltration and vasculitis in rejecting WT grafts but less infiltrates and less vasculitis in all anti-VEGF-treated chemokine deficient recipients. To further address the role of intragraft chemokines for VEGF function, we also transplanted C57BL/6 WT, MCP-1$^{-/-}$, or IP-10$^{-/-}$ grafts into BALB/c recipients that were untreated or treated with anti-VEGF. Surprisingly, while IP-10$^{-/-}$ grafts had a prolonged survival, MCP-1$^{-/-}$ grafts were rejected at a similar rate as WT grafts (MST 9 days, n=6). Moreover, whereas anti-VEGF further prolonged survival of IP-10$^{-/-}$ grafts (MST 19 days, n=6, -> 34 days, n=7), in initial studies, anti-VEGF failed to prolong survival of MCP-1$^{-/-}$ grafts (MST 10 days, n=5). Taken together these data suggest that VEGF function *in vivo* is independent of recipient MCP-1 and CXCR3 interactions. Moreover, it appears that donor MCP-1 alone is not sufficient or necessary for the development of acute rejection of cardiac allografts; and that intragraft MCP-1 may be important for VEGF function *in vivo*. Our findings are consistent with the possibility that VEGF is a potent pro-inflammatory cytokine in rejection and that it is a potential therapeutic target. Importantly, these findings define VEGF as functional for leukocyte trafficking *in vivo* in both a chemokine-dependent and a chemokine-independent manner.

Abstract# 1467

CD103 PROMOTES RETENTION OF HOST-SPECIFIC CD8 EFFECTORS IN EPITHELIAL COMPARTMENTS DURING GRAFT-VERSUS-HOST DISEASE. Riham El-Asady,[2] Cinthia B. Drachenberg,[3] Gregg A. Hadley.[1,2] *[1]Surgery, University of Maryland, Baltimore, MD; [2]Microbiology and Immunology, University of Maryland, Baltimore, MD; [3]Pathology, University of Maryland, Baltimore, MD.*

Introduction: The destruction of the host intestinal epithelium by alloreactive CD8 effectors is a hallmark of GVHD pathology. We previously reported that host-specific CD8 cells dramatically upregulate expression of the integrin, CD103, following entry into the gut epithelium in GVHD. Given that CD103 recognizes an epithelial ligand (E-cadherin), these data suggested that CD103 may play a key role in targeting host epithelial compartments for destruction by host-specific CD8 cells. To test this hypothesis, we herein assessed the capacity of host-specific CD8 effectors with targeted disruption of CD103 to mediate GVHD pathology. **Methods**: Equal numbers of wild type Thy1.1^{+} and CD103$^{-/-}$ Thy1.2^{+} 2C TCR transgenic (H-2L^{d}-specific) spleen cells (SC) (H-2^{b}) were adoptively transferred, along with C57BL/6 (B6) BM and SC (H-2^{b}), into lethally-irradiated Balb/c (H-2L^{d}) hosts. Lymphocytes isolated from host organs were subjected to 4-color FACS analyses using mAb to the clonotypic TCR (1B2), Thy1.1 and Thy1.2. The percentages of host-specific wild type (2C-WT) and CD103$^{-/-}$ (2C-KO) lymphocytes infiltrating the gut epithelium among gated 1B2^{+} CD8^{+} effectors, were compared at days 7, 14, 21 and 28 post-BMT. To determine the contribution of CD103^{+} CD8^{+} effectors to GVHD pathology, lethally-irradiated Balb/c hosts were transplanted with BM and CD8 cells from WT or CD103$^{-/-}$ B6 donors. **Results:** 2C-WT and 2C-KO CD8 effectors (CD44^{hi} CD62L^{lo}) migrated with comparable efficiency into the gut epithelium by day 7 post-transfer. Thus, CD103 is not required for initial migration of CD8 effectors to the intestinal epithelium. However, the proportion of 2C-KO cells declined precipitously with time compared to 2C-WT cells: by day 28 post-transfer 2C-WT cells (comprising 50% of the total CD8 effectors) vastly outnumbered the 2C-KO cells (15% of the total CD8 effectors), indicating that CD103 is required for CD8 effector retention in the gut epithelium. The significance of such retention is supported by the finding that recipients of WT CD8 cells died more rapidly (MST = 11 days) than recipients of CD103$^{-/-}$ CD8 cells (MST >30 days) and exhibited more severe epithelial lesions as evidenced by increased apoptotic bodies within the crypt epithelium. **Conclusion:** These data document an important role for CD103 in retention of host-specific CD8 effectors in epithelial compartments and demonstrate that such retention is causally related to the destruction of epithelial compartments, a cardinal feature of GVHD.

Abstract# 1468

A LOW MOLECULAR WEIGHT CCR5 CHEMOKINE RECEPTOR ANTAGONIST IN COMBINATION WITH CSA PROLONGS GRAFT SURVIVAL IN A LIFE-SUPPORTING KIDNEY TX MODEL IN CYNOMOLGUS MONKEYS. Hans-Guenter Zerwes, Markus B. Streiff, Christian Buns, Marc Bigaud, Klaus Menninger, Adrien Kunkler, Sabine Riesen, Grazyna Wieczorek, Nigel Cooke, Rainer Albert, Marcel Luyten, Christian Beerli, Randall E. Morris, Gebhard Thoma. *Transplantation and Immunology Research, Novartis Institutes for BioMedical Research, Basel, Switzerland.*

Introduction. Both epidemiological studies in renal allograft recipients and *in vivo* experiments using CCR5 KO mice indicate that the chemokine receptor CCR5 plays an important role in transplant rejection by affecting trafficking of T cells and monocytes. Thus, antagonists of CCR5 are considered to be of therapeutic value. We report here on the pharmacological properties of NIBR-1282, a highly potent and selective competitive CCR5 antagonist.

Methods: NIBR-1282 was selected based on potency in *in vitro* assays using CCR5 transfected cell lines and human T cells. Assays were designed to provide information on molecular events (i) at the extracellular side of the receptor (radioligand binding assay), (ii) at intracellular sites i.e. G-protein association with the intracellular domains of CCR5 following receptor engagement; (GTPγS assay), (iii) downstream in the signal transduction pathway (Ca^{2+} mobilization assay) and (iv) cell migration (chemotaxis assays). NIBR-1282 was tested for immunosuppressive activity in cynomolgus monkeys with and without co-administration of cyclosporine (CsA).

Results: One of the best CCR5 antagonist designated NIBR-1282 had IC_{50} values between 2 and 40 nM in *in vitro* assays, was about equipotent on human and cynomolgus CCR5 and acted as a competitive antagonist of MIP-1α binding to CCR5. NIBR-1282 had an oral bioavailability of 43% in cynomolgus monkeys. The compound was tested in a model of life-supporting renal allograft rejection in cynomolgus monkeys as monotherapy or in combination with a dose of CsA which was ineffective vs vehicle controls (MST=7 d; p=n.s.). NIBR-1282 was not efficacious as monotherapy but when administered in combination with the non-effective dose of CsA graft survival was statistically significantly prolonged (MST=51.5 d; p< 0.05 vs. CsA). No PK interaction between NIBR-1282 and CsA was observed demonstrating that the graft prolongation was compound related.

Conclusion: We report here for the first time that administration of a low molecular weight CCR5 inhibitor can lead to significant prolongation of kidney allograft survival in cynomolgus monkeys in combination with a non-effective dose of CsA. Inhibition of CCR5 may offer new therapeutic opportunities for transplant patients.

Abstract# 1469

ADHESION MOLECULE EXPRESSION PROFILES OF REJECTING SKIN ALLOGRAFTS. Thomas R. Jones,[1] Andrew B. Adams,[1] Rose Hendrix,[1] Thomas C. Pearson,[1] Christian P. Larsen.[1] *[1]Department of Surgery and Emory Transplant Center, Emory University, Atlanta, GA.*

Several studies have demonstrated roles for chemokines and integrin ligand/receptor pairs in the effector response to the allograft. In particular, studies have repeatedly demonstrated the importance of the IFN-γ dependent chemokines IP-10 and Mig. However, due to overlapping costimulatory and homing functions of these molecules, it has been difficult to determine the mechanisms of prolonged allograft survival. In addition, it is important to distinguish between non-specific inflammatory events and antigen-driven chemoattractant expression. In this study, C57BL/6 SCID mice were engrafted with either an allogeneic or syngeneic skin graft. After 1 month, mice were reconstituted with T-cells from wild type C57BL/6 or from IFN-γ -/- C57BL/6 mice. Grafts were harvested at various time points after reconstitution, then processed to isolate mRNA for quantitative PCR analysis. We found that MCP-1 (13.16-fold increase relative to unreconstituted grafts, $p<0.05$ v. syngeneic) and the IFN-γ dependent chemokines Mig (114-fold increase), IP-10 (58.7-fold increase), and I-TAC (71.8-fold increase, all $p<0.04$ v. syngeneic controls) are significantly upregulated 4 days after T-cell reconstitution, and decline rapidly thereafter. CD4 and CD8 T-cell transcripts were not significantly detected until day 8 of rejection. Several chemokine receptor transcripts became detectable at this time, but did not clearly correlate with the impressive expression of MCP-1 or the CXCR3 ligands. Grafts from animals reconstituted with T-cells from IFN-γ knock-out mice did not display upregulation of the IFN-γ dependent chemokines Mig, IP-10, and I-TAC, suggesting that IFN-γ production from alloreactive T-cells is required for this process. Interestingly, we also found that allografts, but not syngeneic grafts, display increased expression of the IFN-γ – dependent chemokines Mig, IP-10, and I-TAC prior to T-cell reconstitution, albeit at much lower levels than observed during rejection (10.9-fold, 4.3-fold, and 7.0 - fold upregulated, $p<0.03$ v. syngeneic controls). This baseline upregulation was eliminated by treatment with anti-IFN-γ monoclonal antibody, but not through depletion of NK cells, suggesting an antigen-specific IFN-γ - dependent inflammatory process in the absence of a T-cell response. In summary, we have demonstrated that an extensive array of signals is upregulated during T-cell mediated rejection of skin allografts, and that the first major events occur shortly after T-cell reconstitution but prior to the appearance of an infiltrate.

Factors Determining Outcomes in Pancreas

Abstract# 1470

CHANGING PATTERN IN THE HISTOLOGICAL FINDINGS IN PANCREAS TRANSPLANT NEEDLE ALLOGRAFT BIOPSIES (PBx). Cinthia B. Drachenberg,[1] John C. Papadimitriou,[1] Emilio Ramos,[2] David K. Klassen,[2] Stephen T. Bartlett.[3] *[1]Pathology, University of Maryland, Baltimore, MD; [2]Medicine, University of Maryland, School of Medicine, Baltimore, MD; [3]Surgery, University of Maryland, School of Medicine, Baltimore, MD.*

Progressive improvement in the outcome of pancreas transplantation has been seen in recent years. An important reason for this improvement is the decrease in the rate of immunological graft loss. The latter has been seen with all types of pancreas transplants but has been more pronounced in the case of pancreas after kidney and pancreas alone transplants. To determine the impact of this clinical trend in the pathological material, we compared the pathology diagnosis of the first 100 and the last 100 biopsies performed in one transplant center. The first biopsies were performed between August 1992 and May 1994 and the later biopsies were performed between January 2002 and March 2003. There has been a marked decrease in the rate of acute rejection in the later biopsies in comparison to the earlier biopsies ($p<.0000$) (table 1). The severity of acute rejection also decreased and severe rejection was not observed in the latter group of biopsies (table 2). Chronic rejection was seen in 4% of the earlier biopsies versus in 23% of the later biopsies ($p=.0001$). Severe chronic rejection/graft sclerosis was only seen in the later biopsies. A comparison of other diagnostic categories in the 2 groups showed that whereas post-transplant lymphoproliferative disorder was seen in 4 instances in the earlier group, no such a diagnosis was found in the latter group. Pancreatitis and peripancreatitis were seen with equal frequency in both groups. Also, from the comparison of the two periods it was found that in the earlier period, coinciding with the implementation of the percutaneous biopsy technique in the program, there was a 12% rate of inadequate biopsies versus 5% in the later period. We conclude that a marked decrease in the rate of acute rejection has been seen in PBx after the introduction of more potent immunosuppressants. Despite this improvement, however, a significant increase in graft sclerosis (chronic rejection) is seen in the recent biopsies. The reasons for this increase remain to be clarified. Due to the 10 year interval between the 2 groups studied, aging of the grafts could account in part for the increase in graft sclerosis.

Table 1 Acute Rejection

Diagnosis	Normal/No rejection	Acute Rejection	Other Process
Earlier PBx	27%	71%	1%
Later PBx	38%	39%	23%

Table 2 Grades of acute rejection

Acute Rej. Grade	II, Minimal Rej.	III, Mild Rej.	IV, Moderate Rej.	V, Severe Rej.
Earlier PBx	12%	44%	7%	5%
Later PBx	8%	28%	5%	0%

Grading based on Transplantation 1997,21;227 and AJT 2003,75;1186

Abstract# 1471

CONVERSION TO A CALCINEURIN INHIBITOR (CI) AND STEROID FREE REGIMEN AFTER PANCREAS (Px) TRANSPLANTATION (Tx). R. Gruessner,[1] A. Humar,[1] R. Kandaswamy,[1] A. Gruessner,[1] D. Sutherland.[1] *[1]Surgery, Univ of MN, Mpls, MN.*

Introduction: Standard maintenance immunosuppression in Px Tx recips (with or without a kidney tx) is a CI plus steroids and an anti-proliferative agent. Both steroids and CIs have significant side effects but at least steroids can be withdrawn in Px Tx recips with a low rejn risk. In pts with nephrotoxicity (common) or other CI side-effects(diabetogenicity, neurotoxicity), CI withdrawal would be desirable if it could be done with a low rejn episode risk. We have tested the ability to do so in conjunction with a course of anti-T (and B)-cell therapy with Campath (alemtuzumab) in pts <1-10 years post Px Tx, with maintenance thereafter by mycophenolate mofetil (MMF) or rapamycin (RAP) alone.

Patients and Methods: Between 1/03 and 11/03, we stopped CI (usually tacrolimus) and steroids (if still on) in 65 Px Tx recips. At the time CI was stopped we gave Campath (30 mg x 3); thereafter the pts were maintained on either MMF (most, target 2-3 g/day, but adjusted by neutrophil count) or RAP (if GI toxicity from MMF, target level 5-8). There were 21 simultaneous Px and kidney (Kd) (SPK), 22 Px after Kd (PAK) and 22 PxTx alone (PTA) recips. The median time of conversion was 32 mos post-tx (range: 2.7 – 151mo) and the median follow-up time is 3 mo (range: 0.1 – 7.7 mos). Reasons for conversion were pancreas or kidney rejn (n=15), nephrotoxicity, severe defined as a serum creatinine >1.9 mg/dl (n= 34) and mild as below this level (n=13), neuropathy (n=2), or diabetogenicity (n= 1).

Results: After conversion, no patient deaths occurred. There were a total of 4 pancreas graft losses (1 SPK, 1 PAK and 2 PTA), all but one from a continuation of the rejn process that preceded the conversion. In contrast to the PAK category, where no kidney grafts were lost, 2 kidneys continued to deteriorate in the SPK category and dialysis was required. We also observed a total of 5 reversible rejn episodes (4 of the pancreas and 1 kidney rejn) after conversion. In pts converted for nephrotoxicity, except for those cited above, serum creatinine levels have remained stable or declined but the followup period is short.

Conclusions: The early results of a CI and steroid withdrawal protocol concomitant with a course of Campath show promise as an a alternative to the standard long-term maintenance strategy for pancreas and pancreas-kidney recips. If rejn does not occur, the elimination of a nephrotoxic agent has the potential to increase the life span of transplanted or native kidneys, especially those with impaired function at the time of conversion.

Abstract# 1472

STEROID AVOIDANCE VERSUS STEROID WITHDRAWAL AFTER SIMULTANEOUS PANCREAS-KIDNEY TRANSPLANTATION: A PROSPECTIVE AND RANDOMISED STUDY. Diego Cantarovich,[1] Georges Karam,[2] Magali Giral,[1] Maryvonne Hourmant,[1] Jaques Dantal,[1] Gilles Blancho,[1] Jean-Paul Soulillou.[1] *[1]Nephrology and Clinical Immunology, ITERT, Nantes University Hospital, Nantes, France; [2]Urology Department, Nantes University Hospital, Nantes, France.*

Sparing steroids, one of the cornerstone immunosuppressive agents currently used, can represent an advantage in the long-term follow-up of recipients of simultaneous pancreas-kidney transplantation (SPKT). We hypothesized that steroids can be safely avoided in the context of T-cell depletion, calcineurin blockade and purine biosynthesis inhibition, without increasing the incidence of acute rejection during the first 12 months after SPKT.

Two steroid sparing immunosuppressive regimens were compared from January 1999 to November 2002 in a prospective, randomised, open label, one centre study. A total of 50 diabetic and uraemic patients received rabbit antithymocyte globulin induction (Thymoglobulin; r-ATG) for 10 days, mycophenolate mofetil (MMF) and cyclosporine microemulsion (CsA), both from the day of surgery. 25 patients (16 males, mean age 42 yrs) did not received steroids (avoidance group) and 25 patients (17 males, mean age 40 yrs) received in addition a 3-month low-dose prednisone taper course (withdrawal group). All patients achieved one year of follow-up.

One patient in each arm (4%) experienced a biopsy-proven acute kidney rejection: 1 Banff 2a rejection on day 105 in the steroid avoidance group and 1 Banff 2a rejection on day 80 in the steroid withdrawal group. No episode of acute pancreatic rejection was diagnosed and no episode of kidney rejection was observed after the fourth month in any of the two groups. r-ATG was tolerated well in all patients (no premature discontinuation), with similar cumulative doses in both groups (656 mg vs 628 mg). One patient in the avoidance group died on day 2 because of cardiac failure. Similar 1-year survival of the patients (96 and 100%), kidney (96 and 100%) and pancreas (84 and 92%) were noted in the avoidance and withdrawal groups, respectively. No statistical differences were observed in the pancreatic and renal functions, lipid profile, insulin levels, and viral infectious episodes. Up to day, no cancer was diagnosed.

Complete steroid avoidance and a short 3-month steroid course, in the context of r-ATG, MMF and CsA, are equally safe and highly efficacious immunosuppressive regimens in diabetic recipients of SPKT.

Abstract# 1473

CAN ACUTE REJECTION BE PREVENTED IN SPK TRANSPLANTATION? A RANDOMIZED, PROSPECTIVE STUDY WITH THYMOGLOBULIN/ZENAPAX INDUCTION, TACROLIMUS AND STEROID MAINTENANCE: COMPARING RAPAMYCIN WITH MYCOPHENOLATE MOFETIL. George W. Burke,[1] Gaetano Ciancio,[1] Adela Mattiazzi,[1] Carmen Gomez,[1] Anne Rosen,[1] Joshua Miller.[1] *[1]Department of Surgery, Division of Transplantation, University of Miami School of Medicine, Miami, FL, 33136.*

INTRODUCTION. The rate of acute rejection (AR) in SPK transplantation has been falling over the past decade raising the possibility that AR may be entirely eliminated.

MATERIALS AND METHODS. From 9/2000 thru 11/2003, 84 SPK have been performed in patients with type 1 insulin dependent diabetes mellitus and end stage renal disease. These patients have been part of a prospective, randomized trial in which they received thymoglobulin (1.0 -1.5 mg/kg intraoperatively, and next 4 days) and zenapax (1 mg/kg x 2 doses, intraoperatively, and two weeks), tacrolimus and steroids as baseline immunosuppression. They were randomized to receive either mycophenolate mofetil (MMF) (1 gm BID), or rapamycin (Rapa) (4 mg/day – target trough level 8 – 10 ng/ml). Forty patients received MMF, and 44 received Rapa. All patients received ganciclovir/cytovene and bactrim prophylaxis for CMV and PCP respectively.

RESULTS. There have been 5 (12.5%) episodes of acute rejection (AR) in the MMF group – all except one in patients who were either off MMF (wound infection, pneumonia), or steroids. The AR occurred at 1, 3, 5, 6 and 8 months post SPK. Each of these episodes was steroid resistant but responsive to antibody therapy (OKT3 or Thymoglobulin). There has been one (2.3%) episode of AR in the Rapa group (off Rapa at the time) at 3 weeks ($p < .05$ vs MMF), responsive to thymoglobulin. There was one instance of CMV hepatitis (Rapa) which responded to ganciclovir. There was one case of Kaposi's sarcoma (Rapa) that responded completely to chemotherapy over a year ago with ongoing normal SPK function. There have been no lymphoproliferative disorders. There have been four deaths (2 MMF, 2 Rapa), two kidney graft losses (MMF group), three pancreas losses (1 MMF/2 Rapa). The lipid profile (cholesterol, triglycerides, HDL, LDL) is essentially normal in both groups, however, a larger proportion of Rapa patients are receiving lipid lowering agents. The overall actuarial survival with 1 1/2 year mean follow-up for patient, kidney and pancreas is 94%, 91% and 90%.

CONCLUSION. In a randomized, prospective study with a mean 1 1/2 year follow-up, the incidence of AR has been limited with one exception to only those instances where recipient's immunosuppression was significantly reduced. There was a smaller percentage of AR in the Rapa vs MMF group. There were no graft losses associated with AR.

Abstract# 1474

STEROID WITHDRAWAL IMPROVES ONE-YEAR OUTCOMES IN SOLITARY PANCREAS TRANSPLANTATION. Anne M. Wiland,[1] Benjamin Philosophe,[2] Matthew R. Weir.[3] *[1]Pharmacy Services, University of Maryland Medical Center, Baltimore, MD; [2]Transplant Surgery; [3]Nephrology, University of Maryland School of Medicine, Baltimore, MD.*

Steroid elimination has been successful using Thymoglobulin (RATG) induction therapy in both renal and simultaneous pancreas-kidney (SPK) transplantation; however, little has been reported in solitary pancreas transplantation. We introduced a steroid withdrawal protocol in February 2002 in solitary pancreas transplant recipients. This study compares 22 solitary pancreas transplant recipients withdrawn from steroids to a historical control group of 37 solitary pancreas transplants who remained on prednisone maintenance therapy. This analysis included 18 Pancreas Transplant Alone (PTA), 20 Pancreas After Kidney (PAK) and 21 Simultaneous Pancreas-Living Donor Kidney (SPLK) transplant recipients transplanted between January 2001 and August 2003. Both groups received 7-10 days of RATG induction therapy, tacrolimus (FK506) with a goal trough level of 12 ng/mL and mycophenolate mofetil (MMF). There was no difference in sex, previous transplant history, CMV status, peak PRA or AB and DR mismatches between the groups. All patients were non-African American. There was no difference in doses of MMF and FK506 between groups. Overall and 1-year rejection rates were lower in the prednisone withdrawal group and graft survival was improved.

	Prednisone Withdrawal (N=22)	Prednisone Maintenance (N=37)	P-Value
1-Yr Rejection Rate	0 (0%)	11 (29.7%)	0.01
Biopsy-Proven RR	0 (0%)	11 (29.7%)	0.01
1-Yr Graft Survival	22 (100%)	28 (75.7%)	0.01
1-Yr Patient Survival	22 (100%)	34 (91.9%)	0.17

CMV infection occurred in 4 patients in the prednisone maintenance group compared to none in the prednisone withdrawal group. Mean trough levels of FK506 were 9.2±5.1 ng/mL in the prednisone withdrawal group compared to 10.4±3.3 ng/mL in the prednisone maintenance group (p=0.40). Seventy-seven percent (17/22) of patients remained prednisone-free. Four patients were restarted on prednisone due to intolerance of FK506 or MMF and 1 patient was restarted for renal rejection (SPLK recipient }. Median total hospital readmission days 1-year post transplant were lower in the prednisone withdrawal group (4±1.4 vs. 6±3.3 days, p=0.075). Prednisone withdrawal at 21-days post-transplant is associated with improved outcomes in solitary pancreas transplantation.

Abstract# 1475

REDUCTION OF CMV DISEASE WITH STEROID-FREE IMMUNOSUPPRESSION IN SPK TRANSPLANT RECIPIENTS. Dixon B. Kaufman,[1] Joseph R. Leventhal,[1] Lorenzo G. Gallon,[2] Michele A. Parker.[3] *[1]Surgery, Feinberg School of Medicine, Chicago, IL; [2]Medicine, Feinberg School of Medicine, Chicago, IL; [3]Medicine, Duke University, Durham, NC.*

Lymphocyte depletion induction therapy allows for prednisone-free immunosuppresion in SPK tx. However, lymhocyte depletion may increase the risk of viral infections, especially CMV. We have conducted a retrospective analysis examining the relationship between immunosuppression and the risk of acquiring CMV in 200 consecutive SPK tx at a single center spanning two eras: Era I: Prednisone + (n=100, Tx 7/95-12/99, mean f/u 32.4 mo). Tacrolimus/MMF/Pred. ± induction. Era II: Pred-free (n=100, Tx 1/00-4/03, mean f/u 27.1 mo). Tacrolimus /MMF or sirolimus, Ø pred. and Thymo or CAMPATH induction. Pertinent recipient and donor demographics were similar among pts in both eras. All pts received CMV prophylaxis except the D-/R- group. Table I shows the rates of CMV according to D/R CMV serologic status and use of corticosteroids. The cumulative rate of CMV was markedly reduced in the pred-free cohort. When examined according to D/R status, the rates of CMV in the high risk pts (D+) were reduced by over 50%.

2 Year Actuarial CMV Rates in SPK Transplantation

Group	CMV rate	D+/R- (n)	D+/R+ (n)	D-/R+ (n)	D-/R- (n)
Pred + (100)	17%	36.4% (33)	26.7% (15)	0% (16)	2.8% (36)
Pred-free (100)	8.7%	15.5% (29)	13.1% (31)	0% (14)	0% (26)

Table 2 shows survival and rejection rates, and the overall and tissue invasive rates of CMV according to immunosuppression. Pt. and graft survival rates were similar among all groups. Rejection rates were lowest in the CAMPATH pred-free group. The overall rates of CMV in the high risk subgroups were significantly ($p<0.03$) lower in the pred-free cohort. Within that cohort the CMV rate was lower in pts receiving Campath vs. Thymo. (4.3% vs. 19.5%, p=0.16). The rates of invasive CMV in the high risk subgroups were also significantly (p=0.05) lower in the pred-free cohort. Within that cohort the CMV rate was lower in pts receiving Campath vs. Thymo. (0% vs. 13.9%, p=0.09).

2 Year Actuarial Rejection/Patient/Graft Survival and CMV Rates in SPK Transplantation

Group (n)	Patient	Kidney	Pancreas	Rejection	CMV*	Invasive CMV*
Prednisone + (100)	95.3%	92.3%	89.3%	24.6%	33.3%	22.9%
Pred-free (100)	96.4%	91.9%	92.0%	11.6%	14.6%	9.1%
Thymo (58)	96.1%	91.4%	96.1%	12.1%	19.5%	13.9%
Campath (42)	97.6%	92.3%	90.5%	7.1%	4.3%	0%

* high risk cohort only (D+/R- and D+/R+)

These results show that in SPK tx the rate of CMV can be significantly reduced by steroid avoidance even with lymphocyte depletion induction.

Abstract# 1476

INFLUENCE OF A PANCREAS TRANSPLANT ON RENAL FUNCTION IN PATIENTS WITH A PREVIOUS KIDNEY TRANSPLANT. Joshua J. Augustine,[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.*

An increasing proportion of pancreas transplants (PTx) are being performed sequentially after a previous kidney transplant (KTx). This approach (PAK) generally requires an increase in immunosuppression at the time of PTx and may increase the risk of immunosuppressive side effects including nephrotoxicity. Since 1997, 23 PAKs have been performed at our center. We compared kidney function before and after PTx in 17 of these patients who had sufficient follow-up before and after PTx to allow calculation of the slope of 1/creatinine versus time (SLOPE). Maintenance immunosuppression was heterogeneous but all patients received calcineurin inhibitors. Eleven of the 17 patients were converted from cyclosporine to tacrolimus (FK) at the time of the PTx. Duration of follow-up prior to PTx ranged from 4 to 123 months (mean 30±29 months). Duration of follow-up after PTx ranged from 6 to 65 months (mean 26±17 months). Serum creatinine concentration at the time of PTx ranged from 1.0 to 2.5 mg/dl (mean 1.7±0.5 mg/dl). For the entire duration from the time of KTx to last follow-up, SLOPE was negative in 16 of 17 patients (mean -0.0081±0.0074 dl/mg/month). In 5 of 17 patients, SLOPE was positive for the time interval between KTx and PTx. For the entire cohort, mean SLOPE for the time interval between KTx and PTx was +0.0026±0.0034 dl/mg/month). SLOPE was negative in all 17 patients following PTx (mean -0.0146±0.0121 dl/mg/month). Four of the 17 patients have either been retransplanted (n=2) or returned to dialysis (n=2). Using a paired t-test analysis, post-PTx SLOPE was significantly lower than pre-PTx slope ($p<0.05$). In addition, post-PTx SLOPE was lower than the overall SLOPE measured from the time of KTx (-0.0146±0.0121 versus -0.0081±0.0074 dl/mg/month), suggesting that PTx was associated with a breakpoint in the linear relationship between 1/creatinine and time. Serum creatinine at the time of PTx correlated inversely with post-PTx SLOPE ($R=-0.55$, $p<0.05$). In 6 patients treated with FK before and after PTx, mean trough FK level rose from 6.4±2.1 ng/ml during the 6 months pre-PTx to 9.5±1.5 ng/ml in the 6 months post-PTx ($p=0.002$). Results of this analysis suggest that renal function deteriorates at an accelerated rate after PTx in many patients with a previous KTx. Higher serum creatinine at the time of PTx increases the likelihood of renal deterioration. Whether this change in renal function is a consequence of increased immunosuppression at the time of PTx or other factors remains to be determined.

Abstract# 1477

GRAFT PANCREATITIS: SIGNIFICANT IMPACT OF DONOR FACTORS AND TYPE OF IMMUNOSUPPRESSION ON OUTCOME. A UNOS ANALYSIS. Christoph Troppmann,[1] R. Perez,[1] A. Gruessner,[2] D. Sutherland,[2] R. Gruessner.[2] *[1]Dept. of Surgery, Univ. of California, Davis, Sacramento, CA; [2]Dept. of Surgery, Univ. of Minnesota, Minneapolis, MN.*

Surgical complications after pancreas transplantation (PxTx) have significantly decreased over the past decade. But the impact of the multiple changes in practice (eg, increased use of enteric [ED] over bladder drainage [BD]) on graft pancreatitis (Pxitis) is unknown. **METHODS:** We reviewed all 6127 primary PxTx done between 1/98 and 5/03 (76% SPK, 17% pancreas-after-kidney Tx [PAK], and 7% pancreas Tx alone [PTA]) that were reported to UNOS. **RESULTS:** Pxitis incidence was similar for combined and solitary PxTx (SPK, 3.4%; PAK, 3.7%; PTA, 4.0%). Donor age, preservation time, as well as Pxitis during the initial admission (as reported by the Tx centers) by exocrine drainage technique were:

RECIPIENT CATEGORY		Mean Donor Age [yr]	Mean Preservation Time [hr]	Pancreatitis Incidence
SPK	**BD (n=1100)**	26.9	13.7	5.0%^
	ED (n=3556)	26.9	13.8	2.9%^
PAK	**BD (n=434)**	28.2#	16.4*	3.8%
	ED (n=599)	26.2#	14.1*	3.3%
PTA	**BD (n=222)**	29.3@	16.9	4.1%
	ED (n=216)	26.8@	15.8	3.5%

Pairwise comparisons (Chi-square): ^:p=0.001; #:p=0.04; *:p<0.0001; @:p=0.04

Overall, we noted 72 Px losses due to Pxitis (SPK, 43 [0.9%]; PAK, 21 [2.0%]; PTA, 8[1.8%]). Pxitis-associated Px loss occurred significantly more frequently in recipients of solitary PxTx (p=0.002 for PAK+PTA vs SPK). We studied risk factors for Px loss from Pxitis in a multivariate analysis:

COVARIATE	Relative Risk for Graft Loss from Pancreatitis Odds Ratio	*p*-Value
Donor Age >45 yr or CCV* Cause of Death (Yes vs No)	**1.72**	**0.11**
Preservation Time >24hr vs <24hr	**1.98**	**0.14**
SPK vs (PAK+PTA)	1.40	0.40
Recipient Age <45yr vs >45yr	0.69	0.33
BD vs ED	1.35	0.46
Induction with Depleting AB	**2.03**	**0.07**
Induction with Nondepleting AB	1.53	0.25
Induction with Tacrolimus+MMF	**0.20**	**0.009**

*CCV: cardio-cerebrovascular

SUMMARY: In SPK recipients, BD was significantly associated with increased Pxitis, but Px loss due to Pxitis occurred significantly more frequently in solitary PxTx. Donor risk factors, preservation time as well as induction with depleting AB or agents *other* than tacrolimus/MMF were all independent risk factors for Px loss from Pxitis. **CONCLUSIONS:** Incidence of Pxitis in SPK can be further lowered through the preferential use of ED over BD. Minimization of Pxitis graft losses requires judicious donor selection, short preservation times, and the avoidance of depleting AB. Further investigations are warranted to devise appropriate immunosuppressive strategies for PxTx recipients at high immunologic *and* Pxitis risk.

Abstract# 1478

EFFECT OF CENTER VOLUME ON PANCREAS TRANSPLANT OUTCOMES. Aloke K. Mandal,[1] Nicholas Drew,[2] Jodi A. Lapidus.[2] *[1]Division of Liver & Pancreas Transplantation, Oregon Health & Science University, Portland, OR; [2]Department of Public Health & Preventive Medicine, Oregon Health & Science University, Portland, OR.*

Background: The number of cases often correlates with improved outcomes for several procedures, including abdominal organ transplantation. Given the unique nature of pancreas transplantation and the large variation in transplant center volumes, this study explores whether center volume affects patient and graft survival following pancreas transplantation. *Methods:* Registry data on all forms of solid organ pancreas transplants performed between 1995 and 2000 were obtained from the United Network for Organ Sharing. Patient and graft survival rates were followed until 2002. Based on data from United States transplant centers, center volume was categorized as: low (≤10/yr), medium (11-20/yr), high (21-50/yr), and very high (>50/yr). *Results:* From 1995 to 2000, 6,876 pancreas transplants were performed: 2,143 at 87 low, 1,886 at 25 medium, 1,415 at 8 high, and 1,432 at 3 very high volume centers. Very high volume centers were more likely to do pancreas after kidney (PAK), pancreas transplant alone (PTA), pancreas with kidney (PWK), and repeat transplants while other centers more frequently performed simultaneous pancreas-kidney (SPK) transplants ($p<.001$, Log Rank test). In terms of recipient characteristics, very high volume centers were more likely to transplant older recipients and less likely to transplant minorities. While a quarter of the recipients at low, medium, and high volume centers had Medicaid; only 9% of patients at very high volume centers received Medicaid ($p<.001$). Low volume centers accepted pancreatic allografts from younger donors and had the longest waiting times. Very high volume centers used better HLA-matched organs. There were no differences in patient survival; however, both medium and high volume centers had better pancreas graft survival than low or very high centers. Female recipient, Medicaid coverage, and older donors all coincided with poorer graft survival ($p<.001$). Medium volume centers had the most late (>1 yr) graft failures ($p<.001$). Kidney transplant volume did not correlate with pancreas outcomes. *Conclusions:* Specific recipient and donor characteristics rather than just center volume affect pancreas transplant outcomes.

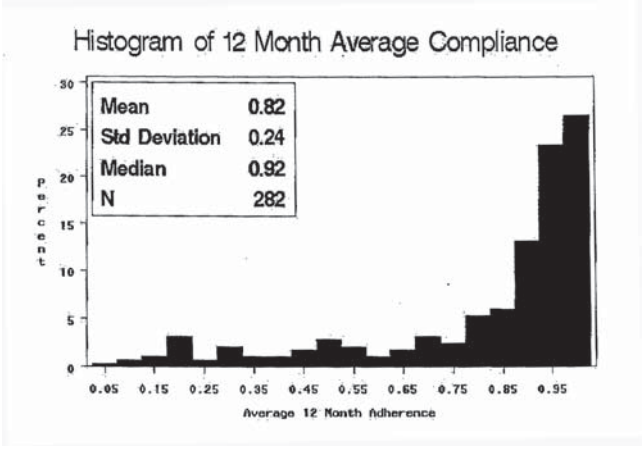
Abstract# 1479

PREDICTORS OF ADHERENCE TO IMMUNOSUPPRESSION DRUG REGIMENS DURING THE FIRST YEAR AFTER CADAVERIC RENAL TRANSPLANTATION (CRT). Ajay K. Israni,[1,2] Christina Gaughan,[2] Tracey Hoy,[2] Marshall Joffe,[2] Kevin C. Mange,[1,2] Sylvia Rosas,[1] Kenneth Brayman,[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]Renal, Pancreas & Human Islet Transplant Program, UVa Medical Center, Gordansville, VA.*

Lower levels of adherence to an immunosuppression regimen after CRT have been hypothesized as a risk factor for foreshortened allograft survival. We prospectively studied 282 CRT recipients to examine risk factors for poor immunosuppressive drug adherence measured using electronic pill bottle cap monitors (Aardex, Inc.) over the 12 months following transplantation. Adherence was expressed as the mean daily proportion of medication doses taken. A single medication was monitored for each subject.

Results: 70 (25%) of subjects were African-American(AA) and 175 (62 %) were female. The distribution of average adherence (Figure) illustrates that 41% of subjects had a 12-month average adherence >95%, 31% had 80-95% adherence, 15% had 50-80% adherence and 13% had less then 50% adherence. The only demographic or socio-economic factor associated with lower levels of adherence was AA race (p=0.02). There was no association between depressive symptoms (0R=1.06, 95% C.I. 0.59-1.90, p=0.86) as measured by CES-D questionnaire, transplant related stress (OR=0.99, 95% C.I. 0.97-1.02, p=0.54), internal locus of control (i.e. belief that the subject is in-charge of their own health) (OR=1.01, 95% C.I. 0.95-1.07, p=0.72) chance locus of control (i.e. belief that no one can be in-charge of the subject's health) (OR=0.99, 95% C.I. 0.95-1.04, p=0.64) or other locus of control (someone else is in-charge of the subject's health) (OR=1.03, 95% C.I. 0.97-1.08, p=0.34) as measured by MHLC questionnaire.

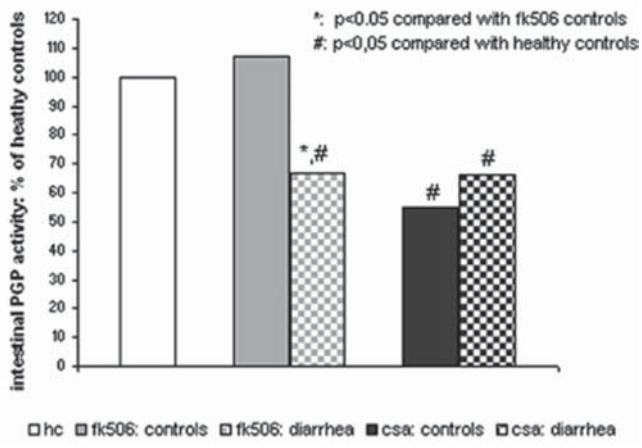
Conclusion: AA subjects were associated with lower levels of adherence, even after adjusting for other demographic and socio-economic factors. There was no detectably significant difference in depressive symptoms, transplant related stress or locus of control between subjects who had high levels of adherence compared to those with lower levels.



Abstract# 1480

DIARRHEA DIFFERENTIALLY AFFECTS INTESTINAL PGP ACTIVITY IN PATIENTS ON MAINTENANCE IMMUNOSUPPRESSION WITH TACROLIMUS VERSUS CICLOSPORIN A. Wim Lemahieu,[1] Bart Maes,[1] Kristin Verbeke,[2] Yves Vanrenterghem.[1] *[1]Internal Medicine, Division of Nephrology, University Hospitals Gasthuisberg, Leuven, Belgium; [2]Laboratory of Radiopharmaceutical Chemistry, University Hospitals Gasthuisberg, Leuven, Belgium.*

Diarrhea is a frequent adverse event in transplant patients and has been associated with an increase of trough levels of tacrolimus (FK506). In contrast, this has not been observed for cyclosporin A (CsA). The aim of this study was to evaluate whether intestinal CYP3A4 and PGP activities were differently affected by diarrhea in patients on FK506 versus CsA. Intestinal CYP3A4/PGP activity in vivo was assessed by the recently validated combined per oral and intravenous ^{14}C Erythromycin breath and urine test. Twenty-one renal transplant patients on immunosuppression with MMF + steroids + FK506 (n=15) or CsA (n=6) with afebrile and persistent diarrhea (faecal output > 200g/day, duration ≥ 2 weeks) were examined. Each group was compared with a corresponding group of stable controls taking FK506 (n=27) or CsA (n=9). In addition, all patient groups were compared to a group of 27 healthy controls (hc). Diarrhea did not alter CYP3A4 activity, but strongly impaired intestinal PGP activity in patients on FK506 compared to stable controls and hc. In contrast, all patients on CsA had a diminished PGP activity compared to hc, irrespective of the occurrence of diarrhea (figure). These data suggest that the impact of diarrhea on the oral bioavailability of FK506 is (at least partially) mediated by diminished intestinal PGP activity. The observed decreased intestinal PGP activity in patients on CsA is a plausible explanation for the lack of any additional impact of diarrhea on CsA trough levels.



Abstract# 1481

IMPACT OF ANTACIDS AND STATINS ON OVERALL CYP, CYP3A4 AND PGP ACTIVITY IN VIVO. Wim Lemahieu,[1] Bart Maes,[1] Kristin Verbeke,[2] Yves Vanrenterghem.[1] *[1]Internal Medicine, Division of Nephrology, University Hospitals Gasthuisberg, Leuven, Belgium; [2]Laboratory of Radiopharmaceutical Chemistry, University Hospitals Gasthuisberg, Leuven, Belgium.*

Bioavailability of various immunosuppressants such as ciclosporin A, tacrolimus and sirolimus, is strongly determined by hepatic and intestinal CYP3A4 and PGP activity, hence a considerable risk for drug-interactions. In clinical practice, transplant recipients receive ulcer prophylaxis and often lipid-lowering drugs. This study assessed the in vivo activity of overall cytochrome P450 (CYP), CYP3A4 and PGP in healthy volunteers after a 5 days' course of 3 different anti-ulcer drugs (omeprazole: Omp; cimetidine: Cim; ranitidine: Ran) in a randomized cross-over design. Likewise, the effects of 4 statins (simvastatin: Simva; pravastatin: Prava; atorvastatin: Atva; fluvastatin: Fluva) were evaluated. CYP activity was measured with the aminopyrin breath test and CYP3A4/PGP activity by the combined per oral and intravenous ^{14}C-Erythromycin breath and urine test at base-line and completion of each treatment. ANOVA was used for statistical comparisons. The results are expressed as %change from baseline activity (table). Only Cim had a significantly negative effect on overall CYP (-28%). Intestinal CYP3A4 activity was significantly increased by Omp (+63%) and Simva (+52%). Except for a tendency (p=0.08) of Simva to inhibit intestinal PGP (-21%), no clear impact on hepatic CYP3A4 or PGP activity was retained. This preliminary study confirms earlier reports on the inhibition of cytochrome P450 activity by cimetidine. In contrast, cimetidine did not specifically influence CYP3A4, as opposed to omeprazole and simvastatin, which induced intestinal CYP3A4 activity. Ranitidine and pravastatin overall had the smallest difference with baseline, implicating they are least likely to provoke drug interactions with other CYP3A4/PGP metabolized drugs like calcineurin inhibitors or sirolimus.

Treatment with different antacids and statins: % change of baseline activity

	Overall CYP	Hepatic CYP3A4	Intestinal CYP3A4	Hepatic PGP	Intestinal PGP
Cim	***-28%****	-1%	-4%	-8%	-6%
Omp	+2%	+14%	***+63%****	+2%	-1%
Ran	-2%	+2%	+26%	-2%	+26%
Atva	+3%	+3%	+21%	-5%	+5%
Fluva	-11%	-13%	+37%	-2%	-18%
Prava	-4%	+1%	-12%	-1%	+5%
Simva	-6%	-1%	***+52%****	-2%	*-21%*§

*: p<0.05; §: p=0.08

Abstract# 1482

EARLY STEROID WITHDRAWAL DOES NOT INCREASE RISK FOR RECURRENT FOCAL SEGMENTAL GLOMERULOSCLEROSIS. R. E. Boardman,[1] J. Trofe,[1] R. R. Alloway,[1] C. Rogers,[1] P. Roy-Chaudhury,[1] M. R. Cardi,[2] S. Safdar,[2] B. Groene,[2] J. F. Buell,[1] M. J. Hanaway,[1] M. Thomas,[1] W. J. Alexander,[2] R. Munda,[2] E. S. Woodle.[1] *[1]Division of Transplantation, University of Cincinnati; [2]Christ Hospital, Cincinnati, OH.*

The recurrence rate of focal segmental glomerulosclerosis (FSGS) in kidney transplantation (KTX) ranges from 20%-40%, with graft failure resulting in 40%-50% of these cases. Multiple risk factors for recurrent FSGS have been established. However, recurrence rates in pts undergoing early corticosteroid withdrawal (ECSW) (≤7 days) has not been determined. We present results of 49 pts that underwent KTX b/t 1999-2003 for FSGS, and compared pts that were enrolled into ECSW regimens to those that received long-term steroids. **Demograpics:** Pts include 51% male, 65% Caucasian, 35% African American, 57% CAD KTX, and 16% repeat KTX at mean age of 40 ± 12.5 yo at time of KTX. Of the 8 pts that had repeat KTX, 35% lost 1st allograft from recurrent FSGS. **Results:** Median time from dx of FSGS to ESRD (dialysis or KTX) was 4.3 (0-24) yrs. 27% of pts had documented use of immunosuppressive medication (prednisone ± CSA) to treat FSGS prior to KTX. Mean pre-KTX proteinuria was 6.3 ± 4.9 gm/24 hrs. Permeability factor was measured in 27% of pts with 2 pts having a value ≥ to control. Immunosuppression (IS) consisted of 74% Thymoglobulin induction, 49% CSA, 51% FK, 100% MMF, and 43% Rapa. 2 pts were lost to f/u and 4 pts received blinded study drug (prednisone or placebo) and were excluded from analysis. The remaining results are for 43/49 pts with a median f/u of 569 (18-1737) days. Results from the two groups are presented below.

		Early Steroid Withdrawal (n=21)	Long-Term Steroids (n=22)	*P* value
Median Follow-Up		359 (18-1442)	828 (10-1737)	**0.02**
Gender (M:F)		14:7	7:15	**0.02**
Race (C:AA)		14:7	15:7	0.91
Mean Age (yr)		38 ± 12	42 ± 14	0.37
Median Time from Dx of FSGS to ESRD (yrs)		4 (0-24)	4 (0-17)	0.52
Repeat Txp		24% (5/21)	14% (3/22)	0.39
Pre-KTX Proteinuria gms/24 hrs		6.4 ± 4	7.3 ± 6.8	0.74
Permeability Factor (≥ control)		0% (0/5)	29% (2/7)	0/19
Immunosuppression	CSA:FK	10:11	12:10	0.65
	Rapa	86% (18/21)	14% (3/22)	**<0.05**
Creatinine Clearance	0	13	13	0.86
	30	60	73	0.14
	90	65	83	0.09
	180	63	71	0.35
	365	84	71	0.37
	Last f/u	64	68	0.74
Recurrent FSGS		24% (5/21)	27% (6/22)	0.79
Median Time to Recurrent FSGS (days)		26 (19-205)	84 (20-482)	0.38
Graft Loss		14% (3/21)	14% (3/22)	0.95
Graft Loss d/t Recurrent FSGS		10% (2/21)	9% (1/22)	0.52

When comparing pts with recurrence vs. those without recurrence there was no statistical difference found with respect to gender, race, age, time from dx to ESRD, permeability factor, # of KTX, and IS. **Conclusion:** ECSW does not increase risk for recurrence of FSGS. These observations indicate that ECSW can be achieved in FSGS pts, thereby affording these pts the benifits of steroid elimination.

Abstract# 1483

IMMUNOSUPPRESSIVE PROPERTIES OF MYCOPHENOLATE MOFETIL AND VALGANCICLOVIR USED IN COMBINATION IN RENAL TRANSPLANT PATIENTS. Christopher M. Bearden,[1] Benita K. Book,[1] Richard A. Sidner,[1] Mark D. Pescovitz.[1] *[1]Surgery, Indiana University School of Medicine, Indianapolis, IN.*

Cytomegalovirus (CMV) infection has been implicated as a direct cause of acute rejection in kidney transplant patients. Patients treated prophylactically with ganciclovir (GCV) have lower rates of CMV infection and acute rejection. Valganciclovir (VGCV) is becoming the standard for prevention of CMV disease. *In vitro* studies of mycophenolate mofetil (MMF) and GCV show suppression of lymphocyte proliferation and production of monocytes. *In vivo* effects of the combination of these two drugs has not been studied. Cylex® Inc. is an *ex vivo* test for measuring overall immune system function.

20 primary kidney recipients underwent an IRB approved, prospective, non-randomized protocol. Subjects were immunosuppresed with MMF, prednisone and a calcineurin or mTor inhibitor. Per standard of care, patients were placed on VGCV postoperatively and dose adjusted for renal function. Each subject was tested on postoperative weeks 10, 12, 14, and 16. VCGV was stopped on the 12th week, immunosuppressive drugs doses were not changed. Testing included CBC, MMF and GCV drug levels, Cylex immunosuppressive activity, and flow cytometry for expression of CD3, CD4, CD8, CD20, IL2R, HLA-DR, CD69. Data analyses was performed by averaging data on and off drug and comparisons were done with paired Students t-Test.

Twenty subjects mean age 51y (range 31 to 68), 11M 9F, completed the protocol. WBC was significantly increased from 4.96±2.04 K/cumm to 5.77±2.13 ($p<0.01$) after discontinuation of VCG. Total granulocytes, lymphocytes and NK cells were not significantly altered during the study. Total monocytes counts significantly increased from 0.236±0.175 K/cumm to 0.702±0.278 ($p<0.001$). Total ATP production by CD4+ mononuclear cells measured by the Cylex assay was not changed during the study. However, when adjusted for the change in ratio of CD4+ lymphocytes and CD4+ monocytes that occurred during the study the Cylex study showed an increase in ATP production from 9.47±7.62 pg/cell to 21.44±19.87 ($p<0.001$). Percentage of CD4+ cells expressing HLA-DR increased from 1.26±1.31% to 2.72±1.99% ($p<0.001$) and those expressing CD69 increased from 0.40±0.21% to 0.52±0.28% ($p<0.05$). Il2R expression did not change.

Combining MMF and VCGV have an added immunosuppressive effect in transplanted patients similar to *in vitro* studies presented before. Close monitoring of patients blood counts are necessary when using these two drugs. Neutropenia may be reversed with discontinuation of VCGV rather than altering MMF as is often done.

Abstract# 1484

CELL SURFACE AND INTRACELLULAR CTLA4 AND CD40L EXPRESSION ON CD4+ T CELLS IN KIDNEY ALLOGRAFT RECIPIENTS - A POSSIBLE LINK TO FAVOURABLE OUTCOME AND REJECTION. Maria Magott-Procelewska,[1] Agata Kosmaczewska,[2] Irena Frydecka,[2] Lidia Ciszak,[2] Dariusz Patrzalek,[3] Piotr Szyber,[3] Marian Klinger.[1] *[1]Department of Nephrology and Transplantation Medicine, Medical University, Wroclaw, Lower Silesia, Poland; [2]Laboratory of Immunopathology, Institute of Immunology and Experimental Therapy, Wroclaw, Lower Silesia, Poland; [3]Department of Vascular Surgery and Transplantation, Medical University, Wroclaw, Lower Silesia, Poland.*

Experimental data suggest that an increased CTLA4 expression on CD4+ T cells can contribute to peripheral transplantation tolerance whereas CD40L engagement provides a costimulatory signal for T cell activation and ongoing graft rejection. The aim of the present study is to clarify the expression of costimulatory molecules in different groups of kidney graft recipients. CTLA4 (cell surface and intracellular) and CD40L expression were measured on unstimulated and anti-CD3 MoAb+rIL-2 stimulated peripheral blood CD4+ lymphocytes by dual immunofluorescence method and expressed as a percentage of double positive cells.

The study were performed in 72 kidney graft recipients and in healthy controls. In patients with acute rejection (AR) (group 1, n=15) and without AR (group 2, n=23) analysis was done during 30 days after transplantation (Tx). Patients with good graft function in long-term follow up (Scr<1,5mg/dl, group 3, n=23) and patients with biopsy proven chronic graft rejection (group 4, n=11) has undergone assessment 18± 5 months after engraftment. The percentage of cell surface and intracellular unstimulated CTLA4/CD4+ cells and unstimulated CD40L/CD4+ cells were significantly higher in all groups of kidney recipients as compared to controls. The detailed analysis revealed significantly elevated percentage of surface unstimulated and intracellular stimulated CTLA4 /CD4+ T cells in kidney allograft recipients with good long-term graft function (group 3) as compared to recipients with AR (group 1) during first month after kidney Tx (7.5±6.4vs 3.3±5.4 and 19±17 vs 6.4±6.8 respectively, p=0.01and p=0.02). After ex vivo stimulation the percentage of surface CTLA4/CD4+ T cells were significantly higher in kidney recipients with good graft function long-term after Tx than in patients without AR (group 2) tested during first month after Tx. Allograft recipients with good long-term kidney function also exhibited the significantly lower percentage of unstimulated CD40L/CD4+ cells in comparison to recipients with biopsy proven CR (group 4)(3.3±3.3vs6.9±4.7,p=0.01).We can conclude that CTLA4 expression on CD4+ T cells is modified during the follow up. Up-regulation of surface and intracellular CTLA4 can influence the favourable outcome of kidney transplantation while increased CD40L expression can sustain rejection.

Abstract# 1485

WEAKER AND WEAKER STILL: STEROID AVOIDANCE DOES NOT PREVENT BONE LOSS FOLLOWING KIDNEY TRANSPLANTATION. Christine E. Chamberlain,[1] Eric Elster,[2] Douglas Hale,[2] Allan D. Kirk,[2] Charles E. Daniels,[1] Roslyn B. Mannon.[2] *[1]Pharmacy Dept, CC, NIH, DHHS, Bethesda, MD; [2]Transplantation Branch, NIDDK, NIH, DHHS, Bethesda, MD.*

Metabolic bone loss is a common adverse event following kidney transplantation. Corticosteroids used in most immunosuppressive cocktails have long been associated with loss of bone density. With the recent utilization of steroid-free transplant strategies, we wished to determine the effect of steroid avoidance on bone mineral density. We evaluated changes in bone mineral density based on DEXA bone scans obtained prior to and at 6 to 24 months post transplant in recipients enrolled in kidney transplant protocols. We compared patients that had received lymphocyte depletional induction followed by sirolimus or tacrolimus monotherapy (steroid free; n=10) with those on standard triple immunosuppression with chronic steroids (steroid; n=8). The steroid free group only received methylprednisolone during induction therapy (mean = 790mg). Patient age was similar between groups (42y versus 38y, respectively). Parathyroid hormone levels were similar between groups pre-transplant (mean 425 versus 600). Both groups showed a progressive worsening in T-score in all areas. The magnitude of changes in T-scores was similar for total spine in both groups. There was a trend of worsening T-scores in radius (6 of 10 versus 3 of 8) , and femoral neck (7 of 10 versus 3 of 8) measurements in the steroid free group versus the steroid group. In contrast, the change in T-score for total hip was slightly worse in the steroid group (5 of 10 steroid free group versus 7 of 8 in steroid group). Thus, the absence of steroids did not appear to stop the decline in bone loss in our population. This decline may be a result of other immunosuppressants such as tacrolimus and sirolimus. Bone loss following transplantation should be carefully monitored. The impact of biphosphonates in this population is being explored.

T-Scores (mean ± SEM)

	T-score Pre-tx steroid free	T-score Post-tx steroid free	T-score Pre-tx w/steroids	T-score Post-tx with steroids
Radius	-0.79 ±0.23	-1.13 ±0.31	-1.39 ±0.58	-1.41 ±0.64
Spine	-0.47 ±0.42	-0.91±0.45	-1.36±0.63	-1.74 ±0.63
Total Hip	-0.59 ±0.32	-0.75 ±0.36	-0.51 ±0.48	-0.95 ±0.45
Femoral Neck	-0.97 ±0.35	-1.19 ±0.43	-0.61±0.43	-0.93 ±0.4

Abstract# 1486

SIROLIMUS-BASED THERAPY AFTER EARLY CYCLOSPORINE WITHDRAWAL RESULTS IN SIGNIFICANTLY BETTER RENAL HISTOLOGY AND FUNCTION AT 3 YEARS FOLLOWING KIDNEY TRANSPLANTATION. Alfredo Mota,[1] Manuel Arias, Eero I. Taskinen, Timo Paavonen, Christophe Legendre, Kerstin Claesson, Josep M. Campistol, Brian Hutchison, James T. Burke, Pekka Hayry, John F. Neylan, the Rapamune Maintenance Regimen Study Group. *[1]Serviço de Urologia e Transplantaçao, Hospitais da Universidade de Coimbra, Coimbra, Portugal.*

Introduction: Graft function and histology are predictive of renal transplant survival. We report renal function and protocol-mandated biopsy results from the Rapamune Maintenance Regimen trial.

Methods: Patients receiving cyclosporine (CsA), sirolimus (SRL), and steroids were randomly assigned at 3 months to remain on triple therapy (SRL+CsA, n=215) or to have CsA withdrawn (SRL, n=215). Protocol-mandated biopsies were performed at engraftment and at 12 and 36 months. Chronic Allograft Damage Index (CADI) scores were determined by 2 pathologists, blinded to treatment and clinical outcome.

Results: 484 biopsies were evaluated. The table shows mean CADI and component scores for patients with all 3 serial biopsies. These patients were representative of those remaining on assigned therapy through month 36. Calculated GFR was significantly higher in SRL patients (68.2 vs 54.8 mL/min, p=0.009).

Mean (± SD) CADI and Component Scores

	Engraftment		12 Months		36 Months	
Score	**SRL+CsA**	**SRL**	**SRL+CsA**	**SRL**	**SRL+CsA**	**SRL**
CADI	1.26±1.60	0.99±0.92	3.74±1.84	3.53±1.66	4.70±1.86*	3.20±1.85*
Inflammation	0.11±0.35	0.01±0.06	0.59±0.43*	0.82±0.51*	0.64±0.51†	0.50±0.51†
Tubular atrophy	0.08±0.27	0.08±0.19	0.70±0.42	0.56±0.52	0.77±0.50*†	0.32±0.41*†
Intimal proliferation	0.10±0.24	0.14±0.38	0.46±0.52	0.34±0.39	0.67±0.70	0.41±0.52
Glomerular sclerosis	0.33±0.41	0.23±0.38	0.27±0.54	0.18±0.28	0.64±0.57	0.37±0.57
Mesangial matrix	0.27±0.34	0.20±0.27	0.47±0.40	0.49±0.36	0.78±0.38	0.62±0.48
Fibrosis	0.37±0.52	0.33±0.38	1.26±0.63	1.13±0.70	1.21±0.53	0.99±0.61

*p<0.05 between groups, ANCOVA change from engraftment; †p<0.05 between groups, ANCOVA change 12 to 36 months

Conclusion: CADI scores are useful for evaluating treatment-related differences in the progression of histologic changes during a multicenter trial. Protocol biopsies showed significantly better renal histology in patients receiving SRL-based therapy after early CsA withdrawal.

Abstract# 1487

RAPAMYCIN-ASSOCIATED POSTTRANSPLANT GLOMERULONEPHRITIS AND REMISSION AFTER REINTRODUCTION OF CALCINEURIN-INHIBITORS. Elisabeth Dittrich,[1] Sabine Schmaldienst,[1] Afschin Soleiman,[2] Walter H. Hoerl,[1] Erich Pohanka.[1] *[1]Dept.of Medicine III, Div. of. Nephrology, University of Vienna, Vienna, Austria; [2]Dept. of Pathology, University of Vienna, Vienna, Austria.*

Background: Posttransplant glomerulonephritis (GN) is a common feature which may develop in renal grafts *de novo* or as recurrence of the original disease. It can be observed in all immunosuppressive drug combinations and is unlikely to respond to therapy modifications.

Methods: Renal transplant recipients were investigated for possible complications or side effects after conversion from calcineurin-inhibitor (CNI) based immunosuppression to rapamycin/steroids. We report on 4 patients developing heavy proteinuria, edema and decline of graft function, respectively.

Results: Proteinuria of 2.5-8 g/d occurred 2-9 months after converion to rapamycin. Renal biopsy revealed IgA nephropathy (2 cases), membranoproliferative GN type I and membranous GN (1 case each). Withdrawal of rapamycin and reintroduction of cyclosporine or tacrolimus was followed by compelete remission of proteinuria after 2-5 months in all patients. This effect was maintained for an observation period of up to 21 months. The complete remission of posttransplant GN was further confirmed by a second biopsy in 2 cases with disappearance of floride GN-associated lesions and by concomittant improval or stabilisation of renal graft function.

Discussion: The occurrence of reversible posttransplant GN is a quite uncommon observation. At present it is unclear, if rapamycin itself may have triggered the process by possible induction of proinflammatory effects, which resolved after its withdrawal. Alternatively, the substance may have been unable to sufficiently suppress GN activity in the graft. Withdrawal of CNI could then have allowed immune activation, which was finally terminated by reintroduction of cyclosporine or tacrolimus. We therefore recommend careful monitoring of urinary protein excretion in renal allograft recipients with rapamycin/steroid dual therapy.

KIDNEY: NON-IMMUNOLOGICAL COMPLICATIONS

Abstract# 1488

HISTOLOGIC FINDINGS ON SURVEILLANCE PROTOCOL BIOPSIES AFTER RENAL TRANSPLANATION. David J. Rea,[1] Mark D. Stegall,[1] Fernando G. Cosio,[2] Joseph P. Grande,[3] James M. Gloor,[2] Timothy S. Larson.[2] *[1]Department of Surgery, Division of Transplantation, Mayo Clinic College of Medicine, Rochester, MN; [2]Department of Internal Medicine, Division of Nephrology, Mayo Clinic College of Medicine, Rochester, MN; [3]Department of Pathology and Laboratory Medicine, Mayo Clinic College of Medicine, Rochester, MN.*

Introduction. The desire to detect histologic changes that precede functional renal allograft decline has stimulated our group to perform protocol surveillance biopsies (bx) in all patients. The aim of this study was to evaluate the histologic abnormalities found on protocol surveillance bx in both living donor (LD) and deceased donor (DD) renal transplant recipients.

Methods. We prospectively studied 516 renal transplants (401 LD and 115 DD) between Jan 2000 - Oct 2002. ABO incompatible and positive crossmatch renal transplants were excluded. Protocol bx were performed at 4, 12 and 24 mo after transplantation and were assessed for subclinical rejection (using Banff criteria), polyoma virus infection (using in-situ PCR) and for intragraft fibrosis (using the Banff ci score). A 30 min post-reperfusion (time 0) bx was performed in some patients to assess baseline histology.

Results. 975 protocol bx were performed in 516 patients during the study period. For the percutaneous biopsies the overall complication rate was 1.5% (10/752) with 7 patients requiring admission for bleeding. No graft was lost from a bx. Of all the patients transplanted, 69% underwent 4 mo bx, 56% underwent 12 mo bx and 15% underwent 24 mo bx. The reasons for not biopsying were anticoagulation treatment, patient refusal, graft loss, or recent clinical biopsy. Histologic abnormalities were noted on 66.8% of bx, however 16.8% of bx had incomplete Banff data. The histologic findings are shown in Table 1. The incidence of BKV nephropathy increased over time (4 vs. 24 mo, p=0.03), as did findings of interstitial fibrosis. Subclinical rejection rates were not statististically different among the time points.

Table 1.

Time (months)	Normal	Subclinical Rejection	BKV	Chronic Fibrosis ci1	ci2	ci3
0 (n=198)	185 (93%)	-	-	11 (6%)	2 (1%)	0
4 (n=366)†	237 (65%)	12 (3%)	11 (3%)	98 (27%)	16 (4%)	0
12 (n=298)†	114 (38%)	17 (1%)	17 (6%)	131 (44%)	39 (13%)	10 (3%)
24 (n=51)†	7 (14%)	3 (6%)	5 (10%)	26 (51%)	12 (24%)	3 (6%)

† The sum at each time point is greater than the number of biopsies due to multiple abnormalities.

Conclusions. Histologic abnormalities are not uncommon in biopsies of otherwise well-functioning kidneys. Our experiences with subclinical polyoma virus suggest that early intervention may be beneficial. The utility of treating subclinical histologic abnormalities is unclear, yet their presence is worrisome and suggest that protocol bx may provide a means to improve long-term graft survival.

Abstract# 1489

LONG TERM SURVIVAL DISPARITIES AFTER CADAVERIC VS LIVING DONOR RENAL TRANSPLANTATION IN PREVIOUS LIVER TRANSPLANT RECIPIENTS. John C. Magee,[1] Sarah H. Rush,[2] John R. Lake,[3] Friedrich K. Port,[2] Akinlolu O. Ojo.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI; [3]University of Minnesota, Minneapolis, MN.*

Background: Progressive renal insufficiency following liver transplantation is a growing concern. Renal transplantation is increasingly used to treat ESRD in liver recipients, but there is little data on the long-term efficacy of this therapy.

Methods: Using SRTR/OPTN data, a retrospective cohort of all adult liver transplant recipients who received a first renal transplant between 1995 and 2001 (n=1,156) was grouped into three categories: cadaveric renal transplantation (CRT) following liver transplantation (n=290), living donor renal transplantation (LDRT) following liver transplantation (n=163), and simultaneous liver-kidney transplantation (n=703). The Kaplan-Meier method assessed differences in death-censored renal allograft failure and patient survival. Variables and outcomes associated with CRT and LDRT were compared; the simultaneous liver-kidney transplant data are provided for reference.

Results: In the CRT and LDRT patients, the primary liver disease responsible for initial liver transplantation included hepatitis C (13.5 vs 11.7%, p=0.58), non-A, non-B hepatitis (4.8 vs 4.9%, p=0.97), cryptogenic cirrhosis (14.5 vs 7.4%, p=0.02), and alcohol (23.1 vs 15.3%, p=0.05). Primary renal disease in CRT and LDRT patients included cyclosporine nephrotoxicity (37.5 vs 38.7%, p=0.82), diabetes (9.0 vs 9.2%, p=0.02), and hypertension (5.2 vs 4.9%, p=0.90).

	CRT Following Liver Txp	LDRT Following Liver Txp	P-value CRT vs LDRT	Simultaneous Liver-Kidney Txp
Age at renal transplant (yrs, mean ± s.d./median)	51.6 ± 11.4/53	48.8 ± 13.1/50	0.02	49.9 ± 10.5/51
% Male	67.2	55.2	0.01	62.5
% White	91.4	93.3	0.48	83.4
1-yr Patient survival	89.7%	92.0%	0.20	78.1%
5-yr Patient survival	63.7%	76.0%	0.02	64.0%
1-yr Kidney survival*	90.8%	94.9%	0.05	87.8%
5-yr Kidney survival*	67.3%	79.4%	0.04	80.1%
Mean interval of liver transplant to renal transplant (yrs)	5.4	5.4	0.91	N/A
Mean time / median time on kidney waitlist (days)	459/317	213/152	<0.001	Not calculated

*Kidney survival censored at death

Conclusions: Among prior liver recipients, patient survival following both CRT and LDRT is good at 1 year but relatively poor at 5 years. CRT recipients have significantly lower 5-year survival rates than LDRT recipients. Renal transplantation may present different risk-benefit ratios for liver recipients and ESRD patients with no prior history of liver transplantation.

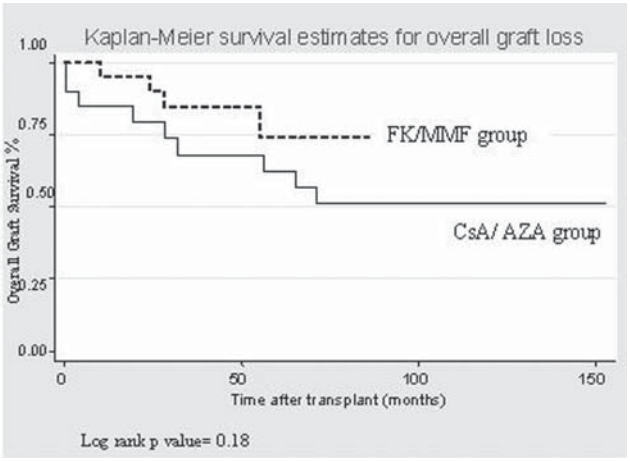
Abstract# 1490

LONG TERM OUTCOME AFTER RENAL TRANSPLANTATION (RTx) IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). Rajendra S. Baliga,[1] Pradeep V. Kadambi,[1] Amandeep Khurana,[1] Kristen Kasza,[2] Michelle A. Josephson,[1] Basit Javaid.[1] *[1]Section of Nephrology, University of Chicago, Chicago, IL; [2]Department of Health Studies, University of Chicago, Chicago, IL.*

Aim: To determine the outcome of RTx in patients with SLE, and predictors of graft and patient survival.

Methods: We studied all SLE patients who received RTx at our institution from 1991 to 2003. Primary endpoints were graft loss and patient death. Secondary endpoint was recurrence of lupus nephritis (RLN) in the allograft.

Results: 47 patients with a mean age of 34.6±11.6 yrs received 49 RTx. Mean duration of SLE prior to RTx was 11.8 years, with a median of 31 months on dialysis. 36 recipients were female and 11 were male. 32 of 49 grafts (65%) were cadaveric (CRT). In addition to prednisone, 20 received cyclosporine (CSA) + azathioprine (AZA), and 27 tacrolimus (FK) + mycophenolate mofetil (MMF). 10 patients had delayed graft function (DGF). Graft survival at 1 and 5 years was 87% and 66% and patient survival at 1 and 5 years was 96% and 85%, respectively. Infections accounted for 5 of 6 patient deaths. Of 16 graft losses, patient death and medical noncompliance accounted for 4 of each. DGF was associated with higher likelihood of overall graft loss (p=0.003), and death censored graft loss (p=0.004). There was no difference in graft survival in the FK/MMF compared to CSA/AZA group.



7 of 49 (14%) grafts have developed RLN, after a mean duration of 23.6±18.2 mos post RTx. 4 of the 7 patients had sudden onset of nephrotic syndrome. Living donor transplants (LRT) accounted for 6 of 7 RLN (p=0.01). There was no correlation between primary renal pathology, and that of the recurrent disease. 6 patients were treated conservative measures only, while one recieved plasmapheresis as well. Graft loss occured in 2 of these 7 patients.

Conclusions: 1. Patients with SLE have satisfactory clinical outcomes after RTx **2.** Infections account for significant mortality after RTx in SLE **3.** Incidence of RLN was 14% **3.** LRTs were more likely to develop RLN **4.** Medical noncompliance is a significant cause of graft loss.

Abstract# 1491

THE SIGNIFICANCE OF POST-TRANSPLANT MICROSCOPIC HEMATURIA IN RENAL TRANSPLANT PATIENT. Soon I. Kim,[1] Jong H. Lee,[1] Kyu H. Huh,[1] Kyu H. Choi,[2] Yu S. Kim,[1] Seung C. Yang,[3] Hyun J. Jeong,[4] Kiil Park.[1] *[1]Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea; [2]Nephrology, Yonsei University College of Medicine, Seoul, Republic of Korea; [3]Urology, Yonsei University College of Medicine, Seoul, Republic of Korea; [4]Pathology, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Purpose: Microscopic hematuria is a frequently encountered clinical problem in kidney graft recipients. The causes vary and could be benign or malignant. Differential diagnosis is difficult but imperative for patient with chronic relapsing hematuria and graft survival. We evaluated the renal recipients who developed hematuria treated with a defined algorithm.

Materials & methods: We evaluated 1,300 renal transplant recipients who underwent renal transplantation from March 1, 1992 to December 31, 2002. Plain radiography, sonography, and cystoscopic examination were performed sequentially to evaluate these patients. Graft biopsy was performed in patients with negative results after taking those studies.

Results: The mean duration after transplantation was 40.81±14.5 months (6-118 months). Microscopic hematuria was developed in 245 recipients. In 99 recipients, hematuria was transitory and spontaneously cleared-up within 3 months. We tried to identify the causes of hematuria in 136 recipients with persisting condition. The known causes of hematuria were urolithiasis in 16, benign bladder mucosal bleeding in 5, bladder cancer in 5 and kidney cancer from the original kidney in 2. Graft kidney biopsy was performed in 108 patients and the results were as follows: chronic rejection in 31, IgA nephropathy in 18, cyclosporine toxicity in 15, acute rejection in 7, focal segmental glomerulosclerosis in 5, other glomerulonephritis in 3, and tubular atrophy and interstitial fibrosis in 19 patients. No pathological diagnosis could be made in 10 patients. Combined pathologic findings were detected in 17 patients.

Conclusions: The causes of hematuria after kidney transplantation varied from benign to malignant disease. When ultrasonographic and/or cystoscopic examination could not determine the cause, graft biopsy should be performed to identify the intra- and extra-graft causes of hematuria.

Abstract# 1492

URINE CONCENTRATING CAPACITY AND SODIUM EXCRETION FOLLOWING RENAL TRANSPLANTATION.

Shilpanjali Prasad,[1] Jen Van Tromp,[1] Rawya Charif,[1] Seema Singh,[1] Tom Cairns,[1] Megan Griffith,[1] Nadey Hakim,[1] Adam McLean,[1] Andrew Palmer,[1] Vassilios Papalois,[1] David Taube.[1] *[1]Renal Unit, St Mary's NHS Trust, London, United Kingdom.*

Background: Impaired tubular concentrating capacity after renal transplantation may occur due to tubular injury, renal denervation and drugs, resulting in altered sodium and water homeostasis. This study assessed sodium and water intake and excretion, and responses to fluid restriction in stable renal allograft recipients on steroid-free immunosuppression. **Method and Results:** 35 patients (mean age 47.1 years, 17.8 months post-transplant, 8 diabetic) completed 3-day food diaries and 24-hour urine collection. Mean creatinine clearance (CrCl) was 59 ± 21.8 ml/min. Mean sodium intake was high (142.5 ± 92.3 mmol/day) with urinary sodium excretion of 167.5 ± 76 mmol/day. Water intake ranged from 0.8-5.3 L/day and urine volume 1.2-5.2 L/day. Mean urine osmolality (UOsm) was 321 ± 117.9 mosmol/kg. 15 of 35 patients produced isotonic or hypotonic urine, with higher urine volume (3.4 L/day vs. 2.7 L/day, p=0.02) and lower CrCl (51 ml/min vs. 68.2 ml/min, p = 0.01) than patients producing concentrated urine. 19 of 35 patients (mean age 47.1 years, 18.6 months post-transplant, 5 diabetic, CrCl 65.8 ± 18.2 ml/min) underwent total fluid restriction for 8 hours, followed by subcutaneous injection of Desmopressin, with hourly blood and urine samples taken. Results are shown below. Plasma osmolality (POsm) and sodium concentration (P Na) rose significantly, indicating mild dehydration. UOsm plateaued in the last two hours of restriction. We expected UOsm to rise above 700 mosmol/kg after restriction based on studies in normal patients, but the majority of subjects did not achieve this concentration. Urine sodium excretion (U Na) increased, possibly in response to the rise in PNa. After Desmopressin a small rise in UOsm was observed (632.7 ± 146.8 mosmol / kg, p=0.02). Analysis of anti-diuretic hormone levels pre- and post-restriction is pending. **Conclusions:** Renal allograft recipients have high sodium intake and excretion, with a tendency to produce poorly concentrated urine, which may be dependent on renal function. Sub-maximal renal concentrating capacity is seen in response to mild dehydration.

	Baseline	After Restriction	p value
POsm (mosmol / kg)	294 ± 7.4	298 ± 7.3	< 0.001
Urine output (ml / min)	2.4 ± 2.4	0.8 ± 0.5	0.01
PNa (mmol / L)	137.6 ± 2.8	139.4 ± 2.3	< 0.001
UOsm (mosmol / kg)	476 ± 156	619 ± 151	< 0.001
U Na (mmol / min)	1.2 ± 0.4	1.5 ± 0.9	0.02

Abstract# 1493

CALCIFICATION OF RENAL ALLOGRAFTS EARLY AFTER TRANSPLANTATION IS AN IMPORTANT FACTOR FOR GRAFT OUTCOME.

Wilfried Gwinner,[1] Michael Mengel,[2] Sylvia Suppa,[1] Hans H. Kreipe,[2] Hermann Haller.[1] *[1]Nephrology, Medical School Hannover, Hannover, Germany; [2]Pathology, Medical School Hannover, Hannover, Germany.*

Tubular calcification can be found in some renal allografts. Aim of this study was to analyze calcification in serial protocol biopsies (bx) obtained 6 weeks (wk), 3 and 6 months (mo) after transplantation (tx) in relation to clinical and labaratory variables, and to graft outcome at one year after tx.

Analyses included 320 patients, with a total number of 275 bx at 6 wk, 303 bx at 3 mo, and 260 bx at 6 mo post tx. Biopsies were evaluated according to the BANFF 1997 classification. Calcification was scored with grade 0=none, grade I=mild and focal, and grade II=severe and diffuse.

The incidence of tubular calcification increased over time, with 5.5% in the 6 wk bx, 8.6% in the 3 mo bx, and 16.1% in the 6 mo bx (p<0.01). Grade II calcification was present in 1 patient 3 mo post tx and in 5 patients 6 mo post tx. Mean serum calcium levels were higher in patients with calcification (2.56±0.21 vs. 2.39±0.18 mmol/l in patients without calcification; p<0.01). Serum phosphate levels were not different. Mean alkaline phosphatase activity was higher in patients with calcification (172±75 vs. 131±69 U/l in patients without calcification; p<0.01). Mean levels of intact parathormone were higher in patients with calcification at each bx time point (6 wk: 343 vs. 231, 3 mo: 432 vs. 258, 6 mo: 518 vs. 207 pg/ml; p<0.01). Biopsies with tubular calcification had significantly less often isometric vacuolization (8.4% vs. 19.1%; p<0.02) and more frequently tubular atrophy and fibrosis (49.4% vs. 29.2%; p<0.0005), whereas incidences of acute rejection, borderline findings, and acute tubular necrosis were not different. Number of patients with vitamin D treatment was not different in the two groups, however, patients with calcification received more often bisphosphonates (7.2% vs. 1.6%). Parathyreoidectomy was performed in 3 patients with calcification. Compared to the lowest serum creatinine observed within the first 6 wk after tx, patients with calcification had an increase at one year after tx (119 to 143 µmol/l; p<0.01), whereas patients without calcification showed no significant increase (125 to 136 µmol/l).

Tubular calcification within the first 6 months after tx is associated with interstitial fibrosis and tubular atrophy resulting in an inferior outcome of graft function at one year. Because of the relation to hyperparathyreoidism and high serum calcium levels, bisphosphonate therapy or parathyreoidectomy should be considered earlier and more often in these patients.

Abstract# 1494

CALCIUM OXALATE DEPOSITION IN KIDNEY ALLOGRAFTS STRONGLY CORRELATES WITH POOR LONG-TERM GRAFT SURVIVAL.

Helady S. Pinheiro,[1] Niels O. S. Camara,[2] Kikumi S. Ozaki,[1] Luiz A. Moura,[3] Alvaro Pacheco-Silva.[1] *[1]Internal Medicine, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil; [2]Nephrology, Federal University of Sao Paulo, Sao Paulo, Brazil; [3]Pathology, Federal University of Sao Paulo, Sao Paulo, Brazil.*

Oxalate accumulates as a consequence of its reduced excretion during end-stage renal disease. After kidney transplantation, it will be promptly excreted creating a risk of supersaturation and tubular deposition. Although it's well established the impact of primary hyperoxaluria on isolated kidney transplant, the influence of secondary oxalosis deposition on graft outcome remains to be determined. We quantified the presence of calcium oxalate (CaOxa) deposits in renal biopsy of kidney transplant recipients with acute graft dysfunction and analyzed its impact on graft survival. Ninety-seven patients were included and prospectively studied for 12 years. CaOxa deposits were present in 52.6%; 26.8% were mild and 25.8% moderate to severe. The deposits were more frequent in cadaveric grafts with longer ischemic time. The incidence of acute tubular necrosis (ATN) was greater when CaOxa deposits were present (47.1 % vs. 22.9 %, p<0.05). In a multivariate analysis, by logistic regression, the moderate-severe CaOxa deposit was an independent variable to ATN development, with an increased risk of 18.6 times. 12-year graft survival was worse in patients with CaOxa deposits during graft dysfunction compared to those free of deposits (49.7 vs. 74.1 %, p=0.013). Further stratification of oxalate deposits showed that 12-year graft survival was also worse in those patients with moderate-severe deposits compared to those free or with mild deposits (38.1 vs 69.1 %, p=0.0016). Our data strongly suggest CaOxa deposits presence and severity positively correlate with poor graft survival and its evaluation by microscopy under polarized light should be included in the routine histopathological analyses.

Abstract# 1495

SUBURETERIC COLLAGEN INJECTION TREATMENT FOR VESICOURETERAL REFLUX IN RENAL TRANSPLANTATION.

Piyush N. Joshi,[1] Amalanshu Jha. *[1]Department of Surgey, Div. of Urology and Transplantation, East Tennessee State University, Johnson City, TN.*

Incidence of vesicoureteral reflux (VUR) in the transplanted kidney is about 26%. This may cause recurrent urinary tract infections (UTI) including transplant pyelonephritis, sometimes leading to earlier graft loss. We herein present an endoscopic subureteric injection technique using bovine collagen to treat transplant VUR.

All our patients had Lich-Gregiore ureteroneocystostomy at the time of transplantation. All patients who had 3 or more culture proven post-transplant UTIs in <1 year, were studied by voiding cystourethrography (VCUG). 9 patients (8 with cadaver kidneys) were found to have VUR and underwent this procedure as an outpatient. All had negative Contigen skin test

Of 7 female patients, 4 had grade 2 and 3 had grade 3 VUR. Both male patients had grade 2 VUR. Of these one had simultaneous Kidney-pancreas and another had double renal grafts placed in same fossa with VUR detected in only one kidney.

Technique:

First, cystoscopy is performed and an angled tip glide wireis passed through a double port bridge into the transplant ureteric orifice with the help of Torque Viceunder fluoroscopic control. This helps straighten and align the intramural ureter with the cystoscope. Sometimes it may be necessary to put pressure in the suprapubic area. Using syringe adapters set stabilizes the injection syringe and the long endoscopic injection needle is primed with Contigen to avoid injecting air.

Using transurethral needle sheath for support, the needle is advanced through a point just next to the orifice, parallel to the guide wire into the intramural subureteric area. With steady slow pullout of the needle, approximately 2 to 3 ccs of collagen is injected in the subureteric space. After this an immediate on-table cystogram is performed. If reflux is still visible more Contigen is injected. If there is a concern about causing ureteral obstruction, a 7 X 16 double J ureteral stent is placed immediately as Contigen tends to mold around the stent and thus relieve obstruction.

2 male and 4 female patients required a single treatment and 3 female patients required 2 or more treatments. One of these eventually required ureteroneocystostomy. All patients have remained obstruction or recurrent UTI free after their last treatment with a median follow up of over 1 year.

In conclusion, this is a simple outpatient procedure, which can help prevent further damage to the transplanted kidney, by preventing VUR related recurrent urinary tract infections and also may help avoid open surgery in majority of the patients.

Abstract# 1496

MOLECULAR MECHANISMS OF TRANSPLANT RENAL ARTERY STENOSIS FROM EARLY USE OF ERYTHROPOIETIN.

S. Nagarajan,[1] J. Vidhun,[1] E. Mansfield,[1] S. Hsieh,[1] R. Liu,[1] O. Salvatierra,[2] M. Sarwal.[1] *[1]Pediatric Nephrology, Stanford University, CA; [2]Surgery, Stanford University, CA.*

Purpose

Erythropoietin (EPO) is common therapy for post transplant (Txp) anemia. Our study consisted of 2 pediatric post renal Txp cohorts; one on a steroid free (SF) and the other

on a steroid based (SB) immunosuppression protocol. EPO was used in the early post Txp period, due to a higher observed rate of anemia in the SF cohort. Despite a lower incidence of hypertension in SF patients compared to matched steroid-based (SB) patients (p<0.001), hypertension (HTN) secondary to an unusual pattern of transplant renal artery stenosis (TRAS) was observed in SF patients on EPO in the first post Txp week. The study objective was to identify the association and molecular mechanisms of early EPO therapy induced TRAS.

Methods

Fifty SF patients were evaluated for HTN, transplant bruit, TRAS by magnetic resonance angiography (MRA), anemia, average EPO dose (units/kg/week) and post-transplant timing of EPO use. Blood samples were subjected to DNA microarray analysis. Data was analyzed using Statistical and Predictive Analysis of Microarray, Chi-square and T-tests. Real-time PCR was used to confirm expression of marker genes identified in the microarray screen.

Results

HTN developed in 11/50 patients of whom 82% had received EPO, 67% received EPO in the first week post Txp. 3/11 patients developed atypical distal TRAS, away from the anastamotic site. All TRAS patients were on EPO, started in the first post Txp week and mean time to onset of HTN was 3 weeks, to diagnosis of TRAS by MRA was 6 weeks. TRAS patients were managed with discontinuation of EPO, Clopidogrel, Aspirin and angioplasty with complete resolution of TRAS in 4 months. EPO use in the first week post Txp significantly increases risk of both HTN (p=0.03) and TRAS (p=0.02).

A signature of 134 significant genes defined HTN patients with and without EPO treatment and only 12 genes were required to differentiate TRAS patients from matched controls with 100% concordance (p <0.001). There were 5 ESTs, cell cycle associated markers (H3F3A, DRF1, ITSN2) and c-CBL which regulates receptor protein-tyrosine kinase ubiquitination. Real-time PCR on c-CBL and PDGFR confirmed the expression differences seen by array analysis.

Conclusion

We recommend EPO be avoided in the first week post renal Txp due to the increased risk of TRAS. Early post Txp EPO down regulates c-CBL, which increases activated PDGF, EGF and CSF-1 receptor function by decreasing receptor ubiquitination, resulting in an increased thrombogenic potential in recently transplanted vasculature.

Liver Transplantation: Complications and Disease Recurrence (Non-HCV)

Abstract# 1497

SUCCESSFUL LIVER TRANSPLANTATION OF CHRONIC HEPATITIS B RECIPIENTS IN THE SETTING OF HIV COINFECTION AND LAMIVUDINE RESISTANCE. Jonathan Carter,[1] Norah Terrault,[1] Laurie Carlson,[1] Michelle Roland,[1] Gerald Lipshultz,[1] Chris Freise,[1] John Roberts,[1] Nancy Ascher,[1] Peter Stock.[1] *[1]Division of Transplantation, Univ. of California-San Francisco, San Francisco, CA.*

Background and Purpose: Prophylaxis with lamivudine (LAM) and hepatitis B immunoglobulin (HBIG) is critical to liver transplantation in patients with chronic hepatitis B (HBV). HBV-HIV co-infected patients present a particular challenge, because LAM is widely prescribed for HIV and LAM-resistant HBV develops in 70% of patients. The availability of tenofovir and adefovir as alternatives for LAM-resistant HBV provides an option for post-transplant HBV prophylaxis. We examined the post-transplant course of five HIV-HBV co-infected patients with specific focus on control of HIV and HBV infections.

Methods: From 4/01 to 9/03, five HIV-HBV co-infected patients underwent liver transplantation Four patients presented for transplant with LAM-resistant HBV infection (defined by >1 log inc in HBV DNA on LAM therapy). A second nucleoside analogue was added pre-transplant in 4 patients and all but one had undetectable HBV DNA. All patients received HBIG 10,000 units IV interoperatively, then daily for 7days, then monthly. After 12 months post-transplantation, HBIG doses were reduced to 2500-5000 IU monthly to maintain anti-HBs titers >200 IU/L. All recipients concurrently received LAM and tenofovir/adefovir as part of their anti-HBV therapy. Immunosuppression consisted of neoral, cellcept, and prednisone in all patients.

Results: With a mean follow up of 437 days, all pts are alive with functioning grafts and normal LFTs. There have been no episodes of acute rejection or graft losses. All pts were transplanted with HIV VL<1000meq/ml. No pts experienced HIV viremia and all have VL <75. CD4 counts have remained stable. All pts remain HbsAg negative and anti-HBs positive post-LT on prophylaxis with HBIG, LAM, and tenofovir or adefovir. One pt developed polymicrobial sinisutis resolved with antibiotics and surgical drainage.

Conclusions: Patients with HIV-HBV coinfection may successfully undergo liver transplantation without progression of viral disease or opportunistic infections, even in the setting of lamuvidine resistance. Combination therapy using of nucleos/tide analogues and HBIG is highly effective in the prevention of HBV recurrence post-transplantation. There was no deterioration in HIV control or opportunistic infections. These results support liver transplantation as an effective treatment for HIV-HBV coinfected patients with end-stage liver disease.

Abstract# 1498

SIGNIFICANCE OF HEPATITIS B VIRUS GENOTYPE IN LIVER TRANSPLANTATION FOR CHRONIC HEPATITIS B. Chung-Mau Lo,[1] Cindy Cheung,[1] George K. K. Lau,[2] Man-Fung Yuen,[2] Chi-Leung Liu,[1] Sheung-Tat Fan,[1] John Wong.[1] *[1]Department of Surgery, Centre for the Study of Liver Disease, The University of Hong Kong Medical Center, Hong Kong, China; [2]Department of Medicine, Centre for the Study of Liver Disease, The University of Hong Kong Medical Center, Hong Kong, China.*

Hepatitis B virus (HBV) genotype influences the disease profile in chronic hepatitis B but its significance in patients who undergo liver transplantation (LTx) is not known. One hundred and nineteen patients with chronic HBV infection who underwent LTx using lamivudine prophylaxis had HBV genotype identifiable from the pre-transplant sera by direct sequencing technique (genotype A, 1; B, 43; C, 74; D, 1). The baseline characteristics and outcome after LTx of patients with genotype B (M:F, 38:5; median [range] age, 45 [17-66] years) were compared to those with genotype C (M:F, 68:6; median [range] age, 49 [21-61] years). More genotype B patients underwent LTx for acute decompensation with flare of HBV (15 of 43 Vs 9 of 74; p= 0.003) than genotype C, resulting in a higher serum alanine aminotransferase (58 U/L Vs 40 U/L; p=0.003), serum total bilirubin (426 µmol/L Vs 71 µmol/L); p=0.012), international normalized ratio (2.2 Vs 1.6; p=0.001), and MELD score (34 Vs 18; p=0.004). There were more patients seronegative for HBeAg on listing in genotype B (36 of 41 Vs 41 of 73; p=0.001) than genotype C but the difference in seropositivity for HBVDNA was not significant (26 of 40 Vs 34 of 69; p=0.112). The graft survival at 1, 2, and 3 years after LTx were 93%, 83%, and 83% for genotype B and 100%, 93%, and 89% for genotype C (p=0.2), respectively. Two patients of genotype B and 12 patients of genotype C developed mutants with resistance to lamivudine. The mutant-free survival at 1, 2, and 3 years after LTx were 100%, 100%, and 96% for genotype B and 88%, 82%, and 79% for genotype C (p=0.017), respectively. Liver biopsy taken after the development of mutants showed recurrent hepatitis B in 7 of 10 patients of genotype C, including 2 with fibrosing cholestatic hepatitis and no histologic evidence of hepatitis B in both patients of genotype B. Our data suggest that HBV genotypes B and C are associated with different patterns of end-stage liver disease that required transplantation and genotype C carries a higher risk of recurrence with lamivudine prophylaxis.

Abstract# 1499

VALGAN (GANCICLOVIR HYDROCHLORIDE) PROVIDES INEFFECTIVE PROPHYLAXIS AGAINST CYTOMEGALOVIRAL (CMV) INFECTION IN LIVER TRANSPLANT RECIPIENTS. Ashok Jain,[1] Mark Orloff,[1] Kerrie Lansing,[1] Randeep Kashyap,[1] Mark Kelley,[1] Robert Betts,[3] Marilyn Menegus,[2] Bozorgzadeh Adel.[1] *[1]Surgery, Strong Memorial Hospital, Rochester, NY; [2]Pathology, Strong Memorial Hospital, Rochester, NY; [3]Medicine, Strong Memorial Hospital, Rochester, NY.*

Valganciclovir provides ineffective prophylaxis against CMV infection in liver transplant recipients?

Introduction: CMV infection is the commonest viral infection after solid organ transplant transplantation leading to significant morbidity and mortality. Ganciclovir has been proven to be effective to prevent and treat CMV infection. Recently Valganciclovir (Valgan; Gannciclovir Hydrochloride) has been found to have significantly better absorption compared to ganncicloviir (450 mg valgan absorption is equivalent to 3 gm oral ganciclovir absorption and 900 mg valgan equivalent to 5mg/kg intravenous ganciclovir) and has been suggested as prophylaxis for prevention of CMV infection after solid organ transplantation.

Aim of the present study is to examine the efficacy of valgan prophylaxis after primary liver transplantation.

Material Methods: Between July 2001 to May 2003, 203 consecutive post-liver transplant recipients (129 males, 74 females, mean age 53±11 years) received valgan prophylaxis (900 mg/day to 450 mg/every other day depending on the renal function.) for 3-6 months. All patients were followed for a minimum of 6 months. PCR positive CMV in peripheral blood diagnosed symptomatic CMV. [Routine periodic surveillance of blood PCR was not performed]. The rate of CMV infection was stratified according to donor and recipient status of CMV antibodies. GroupI= Donor +/Recipient +, Group II= Donor -/Recipient +, GroupIII= Donor +/Recipient -, and GroupIV= Donor -/Recipient -.

Result: During the follow up period 29(14.3%) patients developed CMV (Positive by PCR in peripheral blood), of these 5 patients had invasive CMV on liver biopsies. The incidence was highest in Group III -14/54(25.9%) and lowest in Group IV- 0/35. In Group I and II, it was 16.4%(12/73) and 7.3% (3/41) respectively. Mean time to symptomatic CMV infection was 169 ±117 (median174) days post transplantation. The rate of CMV for either donor or recipient positive status (Group I, Group II, and Group III) was 17.3% with an invasive CMV hepatitis rate of 3%.

Conclusion: Overall rate of symptomatic CMV was 17.3% in either donor or recipients positive for CMV antibody despite high dose prophylaxis of valcyte, suggesting ineffectiveness of valcyte oral prophylaxis for prevention of symptomatic CMV in liver transplant recipients.

Abstract# 1500

HEREDITARY CCR5-DELTA–32-POLYMORPHISM - A NEW RISK FAKTOR FOR ISCHEMIC TYPE BILIARY LESIONS AFTER ORTHOTOPIC LIVER TRANSPLANTATION. Christian Moench,[1] Anja Uhrig,[1] Gerd Otto.[1] *[1]Transplantation and Hepatobiliary Surgery, Johannes Gutenberg University Mainz, Mainz, Germany.*

Introduction: Ischemic type biliary lesions (ITBL) are a major complication following orthotopic liver transplantation (OLT). In many cases re-OLT is indicated. Multiple factors have been claimed to be reasonable for ITBL; here we present a new immunological risk factor: CC-Chemokines play a key role in the recruitment of leukocytes during ischemia-reperfusion damage and acute rejection. Therefore the CC-chemokine-receptor 5 (CCR5) and its functionless CCR5-delta-32-polymorphism (CCR5d32) might have an influence on the grafts pathology after OLT.

Material and Methods: In 146 patients after OLT the CCR5 was analyzed with regard to the CCR5d32 by PCR. 120 patients (82,1%) showed a normal receptor (wildtype) whereas 26 patients suffered from the CCR5d32 (17,9%) (25 heterozygote, 1 homozygote). ITBL occurred in 14 patients out of 120 with normal CCR5 and in 8 cases out of 26 patients with CCR5d32 (p=0,01). The univariate analysis of donor and recipient conditions (CCR5 wildtype vs. CCR5d32) showed no differences in donor gamma GT (48 vs. 39 U/l p=0,615), donor age (45 vs. 50 years p=0,210), cold ischemia time (529 vs. 566 min. p=0,382), and warm ischemia time (36 vs. 39 min. p=0,333). Acute rejection occurred in 34% in the wildtype group and in 31% in the CCR5d32 group (p=1,000). The CMV-infection rate was 25% in the wildtype group and in 37,5% in the CCR5d32 group (p=0,356). The multivariate analysis showed a significant influence on the development of ITBL by CCR5d32 (p=0,003, OR 9,35).

Conclusion: The hereditary CCR5d32 has a significant influence on the development of ITBL and should therefore be screened before OLT. This can be subclassified new as immunogenetic-biliary-lesions. Ongoing investigations must show if an increased immunosuppression could be beneficial in patients suffering from CCR5d32.

Abstract# 1501

RAPID RECOVERY OF BILE SALT SECRETION IS ASSOCIATED WITH TRANSIENT BILE DUCT INJURY AFTER HUMAN LIVER TRANSPLANTATION. E. Geuken,[1] D. Visser,[1] F. Kuipers,[2] H. G. D. Leuvenink,[1] K. P. de Jong,[1] P. M. J. G. Peeters,[1] P. L. M. Jansen,[3] M. J. H. Slooff,[1] R. J. Porte.[1] *[1]Hepatobiliary Surgery and Liver Transplantation, Groningen University Medical Center, Groningen, Netherlands; [2]Pediatrics, Groningen University Medical Center, Groningen, Netherlands; [3]Gastroenterology and Hepatology, Groningen University Medical Center, Groningen, Netherlands.*

Biliary complications are a serious cause of morbidity and graft dysfunction after orthotopic liver transplantation (OLT). Ischemia and reperfusion play an important role in the occurrence of bile duct injury. Animal studies have indicated that administration of (hydrophobic) bile salts can seriously amplify preservation injury of the biliary tree. Bile salts have potent detergent properties and their cytotoxic effects are antagonized in normal bile by phospholipids. The role of endogenous bile salt secretion and changes in bile composition on the occurrence of bile duct injury, however, has not been studied before. We investigated changes in the secretion rate of bile salts and phospholipids after human OLT and correlated these with biochemical markers of bile duct injury. In addition, expression of bile salt uptake (NTCP, Na+ -dependent taurocholate cotransporting polypeptide) and secretion (BSEP, bile salt export pump) systems, as well as the phospholipid translocator (MDR3, multidrug resistance protein 3), was studied. Three sequential biopsies were taken from liver grafts in 28 patients: at the end of cold storage, 3 hours after reperfusion, and 1 week after OLT. Blood and bile samples were collected daily until 13 days after OLT. Serum concentrations of alkaline phosphatase (ALP) and γ-glutamyltransferase (γ-GT), as well as the release of these enzymes into the bile, were used as markers of bile duct cell injury. After transplantation, bile salt secretion restored rapidly, whereas phospholipid secretion increased at a slower rate, resulting in a significantly higher bile salt / phospholipid ratio in bile formed on day 1 compared to day 13. In parallel with this, NTCP and BSEP mRNA levels increased significantly after OLT, whereas MDR3 mRNA levels remained unchanged, in comparison with gene expression before OLT or in normal control livers. Bile duct cell injury, as reflected by ALP and γ-GT release into the bile, correlated significantly with bile salt secretion. Conclusion: Bile salt secretion is rapidly restored after OLT and is disproportionately higher than the initial secretion of phospholipids. This results in the formation of bile with a relatively high bile salt concentration, which is cytotoxic and was found to correlate with bile duct cell injury. These findings suggest that endogenous bile salts have a role in pathogenesis of intrahepatic bile duct damage after OLT.

Abstract# 1502

LIVER TRANSPLANTATION FOR DRUG INDUCED LIVER INJURY IN THE UNITED STATES. Mark W. Russo,[1] Roshan Shrestha,[1] Michael W. Fried,[1] Paul Watkins.[1] *[1]Medicine, University of North Carolina, Chapel Hill, NC.*

Severe drug induced liver injury (DILI) is an important medical problem and studies of acute liver failure and DILI have included cases that are mostly from acetaminophen overdose and have not reported post-transplant outcomes (Ann Intern Med 202;137:947).

Aim: To identify the drugs most commonly reported to lead to liver transplantation from DILI and to determine if gender and race are associated with liver transplantation for DILI.

Methods: We used the United Network for Organ Sharing liver transplant database to identify recipients who underwent liver transplantation for DILI from 1989- October 31, 2002. Patients were identified if their diagnosis was acute hepatic necrosis from a drug and if they were UNOS status 1 at the time of transplant. Patients were excluded if they had a prior organ transplant, or if they were diagnosed with DILI, but a specific drug was not identified. The text of the database was manually searched to determine if a specific drug was identified as the cause of acute liver failure.

Results: 267 recipients who had a liver transplant for acute liver failure from DILI were identified. The characteristics of the study group are in the Table. The most common cause of DILI from other drugs (n=146) were isoniazid (INH) n=24 (16%), propylthiouracil n=13 (8.9%), phenytoin n=10 (6.8%) and valproate n=10(6.8%). Complementary-alternative medicine was reported in 13 (8.9%) of this group, with Amanita mushrooms accounting for 7 cases. Women were more prevalent then men in both the acetaminophen and other drug groups. Among recipients receiving a liver transplant due to INH, African- Americans were more prevalent than Whites 16% and 5.9%, p=0.03. Liver transplantation for INH DILI was more common in African-American women compared to White women, OR=3.9 [95%CI 1.3, 11.9], p=0.02. One-year graft survival in the acetaminophen group and other drugs were 68% and 71%, respectively, p=0.17 and one-year patient survival was 78% and 71%, respectively, p=0.15.

Conclusions: Young women are the group most commonly affected by DILI leading to liver transplantation. The drugs most commonly associated with DILI and liver transplantation other than acetaminophen are isoniazid, propylthiouracil, phenytoin and valproate. Liver transplantation for INH DILI is most common in African-American women.

	Acetaminophen n=121	Other drugs n=146
Age	32	35
Female	93 (77%)	108 (74%)
African-American	13 (11%)	36 (25%), p<0.001
Creatinine (mg/dl)	3.5	1.6, p<0.001
Bilirubin (mg/dl)	9.9	23.3, p<0.001
Prothombin time (s)	27.7	27.9

Abstract# 1503

RENAL FUNCTION IN LIVER TRANSPLANT (LT) RECIPIENTS: RESULTS FROM MOST, AN INTERNATIONAL OBSERVATIONAL STUDY. Patrizia Burra,[1] Juan Figueras,[2] Robert Jones,[2] Federico Villamil.[2] *[1]Gastroenterology, University of Padova, Padova, Italy; [2]for the MOST International Study Group.*

Renal dysfunction in non-renal transplant recipients has gained major attention recently (NEJM 349:931, 2003). We used data from MOST, a global, observational trial, to investigate the evolution of renal function and the risk factors for renal impairment after liver transplantation.

Methods: MOST collects data from liver transplant (LT) recipients receiving a Neoral-based immunosuppressive regimen under conditions of normal clinical practice, using an internet-based data collection tool. Out of 2304 patients transplanted in 12 countries between 1985 and 2003 and enrolled into MOST (May 2001-August 2003), GFR at one year was available for 1061 and 1 or more subsequent GFR values for 917 patients. Subpopulations were defined by HCV status (pos 584, neg 1124), and by Neoral dose at year one (<3mg/kg/d, 434; 3-5mg/kg/d, 422; >5mg/kg/d, 96). Yearly GFR-loss was calculated as difference between the most recent available GFR-value and GFR at year one, divided by the number of years. A yearly loss exceeding 2.5ml/min was classified as rapid GFR-decline.

Detailed data at 1, 3, 6 and 12 months post transplantation were collected in 711 patients. These were subdivided by GFR at month 1 (≥60, 30-59, or ≤29mL/min) to assess pretransplant renal function in each of these subgroups.

Results are expressed as median with interquartile ranges (IQR).

Results: A sharp decrease in GFR from 88(67-110) to 71(54-91) mL/min was observed within the first month after liver transplantation. Patients with a GFR ≥60mL/min at month 1 had a median pretransplant GFR of 97 mL/min, while those with a GFR of 30-59mL/min at one month showed 68 mL/min pretransplant.

For all later timepoints up to 10 years post LT, median GFR was at 68 -73 mL/min and individual GFR-loss was 0.6 (IQR -2.3 - +3.9) ml/min/year. HCV-pos patients had significantly lower GFR than HCV-neg patients throughout year 1 (at month 12, 64 vs. 74 mL/min, p=0.002) and at year 3 (67 vs. 73, p=0.009) and 5 (67 vs. 73, p=0.005). A low Neoral dose <3mg/kg was given to 48% of the patients with subsequent rapid GFR-decline, and to 38% of the patients with stable GFR.

Conclusions: A decline in renal function was mainly observed within 1 month post LT.

Neoral doses below 3 mg/kg were not associated with more stable renal function. HCV-pos patients had significantly lower GFR than HCV-neg patients. Optimal immunosuppressive strategies for this particularly vulnerable subgroup of LT recipients may be defined on the basis of accumulating prospective follow-up within MOST.

Abstract# 1504

TREATMENT OF CHOLESTATIC AND NON-CHOLESTATIC RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION.

Jun-ichiro Sageshima,[1] Seigo Nishida,[1] Debbie Weppler,[1] Guy Neff,[2] Tomoaki Kato,[1] Juan Madariaga,[1] David Levi,[1] Jang Moon,[1] Gennaro Selvaggi,[1] Phillip Ruiz,[3] Anthony Gyamfi,[4] Andreas Tzakis.[1] *[1]Surgery, University of Miami/Jackson Memorial Hospital, Miami, FL; [2]Hepatology, University of Miami/Jackson Memorial Hospital, Miami, FL; [3]Pathology, University of Miami/Jackson Memorial Hospital, Miami, FL; [4]Anesthesiology, University of Miami/Jackson Memorial Hospital, Miami, FL.*

Background: Recurrence of hepatitis C (HCV) infection after liver transplantation (LT) is universal. Current treatment option has limited efficacy for this disease, and reduction of baseline immunosuppression (ISP) may increase a risk of rejection after LT. In view of possible T cell mediated graft injury after HCV infection, suppression of the activated cytotoxic T lymphocytes has a potential role of the recurrent HCV treatment. **Aim:** To study the efficacy and the safety of a combination regimen with ISP reduction, antiviral therapy, and IL2R-Ab for recurrent HCV hepatitis. **Patients and Methods:** Thirty-six recurrent HCV patients were treated with this regimen between June 2000 and October 2003. Median follow-up was 9.7 months. Diagnosis of recurrence was made by elevated liver enzymes, liver biopsy, and serum HCV-RNA titers. Maintenance steroid was discontinued and tacrolimus was decreased to the target trough level of 5-7 ng/mL. Interferon and ribavirin were used for antiviral therapy. IL2R-Ab was given for 14 weeks (median). **Results:** The treatment was initiated from 1.1 to 100.9 months (median 15.7 months) after LT. Twenty-one patients were cholestatic (bilirubin ≥ 8 mg/dl) and 15 patients were non-cholestatic (bilirubin < 5 mg/dl). Eight cholestatic patients (38%) and 13 non-cholestatic patients (87%) are alive with biochemical improvement. Pre-treatment biopsy revealed superimposed acute rejection in 72% and chronic rejection in 14% of the patients. Histological changes and HCV-RNA levels were inconclusive. There was no flare-up of HCV infection after treatment. All 3 retransplanted patients, 2 for chronic rejections and 1 for recurrent hepatitis C, are alive at 12 to 22 months after retransplantation. Two patients died of infection at 2 and 3 months after treatment, and one patient died of cancer at 18 months after treatment. **Conclusion:** These results suggest that cholestatic recurrent HCV has poor prognosis. The majority of non-cholestatic recurrent HCV patients responds to this regimen. This treatment does not trigger HCV flare-up. Retransplantation is a reasonable option in selected recurrent HCV patients.

Abstract# 1505

ACCELERATED LIVER TRANSPLANTATION FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC) RESULTS IN INCREASED TUMOR RECURRENCE.

Laura Kulik,[1] David Axelrod,[2] Alan Koffron,[2] Patrice Al-Saden,[2] Mary Mulcahy,[2] Elena Crisan,[1] Jonathan Fryer,[2] Lori Clark,[2] Michael Abecassis.[2] *[1]Division of Hepatology, Department of Medicine, Northwestern University, Chicago, IL; [2]Division of Transplant Surgery, Department of Surgery, Northwestern University, Chicago, IL.*

Background: The advent of living donor liver transplantation (LDLT), partial liver transplants (splits), domino liver transplantation, and the MELD upgrade for patients with HCC has resulted in the potential for "fast-tracking" of patients with HCC. We hypothesized that as a result, patients with more aggressive tumor biology, who would normally drop off the list due to tumor progression, might receive an accelerated transplant, thus increasing the recurrence rate.

Aims: To analyze our experience with liver transplantation (OLT) in patients with HCC to determine whether accelerated transplantation has resulted in an increased in HCC recurrence.

Methods: The 315 OLT which were performed from 1995-2003 were divided into two eras and compared on the basis of tumor stage. In **era 1** (9/94 to 2/98) only full-sized OLT were done. In **era 2** (2/98 to 6/03), LDLT, split, and domino transplants were performed in addition to full sized cadaveric OLT. In 2/02, the MELD system was adopted by UNOS, giving patients with HCC higher allocation priority for organs. We classified all transplants as either "accelerated" or "regular" using both the type and date of transplant. All patients have at least 6 months of follow-up.

Results: 74 liver transplants were performed on patients with HCC. Among these patients, 59 had a pre-operative diagnosis of malignancy and 15 had incidental HCC. In era one, 13 patients were transplanted for HCC, 10 were ≤T2 and 3 were ≥ T3. There was one recurrence in a patient with a T3 lesion. In era two, there were 61 patients transplanted for HCC, 47 patients had ≤ T2 disease and 14 patients had ≥ T3. In this group, there were 9 recurrences (5 ≤ T2 and 4 ≥ T3). 41 of 61 patients in era 2 received accelerated transplant (LDLT-10, splits-8, MELD-22, domino-1). There were 9 recurrences in the accelerated transplant group (LDLT-4, splits-2, MELD-2, domino-1). Interestingly, 5 of these recurrences occurred in patients with ≤ T2 tumors. There were no recurrences in the non-accelerated transplant patients during era 2.

	HCC Recurrence		
Stage	ERA 1	ERA2 (Regular)	ERA 2 (Accelerated)
T1 or T2	0/10	0/15	**5/32**
T3 or T4	1/3	**0/5**	4/9
Total	1/13	0/20	9/41

Conclusions: The recurrence rate following transplant for HCC appears to be higher in patients undergoing accelerated transplantation. Further study is needed to identify markers of tumor biologic behavior.

LIVER TRANSPLANTATION: DONORS, PRESERVATION, AND OUTCOMES

Abstract# 1506

NORMOTHERMIC PRESERVATION WITH A NOVEL MACHINE PERFUSATE REDUCES ISCHEMIA/REPERFUSION INJURY IN A PORCINE LIVER MODEL.

James V. Guarrera, Maximillian Polyak, Ben O'Mar Arrington, Jean C. Emond, William T. Stubenbord, Milan Kinkhabwala. *Organ Preservation Unit, New York Presbyterian Hospital, New York, NY; Center for Liver Disease and Transplantation, College of Physicians and Surgeons, Columbia University, New York, NY; Division of Transplant Surgery, Weill Medical College, Cornell University, New York, NY.*

Introduction: We have reported improved graft function in kidneys preserved with Vasosol® preservation solution. Vasosol® utilizes elements of conventional University of Wisconsin (UW) solution with additional vasodilatory and antioxidant components including PGE1, Nitroglycerine and N-acetylcysteine. We modified Vasosol® for normothermic preservation by altering electrolyte composition and adding bovine hemoglobin and enriched tissue media for as substrate for metabolism. We investigated warm oxygenated preservation with this solution in a porcine liver model of ischemia/reperfusion (I/R) injury. **Methods:** 20 miniature swine (16-22kg) underwent standard liver procurement after 120 min of in-situ warm ischemia via pringle clamping and were randomized to 24h UW cold storage (4°C) or 24h normothermic machine perfusion (35-37°C)(NMP) with modified Vasosol®. NMP was performed using a Waters Mox 100 pulsatile perfusion machine modified with a roller pump for portal perfusion and a membrane oxygenator. After the 24h preservation period both groups were reperfused with autologous blood for 8h and compared. Perfusate and bile assays were performed. Transmission electron microscopy was used to evaluate ultrastructural injury. Data are expressed as mean± SD. P values <0.05 were considered significant. Student's T-test and Mann-Whitney U tests were used for statistical analysis. **Results:** Livers preserved with NMP had superior metabolic, synthetic and hemodynamic function compared to the cold stored livers. Ultrastructural damage was less in the warm perfused group.

Parameters- 8 hours post reperfusion	Cold storage (UW)	Normothermic perfusion (Vasosol)	p value
Flow (ml/min/g)	1.07± 0.15	1.43± 0.12	0.02
Resistance (Flow/mmHg)	0.31± 0.04	0.23± 0.02	0.01
Bile production (microL/g)	1.22± 0.34	3.98± 0.4	0.04
AST (IU/L)	8440± 1121	2817± 561	0.01
cAMP (pmol/g)	101± 23	211± 35	0.02
Lactate (mM/L)	9.8± 3.5	4.8± 1.2	0.04
pH	7.33± 0.02	7.39± 0.02	0.04

Conclusions: Normothermic oxygenated preservation with modified Vasosol® significantly decreases I/R injury in a porcine liver model. Clinical studies of this technique are now indicated. Normothermic perfusion may improve early graft function and increase the donor supply by allowing improved results with liver from "marginal" and nonheartbeating donors.

Abstract# 1507

CELSIOR VERSUS UNIVERSITY OF WISCONSIN PRESERVING SOLUTIONS IN LIVER TRANSPLANTATION: A PROSPECTIVE RANDOMISED CONTROLLED STUDY. Agustín García-Gil,[1] Juan I. Arenas,[1] Antonio Güemes,[1] Esteban Esteban,[1] Eloy Tejero,[1] Felix Lamata,[1] Elena Barrao,[1] Trinidad Serrano,[1] Jesús Larraga,[1] Juan J. Araiz,[1] Alfredo Jiménez,[1] Ramón Sousa.[1] *[1]Liver Transplant Unit, Universitary Clinic Hospital, University of Zaragoza, Zaragoza, Zaragoza, Spain.*

Celsior is a high-sodium, low-potassium and low-viscosity extracellular solution recently introduced for abdominal organ preservation. **AIM:** To compare Celsior solution (CS) with gold standard University of Wisconsin (UW) solution in orthotopic liver transplantation (OLT). **METHODS:** From May 2001 to October 2003, 80 adult liver transplant recipients were randomised prospectively to receive either CS (n=40) or UW(n=40). CS and UW infusion volume was 4 and 3 L through aorta respectively, 2 L through portal vein and 1 L at back table through portal vein. All OLT were done with the piggyback technique. **RESULTS:** Both groups were comparable with regard to donor characteristics: age, cause of death, ICU stay, hypotension episodes, cardiac arrest, vasoactive drug requirement, steatosis, billirubin, AST/ALT, INR, and sodium. Cold ischemia time was 380± 96 minutes for CS and 432 ± 148 for UW (p=0.3). No cases of primary non-function and graft dysfunction occurred in both groups. Reperfusion syndrome happened in 2 patients of the CS group and in 6 patients from the UW group (p=0.2). Other major postoperative course parameters in OLT recipients are shown in table 1. Patient actuarial survival at 3 m, 6m, 12 m and 24 m was 93%, 90%, 86% and 86% for the CS group and 93%, 90%, 80%and 80% for the UW group. Graft actuarial survival at 1m, 3 m, 6m, 12 m and 24 m was 94%, 88%, 85%, 78% and 78% for the CS group and 94%, 88%, 85%, 75% and 66% for the UW group. No significant differences were seeing in both groups regarding graft and patient survival. **CONCLUSIONS:** Celsior solution is as safe as the U. Wisconsin solution in liver graft preservation within the cold ischemic time considered. No primary non-function and graft dysfunction occurred with both solutions. Further studies are needed to confirm the same efficacy with longer cold ischemic times.

Intra and Postoperative course parameters in OLT recipients

	Celsior	U. Wisconsin	p
RBC (units)	4.3 ± 2	5 ± 4	0.678
FFP (units)	4.1 ± 3	6.1 ± 5	0.178
ICU stay (days)	4.1 ± 1.5	4.9 ± 2.4	0.237
Postoperative haemorrhage	4	1	0.2
Biliary complications	9	5	0.219
Vascular complications	0	0	-
Death at 30 days	1	1	-

RBC, red blood cells, FFP, fresh frozen plasma

Abstract# 1508

SIGNIFICANT REDUCTION OF PROINFLAMMATORY CYTOKINES BY TREATMENT OF THE HUMAN BRAIN DEAD DONOR. Johann Pratschke,[1] Onur Kuecuek,[2] Lara Mantouvalou,[1] Ronald Krueger,[2] Sascha Weiss,[1] Roman Klemz,[3] Dietmar Horch,[1] Katia Kotsch,[3] Sven Jonas,[1] Claus Wesslau,[2] Hans-Dieter Volk,[3] Peter Neuhaus,[1] Stefan Tullius.[1] *[1]Department of Surgery, Charite, Campus Virchow, Medical School Berlin, Berlin, Germany; [2]Deutsche Stiftung Organtransplantation, DSO Berlin-Brandenburg, Berlin, Germany; [3]Department of Immunology, Charite, Medical School Berlin, Berlin, Germany.*

Experimentally it was proven that the brain death of the donor has a significant impact on graft quality. No data about the correlation between organ specific cytokine expression and the corresponding serum protein levels in human organ donors are available yet. Furthermore it is unknown, wether the upregulation of proinflammatory cytokines can be reduced by donor treatment and therfor the organ quality optimized.

We investigated the expression pattern of cytokines comparing serum (n=53) and tissue expression (n=25) in human brain dead donors. As controls served living donors (n=25). Additionally 41 donors were treated with steroids before organ harvesting (250 mg initial, afterwards 100 mg/h until laparotomy). Hepatic tissue samples were taken immediately after laparotomy of the donor. Transcription rates of tissue cytokines were assessed by RT-PCR. (IL-6, IL-10, CD3, TGFb , TNFa , BAG, HO-1, Mip1a). Serum samples were gathered after declaration of brain death and before laparotomy. The assessment of serum cytokines was performed by CBA-kits.

The transcription of pro-inflammatory cytokines is increased significantly in brain dead donor livers compared to living donor grafts ($p<0.005$). Donor treatment with steroids lead to significantly decreased tissue and serum expression of proinflammatory cytokines ($p<0.01$) and revealed comparable levels to living donors. Tissue levels of cytokines (IL-6, IL-10) correlated strongly with serum levels of corresponding proteines.

Serum protein levels of proinflammatory cytokines are a valuable and easy accessible marker for defining the immunological graft quality. Our data suggest a beneficial effect of antiinflammatory donor treatment in brain dead organ donors. Standardized donor treatment regimens should be established.

Abstract# 1509

LIVER TRANSPLANTATION FROM NON-HEART-BEATING DONORS: A UNOS ANALYSIS. Yong W. Cho,[1] Rick Selby,[1] Tse-Ling Fong.[1] *[1]Dept. of Surgery, USC Keck School of Medicine, Los Angeles, CA.*

Background: The number of liver transplants from non-heart-beating donors (NHBD) has recently increased in the United States, despite the fact that the patient and graft survival rates are inferior to those from heart-beating donors (HBD). The objectives of this study are to evaluate the transplant outcome from NHBD and the impact of this procedure on the donor pool.

Methods: From Jan, 1994 to Sep, 2003, 355 hepatic allografts from NHBDs were reported to the UNOS. For comparison, we selected 39,094 heart-beating cadaveric and 2,124 living donor (LD) liver transplants during the same period. Partial or split-liver transplants from cadaveric donors were excluded from this study. The log-rank test was used for comparison of two survival curves.

Results: Patient and graft survival of NHBD liver transplants were statistically significantly inferior to those of HBDs ($P=0.01$ for patient and $P<0.001$ graft survival) or LDs ($P<0.001$ for both patient and graft survival). Six percent (n=22) of hepatic grafts from NHBDs were lost due to primary graft failure compared with 4% of HBD ($P=0.06$) and 4% LD ($P=0.06$). Three percent (n=10) of grafts from NHBDs lost resulted from biliary tract complications compared with 1% of HBD ($P<0.001$) and 1% LD ($P=0.03$). Donor warm ischemia time (DWIT) longer than 30 minutes or cold ischemia time (CIT) longer than 10 hours had a deleterious effect on graft survival when compared with shorter DWIT (≤30 min, $P=0.05$) or CIT groups (≤10 hrs, $P=0.03$). We divided NHBD liver transplants into low-risk grafts with DWIT ≤ 30 min and CIT ≤ 10 hrs (n=190) and high-risk grafts, n=166. Significantly superior graft survival was noticed in the low-risk group (84% and 79% at 1- and 3-yr, respectively) compared with the high-risk group (60% and 52% at 1- and 3-yrs, respectively, $P=0.03$). Seven primary graft failures (4%) were observed among the low-risk NHBD group. One- and 3-year patient survival of the low-risk NHBD group were 84% and 79%, respectively, compared with 85% and 78% of HBD group ($P=0.54$). Graft survival of the low-risk NHBD group (75% at 1- and 65% 3-yr) was not statistically significantly inferior to LD group (78% at 1- and 71% 3-yr, $P=0.06$). Inevitable DWIT and subsequent cold ischemia-reperfusion injury may result in higher incidence of primary graft failures and biliary tract complications of NHBD liver transplants. However, transplant outcomes can be improved by shortening the DWIT and CIT of NHBDs.

Conclusion: NHBD may be used to increase the cadaveric donor liver pool especially when those grafts can be performed in a setting that is low risk.

Abstract# 1510

UTILIZATION OF LIVERS FROM CONTROLLED NON-HEARTBEATING DONORS (NHBD)- A SINGLE CENTER EXPERIENCE ON 55 LIVER TRANSPLANTS OVER A 10-YEAR PERIOD. Bijan Eghtesad,[1] Kusum Patel,[1] Michael DeVera,[1] Nogc Thai,[1] Deanna Blisard,[1] Kyle Soltys,[1] Paulo Fontes,[1] Thomas Cacciareli,[1] Timothy Gayowski,[1] Wallis Marsh,[1] Anthony Demetris,[2] Amadeo Marcos,[1] John Fung.[1] *[1]Surgery, University of Pittsburgh, Pittsburgh, PA; [2]Pathology, University of Pittsburgh, Pittsburgh, PA.*

Aim: To assess clinical outcomes using livers from controlled NHBD in the era of organ shortage with report on survival and complications on the largest single center experience with up to10 years of follow-up.

Patients and Methods: 55 liver transplants performed in 54 patients from NHBD between 1994-2003. Donors ranged between 4-68 years old (mean 41±16), with arrest to cross-clamp time of 6±4.7 minutes. Organs were flushed with Viaspan (41) or HTK (14) solutions using aortic cannulation. During this period 26 livers were discarded for documented anatomic and histologic reasons. The age of the recipients ranged from 27-69 years (51±10). Since mid-2002, we accepted NHBD livers recovered by other programs. 13 livers were imported (three transcontinental) and 9 transplanted.

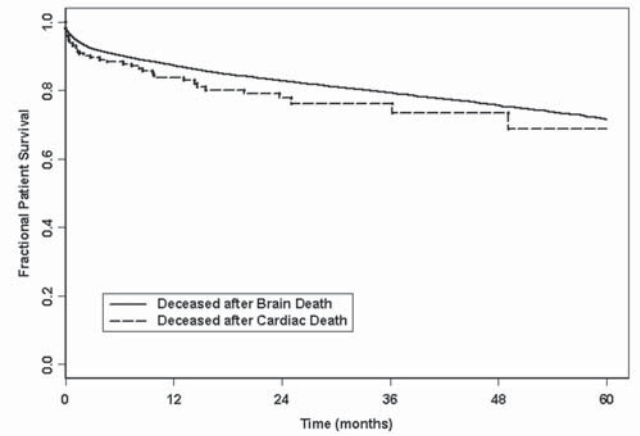
Results: Cold ischemia time was 358-862 (578±149) minutes. Primary Non-Function (PNF) occurred in 6 patients (11%). Four retransplanted and two survived, two other died of sepsis. Hepatic artery thrombosis (HAT) occurred in 6 (11%) patients; three retransplanted and one survived; two died without re-transplantation and one survived with reconstruction. Biliary strictures or leaks were seen in 10 (18%) patients which four were irreversible (two required retransplantation, one died before re-transplantation and one re-listed). Nine patients were re-transplantaed for HAT (3), PNF (4) or biliary complications (2). Livers from NHBD were used to re-transplant 5 emergency patients and three survived. Of the 8 livers recovered from donors 60 years and older, only 2 were utilized - one died of PNF and the other experienced HAT. Overall mortality in the group was 13 patients with 1-, 5-, and 10-year patient and graft survival of 78%, 76%, 76%, and 69%, 67% and 67% respectively. Patient survival in the periods 93-97 and 98-2003 were 45% and 85% respectively. Organs procured by other programs did not show any difference in function or complications.

Conclusion: The rate of PNF and major biliary complications was significantly higher than organs from heartbeating donors, leading to lower early graft survival, although long-term survival was not different. Better management of these potential donors have resulted a better outcome. Thus, controlled NHBD remain an acceptable source of organs in the era of organ shortage.

Abstract# 1511

SHORT AND LONG TERM OUTCOME OF LIVER TRANSPLANTS FROM DONORS AFTER CARDIAC DEATH (DCD). Mona D. Doshi,[1] John D. Rosendale,[2] Lawrence G. Hunsicker.[3] *[1]Internal Medicine, University of Iowa College of Medicine, Iowa City, IA; [2]Research Department, UNOS, Richmond, VA.*

Given the shortage of deceased donor livers for transplantation, new sources of livers must be sought. Livers from DCD donors have been used since 1993, but there is some reluctance to use them due to concerns about short and long term outcomes. Of 30,123 adult recipients of primary deceased donor liver only transplants in U.S. between 1/1/1998 – 6/30/2002, 710 (2.4%) were from the DCD donors and 29,413 from donors after brain death (DBD). Patient and graft survival associated with use of DCD livers was compared to those of recipients of DBD donors. The Kaplan-Meier patient survival curve estimates are shown in the Figure. Patient survival did not differ significantly between DCD and DBD recipients (DCD: 93%, 84%, and 73% vs. DBD: 95%, 87%, and 76% at 30 days and 1 and 4 years respectively; p = 0.17). Graft survival was slightly but significantly less good with DCD donors (DCD: 90%, 76%, and 64% vs. DBD: 92%, 83%, and 69% at 30 days and 1 and 4 years respectively; p = 0.02). Cox proportional hazard models showed no significant difference in patient survival by deceased donor type after correction for recipient creatinine, bilirubin, age, and patient status at transplantation, and cold ischemia time (p = 0.08). The Cox analysis confirmed an increased hazard of graft loss (rel risk 1.5, p = 0.001) with use of DCD livers. Although cold ischemia time is an independent predictor of both patient and graft survival, there was no interaction between these two variables (p = 0.34 and 0.08 respectively). Similarly, there was no interaction between donor liver source and patient Status 1 at the time of transplant (p = 0.40 and 0.19, for patient and graft survival, respectively). There is no apparent need for an altered allocation method for livers from DCD donors. Thus our study demonstrates reasonably good short and long term outcomes associated with the use of livers from DCD donors and supports increased efforts to retrieve these livers.



Abstract# 1512

LONG-TERM SUCCESS FOLLOWING LIVER TRANSPLANTATION USING OLDER CONTROLLED NON-HEART-BEATING DONORS : THE LARGEST REPORTED EXPERIENCE. David J. Reich,[1] Jorge A. Ortiz,[1] Abdaal Khan,[1] Khristian Noto,[1] Victor A. Araya,[1] Kenneth D. Rothstein,[1] Santiago J. Munoz,[1] Cosme Manzarbeitia.[1] *[1]Liver Transplant Program, Albert Einstein Medical Center, Philadelphia, PA.*

PURPOSE: Although 1 group reported short-term success with older controlled non-heart-beating donor (CNHBD) liver transplantation (LTX), several groups have published that they do not use livers from CNHBDs >45 yr old; there are concerns that older donor livers are prone to warm ischemic damage, especially excessive post-LTX biliary complications.

METHODS: We reviewed the long-term experience with all CNHBD livers that we procured and transplanted, and compared results for older and younger CNHBDs (≥50 vs <50 yr old). NHBDs underwent withdrawal of support in the O.R. Organs were rapidly recovered without premortem cannulation techniques.

RESULTS: CNHBDs provided 20/353 (6%) of our cadaver LTXs (8/96 - 9/03). CNHBD recipients have f/u of 40±28 months (15 pts have >2 yr f/u). 7/20 CNHBDs (35%) were older donors (56±6 yr; r 50-66) and 13/20 were younger CNHBDs (23±8 yr; r 11-39). There were no other significant differences between the baseline characteristics of older and younger CNHBDs or the respective recipients:

BASELINE CHARACTERISTICS

	OLD CNHBD	YOUNG
CNHBD		
CNHBD age (mean years)	**56**	**23**
terminal ALT (mean IU)	85	99
CNHBD warm ischemia (mean minutes)[a]	15	14
cold ischemia (mean minutes)	573	567
recipient age (mean years)	52	47

[a]defined as time after withdrawal, from hypotension (MAP<50) until flush.

CNHBD livers from both older and younger donors functioned excellently in both the short and long-term:

RESULTS

	OLD CNHBD (n=7)	YOUNG CNHBD (n=13)
intraop blood (mean units)	6	7
reperfusion syndrome	1	0
poor early function	0	1
PNF	0	0
HAT	0	0
LOS (mean days)	11	14
biliary complications	0	2[a]
K-M survival[b]	100% at 1 yr; 67% at 4 yr	85% at 1 yr; 68% at 4 yr
F/U (mean months)	48	36

[a]2 pts developed ischemic strictures & sepsis; 1 case occurred 4 yr post-LTX, from iatrogenic HAT after balloon angioplasty for HAS. [b]patient and graft survivals were the same.

6/20 recipients died: 2 from the biliary complications (4th mo, 4th yr) and 4 from causes unrelated to the grafts (perforated bowel 2nd mo, MI 2nd yr, noncompliance 3rd yr, trauma 3rd yr).

CONCLUSIONS: This series of CNHBD LTXs is among the largest published and provides long-term results about older CNHBDs. Both older and younger CNHBDs can offer safe donor pool expansion and acceptable long-term outcomes. These recipients of older CNHBD livers have not developed biliary complications. Donor age >50 should not serve as an absolute contraindication to CNHBD LTX.

Abstract# 1513

OBESE DONORS FOR LDLT DO NOT HAVE AN INCREASED RATE OF SERIOUS POSTOPERATIVE COMPLICATIONS. Jennifer L. G. Moss,[1] Dianne Lapointe-Rudow,[1] Jean C. Emond,[2] Milan Kinkhabwala,[2] Doug Marotta,[1] Robert S. Brown, Jr..[1] *[1]Medicine, Columbia University Medical Center, New York, NY; [2]Surgery, Columbia University Medical Center, New York, NY.*

Introduction: Despite the advent of living donor liver transplantation (LDLT), the number of liver transplantations remains limited. Current guidelines indicate that donors (LD) should have a body mass index (BMI) <30, despite lack of evidence that BMI correlates with steatosis or outcome. Obesity is an epidemic; locating nonobese LD may become more difficult.

Hypothesis: Selected donors with a 40>BMI>30 undergo LDLT without an increased the rate of serious complications.

Methods: 68 LD with normal LFT's and without significant comorbidities underwent LDLT from 4/99 to 8/03. Data on postoperative complications in LD and recipients was collected. Postoperative complications for LD were divided into 3 groups: 1) minor complications occurring more often in obese patients (wound infection, pneumonia, and hernia); 2) other minor complications occurring at equal rates in obese and non-obese patients (fever); and 3) major complications (biliary leak, biliary stricture, portal vein thrombosis, bleeding, hepatic dysfunction, and thrombocytopenia). Complication rates for LD with BMI>30 and BMI<30 were compared.

Results: 68 LDLT'S were performed; 65 LD had right lobectomies, and 3 LD had left-sided procedures. 16 LD had BMI>30 and 52 had BMI<30. Major complications, as defined above, occurred in 17% of LD with BMI<30 and 6% of LD with BMI>30 (p=0.43). Rates of wound infection and hernia increased with BMI. 19% of LD with BMI<30 and 25% of LD with BMI>30 had incisional hernias (p=0.73); 3% of LD with BMI<30 and 31% of LD with BMI>30 had wound infections (p=0.006) In both groups, 13% of patients had other minor complications. No LD died. Mortality rates were 0% for recipients with livers from LD with BMI>30 and 21% for recipients with livers from LD with BMI<30, at 0.25-4.6 yrs of follow-up (p=0.055). Of 6 LD with preoperative liver biopsies, 1 had a BMI>30. No patients were excluded based on biopsy. Two morbidly obese LD (BMI>40) successfully donated livers, with a 0% mortality and a 0% serious complication rate.

Conclusions: LD with BMI>30 do not experience a significantly different rate of serious complications when serving as LDLT donors. There is a significantly increased rate of wound infections, known to be associated with obesity and treatable. Updating current guidelines to include selected LD with BMI>30 has the potential to increase the donor pool, without a marked increase in risk. The value of a liver biopsy looking for steatosis requires evaluation.

Abstract# 1514

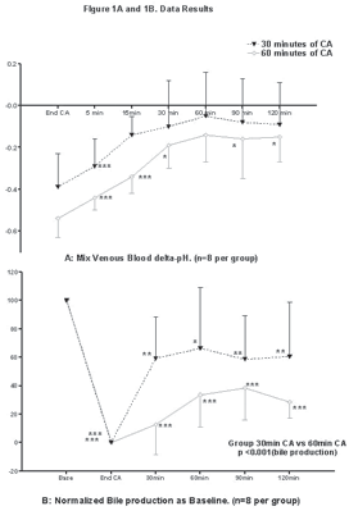
ASSESMENT OF LIVER FUNCTION DURING EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) IN THE NON-HEART BEATING DONOR (NHBD) SWINE. Alvaro Rojas,[1] Ling Chen,[2] Robert H. Bartlett,[3] Juan D. Arenas.[1] *[1]General Surgery Department Division of Transplantation, University of Michigan Health System, Ann Arbor, MI; [2]Physiological Genome Core, University of Maryland, Baltimore, MD; [3]General Surgery Department Division of Critical Care, University of Michigan Health System, Ann Arbor, MI.*

Recently, different programs have been using Non-Heart Beating Donors (NHBD) as an option to decrease the gap between organ demand and supply. Although excellent results have been reported in renal transplants using NHBD organs, the use of livers is still limited, due to warm ischemia (WI), biliary tree injury and inadequate organ assessment. Warm blood reperfusion using ECMO can be a suitable way to reduce WI in organs from NHBD, allowing to determinate hepatic flow characteristics and bile production, facilitating the assessment of organ viability.

Methods: This work evaluates the use of warm blood veno-arterial ECMO reperfusion in NHBD swine. Systemic and hepatic hemodynamics, bile, urine, and mixed venous blood were measured. After baseline data collection, 10kU heparin was given intravenously followed by 1g KCl to elicit cardiac arrest (CA). ECMO was started after 30 or 60 minutes of CA and kept running for 120 min.

Results: One-way RM ANOVA with Turkey test analysis was used within a group. Two-way ANOVA was used between groups. ECMO can restore venous SO_2 and pH in both groups; the values were close to baseline in the 30 minutes CA group. Also, in this group, bile production was >65% from baseline early during reperfusion and its value was lower in the longer CA group <55% ($p<0.001$). AST was doubled at the end of ECMO support in the 60 minutes CA group. ($p<0.05$)

Conclusion: In this pre-heparinized NHBD swine model, ECMO support restores liver perfusion, oxygenation and bile production after 60 minutes of CA in an efficient fashion. Quantification and analysis of bile production could be a determinant of liver function during ECMO support, and it may be a predictor of graft viability before transplantation. Future studies that involve liver transplantation are required to determine the protective effect of warm-preconditioning using ECMO support in the NHBD.



PEDIATRIC KIDNEY

Abstract# 1515

LAPAROSCOPIC (LAP) LIVE DONOR NEPHRECTOMY (DN) IS A SIGNIFICANT RISK FACTOR FOR DELAYED GRAFT FUNCTION (DGF) AND ACUTE REJECTION IN PEDIATRIC RENAL TRANSPLANTATION (PedKtx): A UNOS ANALYSIS. Christoph Troppmann,[1] M. A. McBride,[2] T. Baker,[2] R. V. Perez.[1] *[1]Dept. of Surgery, Univ. of California, Davis, Sacramento, CA; [2]UNOS, Richmond, VA.*

Lap (vs open) DN has been associated with slower early kidney graft function in *adult* live donor Ktx (*Am J Tx 3:1295, 2003*). In *pediatric* Ktx, grafts from adult donors face, in addition to heightened immunoreactivity, significant hemodynamic challenges. We hypothesized that any adverse functional or immunologic impact of Lap procurement might therefore be exacerbated in PedKtx, and reviewed all live donor PedKtx (LapDN or OpenDN [flank incision]) reported to UNOS 1/00-6/03. **RESULTS:** In 2003, 66% of all live DN for PedKtx were Lap (vs 37% Lap in 2000). Outcomes of Ktx done 1/00-6/02 were analyzed for two age groups (0-5 yr [n=212; 44% LapDN] and 6-18 yr [n=778; 49% LapDN]). Demographics were similar within both groups for Lap vs OpenKtx. DGF (ie, dialysis during the first week postTx) and rejection (ie, any treated acute rejection episode) rates were:

RECIPIENT GROUP		DGF	Rejection Incidence at 6 mo	Rejection Incidence at 1 yr
0-5 yr	OpenDN (n=118)	2.5%$^{+}$	5.9%$^{\#}$	7.9%$^{\wedge}$
	LapDN (n=94)	12.8%$^{+}$	18.6%$^{\#}$	24.3%$^{\wedge}$
6-18 yr	OpenDN (n=394)	2.8%$^{@}$	15.6%*	22.7%
	LapDN (n=384)	5.9%$^{@}$	22.5%*	28.1%

Pairwise comparisons (Chi-square): $^{+}$:p=0.004; $^{@}$:p=0.03; $^{\#}$:p=0.01; *:p=0.03; $^{\wedge}$:p=0.004.

Other outcomes, including creatinine levels and graft survival, were similar for Lap and OpenDN Ktx. We studied impact of procurement mode, age, and other potential risk factors on rejection by multivariate analysis:

COVARIATE	Relat. Risk for Reject. at 6 mo		Relat. Risk for Reject. at 1 yr	
	Odds Ratio	*p*-Value	Odds Ratio	*p*-Value
Recipient Age (0-5 vs 6-18 yr)	**0.50**	**0.01**	**0.49**	**0.004**
Recip. Ethnicity (Nonwhite vs White)	1.19	0.38	1.08	0.68
PreTx Dialysis (Yes vs No)	**1.68**	**0.02**	**1.51**	**0.03**
LapDN vs OpenDN (Yes vs. No)	**1.73**	**0.005**	**1.53**	**0.01**
DGF (Yes vs No)	**5.34**	**<0.0001**	**4.49**	**<0.0001**
Antibody Induction (Yes vs No)	0.51	0.56	0.38	0.4

SUMMARY: In an era of increasing frequency of LapDN for PedKtx, Lap procurement is associated with a significant increase in DGF and is an independent risk factor for acute rejection. **CONCLUSIONS:** Our results underscore the importance of renoprotective donor management (eg, volume loading) during LapDN to maximize early Ktx function. Future investigations must (i) determine mechanisms by which LapDN adversely affects immunologic PedKtx outcome and (ii) identify potential strategies to optimize procurement, including the option of OpenDN in selected donors.

Abstract# 1516

LAPAROSCOPIC VS OPEN DONOR NEPHRECTOMY (ODN) IN PEDIATRIC (peds) KIDNEY (KD) TRANPLANTS (Txs). Raja Kandaswamy,[1] Miguel Tan,[1] Abhinav Humar,[1] Rainer W. G. Gruessner,[1] David E. R. Sutherland,[1] Arthur J. Matas.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Background: LDNs are being performed widely in adult KD Tx. However, in peds KD Tx, concerns over delayed graft function, increased acute rejection and technical complications have limited the application of this procedure. We report our experience with LDN (hand-assisted) for peds KD Tx and compare it with ODN. Since Dec 1, '97, we have performed 75 peds KD Tx (45 ODN and 30 LDN). LDN donors were older than ODNs (39±8 vs 35±9) (p=0.03), but they were not different in BMI (26±3 vs 26±5), gender (60% M and 40% F in both groups), parent to child relationship (60% vs 75%) and Caucasian race (87% vs 96%). LDN recipients were older than ODNs (12±4 vs 7±6) (p=0.008), and not different in gender (40% F, 60%M vs 42%F, 58%M), BMI (19±5 vs 21±8) single vessels (93% vs 91%), left kidney (90%vs 78%) and intraperitoneal implantation (33% LND vs 53% ODN) (p=0.1). Induction was with ATG or Thymoglobulin and maintenance with CSA/MMF/steroids. Actuarial 1-year outcomes are presented.

Results: Donor outcomes were similar except length of stay, which was shorter in LDN vs ODN (3.6±1 vs. 4.1±2). Recipient graft (91% vs 89%) and patient survival (95% both groups), delayed graft function 8.1% vs 8.9%, and acute rejection rate 13% vs 27% (p=0.07) were no different between LDN vs ODN. There were 2 graft losses (both recurrent disease) in LDN and 4 (3 technical and 1 late function) in the ODN group. 1 sudden death at 6 months, and in the ODN group, 2 deaths from respiratory failure after 6 months. Creatinine was higher in the LDN group at all time points (table).

Outcomes

Creatinine	LDN	ODN	p value
3 months	1.07 (±0.4)	0.75 (±0.4)	0.006
6 months	1.33 (±0.84)	0.82 (±0.42)	0.01
9 months	1.26 (±0.59)	0.84 (±0.40)	0.01
1 year	1.56 (±1.22)	0..99 (±0.67)	0.01

Conclusion: LDN can be performed in pediatric transplants with donor and recipient outcomes comparable to ODNs. LDN donors have a shorter hospital stay. LDN recipients tended to have a higher creatinine. Learning curve was not a factor since significant LDN experience had been achieved prior to introducing it to peds Tx. The higher creatinine may be due to the older and larger LDN recipients vs ODNs. Longer followup with a larger cohort is necessary to verify this.

Abstract# 1517

A STUDY ABOUT THE SAFETY AND TOLERABILITY OF THE CONVERSION TO ENTERIC-COATED MYCOPHENOLATE SODIUM FROM MYCOPHENOLATE MOFETIL IN STABLE PEDIATRIC RENAL TRANSPLANT RECIPIENTS. E. David-Neto,[1] A. Turconi,[2] F. Giron,[3] M. Adragna,[2] A. Otero,[4] M. L. Pereira.[1] *[1]Htal das Clinicas, Sao Paulo, Brazil; [2]Htal Garrahan, BA, Argentina; [3]Clin Marly, Bogota, Colombia; [4]Novartis SA, Argentina.*

Introduction: Mycophenolate Mofetil (MMF) is widely used in acute rejection (AR) prophylaxis, in organ transplantation, with verified efficacy. Long term application of MMF has been proven safe in children and young adults. Discontinuation or dose adjustments of MMF due to tolerability problems constitute a risk factor increasing the incidence of AR. Enteric-coated mycophenolate sodium (EC-MPS, myfortic®) was designed to improve gastrointestinal (GI) tolerability. EC-MPS 720 mg is equimolar to MMF 1000 mg. **Materials and Methods:** An open, 6-month, multicenter, prospective trial has been conducted to demonstrate the safety and tolerability of the conversion from MMF to EC-MPS among stable renal transplant patients. In the setting of a trial involving several countries, three sites enrolled children. A total of 19 pediatric patients aged 14.2±3.0 years were included, 13 Caucasians, 1 African American, and 5 other, 12 males and 7 females, mean post tx time 3.6±3.1 years. The children were receiving MMF at a dose of 457±147 mg/m² b.i.d. (manufacturer suggested dose 600 mg/m² b.i.d), and were converted to 432±51 mg/m² b.i.d of EC-MPS, which represents a higher amount of mycophenolic acid per m². Patients were also treated with Neoral (mean C2levels 764±400 ng/ml), and steroids as per center standard of practice. **Results:** After the conversion, the incidence of any adverse event (AE) was 63% , 42% gastrointestinal (GI) AEs: 16% upper GI, and 21% diarrhea. The incidence of infections was 52.6%. Neither CMV infection/disease episodes, nor hematological AEs were reported. No patient discontinued the study medication. All safety laboratory parameters remained stable throughout the trial as shown in the table:

	Hb(g/L)	WBC (10^3 cells/mm³)	Neutrophils (10^3cells/mm³)	Platelets (10^3 cells/mm³)	SCreatinine (mg/dl)
Baseline (pre-conversion)	11.62±1.6	8.77±2.52	6.03±1.74	308±80	1.21±.27
Month 6 (EC-MPS)	11.48±1.47	8.67±2.92	6.4±1.33	323±87	1.16±.26
p	.44	.87	.42	.30	.19

Only 4 (21%) patients required dose reduction. AEs subsided upon dose reduction. There were no episodes of acute rejection, death or graft loss.

Conclusions: The conversion to EC-MPS from MMF was safe in this pediatric population. No study drug discontinuation was observed. EC-MPS may be an alternative for stable pediatric renal transplant recipients receiving MMF.

Abstract# 1518

EVEROLIMUS IN *DE NOVO* PEDIATRIC RENAL TRANSPLANTATION: RESULTS AT 24 MONTHS. R. Ettenger,[1] V. M. Moeller,[2] P. F. Hoyer,[3] J. Lemire,[4] J. Mahan,[5] N. Webb,[6] P. Niaudet,[7] J. Jaffe.[2] *[1]Dept. of Pediatric Nephrology, UCLA, Los Angeles, CA; [2]Transplant and Immunology, Novartis Pharmaceuticals, East Hanover, NJ; [3]Zentrum fur Kinderheikkunde, Universitaetsklinikum Essen, Hufelandstr. 55, D-45147 Essen, Germany; [4]Pediatric Nephrology, UC San Diego, San Diego, CA; [5]Dept. of Nephrology, Childrens' Hospital, Columbus, OH; [6]Royal Manchester Children's Hospital, Pendlebury, Manchester M27 4HA, United Kingdom; [7]Service de Nephrologie Pediatrique, Hopital Necker, F-75473 Paris, Cedex 15, France.*

Everolimus is an investigational proliferation signal inhibitor shown to have potent immunosuppressive effects and excellent kidney allograft outcome in adults when combined with cyclosporine microemulsion (CsA-Me). Preliminary data in a *de novo* pediatric patient cohort were promising but information about longer-term outcome is necessary to evaluate the safety, tolerability and efficacy of everolimus in combination with CsA-Me and prednisone. The average starting dose of everolimus was 0.8mg/m²bid (1.6mg/m²/ day). A total of 19 patients were enrolled and stratified by age into 2 groups: 10 patients < 10 years old [Group I] and 9 patients 10-16 years old [Group II]. The mean ages at the time of transplant in Group I and II were 5.7 and 13.2 years respectively. At 24M, 5 patients (50%) in Group I and 7 patients (78%) in Group II were continuing to receive everolimus. Only 3 patients, 2 in Group I, discontinued everolimus because of an adverse event: one due to a urine leak and a second, with an uncomplicated EBV infection, due to an investigator's decision to reduce immunosuppression. The third discontinuation occurred when a patient in Group II experienced a severe acute vascular rejection. There were no graft losses or deaths. Overall, 5 of the 19 patients (26.3%) experienced a biopsy-proven acute rejection episode (BPARE). One patient in Group I and 4 in Group II had BPAREs over the observation period. At 24M, the mean average daily doses of everolimus in Groups I and II were 1.45 and 1.55 mg/m²/day respectively; doses of CsA-Me were 4.7 and 2.3 mg/kg/day and doses of prednisone were 0.1 and 0.2 mg/kg respectively. Adverse events or infections included lymphoceles (1 in Group I and 2 in Group II), urinary tract infections/pyelonephritis (6 in Group I and 3 in Group II), hypercholesterolemia (2 in Group I and 1 in group II) and hypertriglyceridemia (1 in Group I and 2 in group II). As E interferes with testosterone synthesis in preclinical models, blood samples, to obtain additional information relative to endocrine issues, were collected from 9 patients. Testosterone (males only) and FSH and LH showed no abnormal trends when corrected for patients' pubertal development. We conclude that everolimus at a starting dose of 0.8-mg/m² bid is well tolerated in pediatric patients through 2 years post-transplant, with good patient and graft survival.

Abstract# 1519

CAMPATH-1H USE IN PEDIATRIC RENAL TRANSPLANTATION. Sharon M. Bartosh,[1] Hans W. Sollinger.[2] *[1]Pediatrics, University of Wisconsin, Madison, WI; [2]Surgery, University of Wisconsin, Madison, WI.*

Campath-1H is a humanized, monoclonal antibody directed against CD52 determinants on the surface of human B- and T-cells and monocytes. Preliminary reports of Campath-1H use as induction in adult renal transplantation have been encouraging with low rejection rates and minimal adverse side effects. We report 3 pediatric kidney transplant patients who received Campath-1H for unique indications with variable results. Patient #1 was a 10 yr old female who had lost 2 prior cadaveric transplants to poorly controlled, progressive acute rejection episodes. She received a third cadaver kidney (zero HLA match), 1 dose of Campath-1H along with prednisone, mycophenolate and tacrolimus. She is now 7 mos post transplant and has experienced no rejection, or PTLD and has a creat of 0.7 mg/dl. She was treated at 6 mos post transplant for herpes zoster. Patient #2 is a 20 month old male with ESRD secondary to Factor H deficiency-associated atypical HUS. He received a cadaveric kidney, 1 dose of campath-1H, prednisone and mycophenolate mofetil. He experienced severe, antibody-mediated rejection on POD# 21. Rejection was controlled with a combination of corticosteroids, plasmapheresis, thymoglobulin, rituximab and immune globulin. Sirolimus was begun after the rejection was reversed. 6 months post transplant, he has stable renal function (creatinine 0.2 mg/dl) with no infection or PTLD. He has had no hematologic evidence of recurrence of HUS. Patient #3 is a 15 yr old with ESRD secondary to FSGS. She received a cadaveric renal transplant, 1 dose of Campath-1H, prednisone, cyclosporine and mycophenolate mofetil. She experienced immediate recurrence of her FSGS as well as antibody- mediated rejection POD # 35. She was treated with plasmapheresis, thymoglobulin and higher doses of calcineurin inhibitor. Three months post transplant, she has a stable creatinine of 0.9 mg/dl but ongoing heavy proteinuria with random urinary protein:creatinine ratio of 3. Serial flow cytometry was performed on all 3 patients. The patient who recieved only Campath-1H has an absolute lymphocyte count that remain less than 50% of baseline at 7 months post transplant. In addition, CD3, CD4, CD8 and CD20 remain less than 50% of baseline. Conclusions: 1) Use of Campath-1H does not prevent recurrence of FSGS, 2) As seen in adults, lack of calcineurin inhibition when using Campath-1H may increase the risk of antibody-mediated rejection and 3) Prolonged lymphocyte depletion remains even after a single dose of Campath-1H.

Abstract# 1520

DRUG MONITORING OF MYCOPHENOLIC ACID (MPA) IN THE LONG TERM FOLLOW UP OF PEDIATRIC TRANSPLANT RECIPIENTS: TROUGH LEVELS ARE NO GOOD INDICATOR FOR LONG TERM GRAFT FUNCTION. Lars Pape,[1] Jochen H. H. Ehrich,[1] Juergen Strehlau,[1] Gisela Offner.[1] *[1]Department of Pediatric Nephrology, Medical School of Hannover, Hannover, Germany.*

Background: Mycophenolate Mofetil (MMF) has the potential for decreasing acute rejection episodes early after renal transplantation and pharmocokinetic monitoring of MPA trough levels is done by many centers. Mycophenolate Mofetil (MMF) has also been shown to improve long term graft function in patients with chronic allograft nephropathy (CAN), however, no data for long term monitoring of MPA have been published in children.

Methods: MMF therapy with a dose of 600 mg/m²/d was initiated in 42 children (median age 9.4 years, range 1.4-15.1) after a median period of 3.8 years (range 1.0-10.6) post transplantation according to significant increases in serum-creatinine. CAN was diagnosed by renal biopsy and the amount of fibrosis was quantified by PicroSirius Red staining. MMF-therapy was combined with Cyclosporin A and Prednisolone. MPA-C0-levels, measured by HPLC, were taken every 3 months and in 12 children a full MPA - area under the curve concentration (AUC) was measured. The GFR was calculated at the start of MMF therapy and 2 years later.

Results: After initiation of MMF the calculated GFR did not decrease further in 22 children and mean GFR remained stable for 2 years in the whole study group. There was a significant correlation between MPA levels 75 minutes after administration and the full AUC ($r=0.94$, $p<0.001$) but no correlation between trough levels and AUC ($r=-0.07$, $p>0.05$). The mean MPA trough level was 2.8±1.3 ng/ml. The intra-individual coefficient of variation was 2.6±1.4. There was no correlation between mean MPA trough levels and GFR development after two years ($r=0.03$, $p>0.05$) or between MMF-dose and MPA trough level ($r=-0.1$, $p>0.05$). In children with an MPA-level below 1.2 mg/l ($n=5$), the mean GFR was not different from those with a higher level ($p>0.05$).

Conclusions: MMF Therapy had a positive effect on CAN, however, drug monitoring of MPA-trough levels had no impact on long term graft function in kidney recipients. MPA levels taken 75 minutes after administration showed a high correlation with MPA-AUC whereas C0-levels did not correlate. The value of C75 drug measurements for monitoring renal allograft survival will have to be determined in future studies.

Abstract# 1521

INFECTIOUS COMPLICATIONS IN ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION IN CHILDREN. Seiichiro Shishido,[1] Hiroyuki Satoh,[1] Hiroshi Asanuma,[1] Hiroshi Hataya,[1] Masahiro Ikeda,[1] Akira Hasegawa.[2] *[1]Urology and Kidney Transplantation, Tokyo Metropolitan Kiyose Children's Hospital, Kiyose, Tokyo, Japan; [2]Nephrology, Toho University, Ohmori, Tokyo, Japan.*

Background. Due to a severe shortage of suitable cadaveric allografts for children awaiting kidney transplants, we have performed a series of ABO-incompatible living kidney transplantations (LKT) at our institution. It is generally believe that splenectomy is an essential procedure in preventing acute vascular rejection. However, young recipients with splenectomy are at greater risk of serious bacterial infections posttransplant.

Methods. Twenty-three pediatric patients underwent ABO-incompatible LKT between July 1989 and April 2003. The mean age at transplantation was 10.7 ± 4.2 years (range 5.8-17.8 years), with 19 males and 4 females. The donor to recipient ABO blood antigen incompatibility was as follows: A1→O, 7 patients; B→O, 6 patients; A1B→B, 5 patients, A1→B, 3 patients, and A1B →A, 2, patient. All patients received three or four sessions of plasmapheresis and/or immunoadsorption to remove the anti-A and/or anti-B antibodies before transplantation. Immunosuppression initially consisted of cyclosporine, methylprednisolone, cyclophosphamide/MMF, and antilymphocyte globulin. Splenectomy was performed on all recipients at the time of transplantation. In addition to pretransplant pneumococcal vaccine, penicillin prophylaxis was given to patients less than 10 years of age to prevent posttransplant pneumococcal infection.

Results. The patients were followed for 8 to 170 months with a mean follow-up of 78 months. Of 23 patients, 8 recipients (35%) had 11 infectious complications after surgery. One patient developed Klebsiella pneumoniae sepsis at 6 weeks posttransplant, which was treated successfully with antibiotics. Urinary tract infection was also occurred in 2 patients. Cytomegalovirus (CMV) infection was the most frequent infection and occurred in 4 patients (17%). However, no patient had a serious tissue-invasive CMV disease. Herpes zoster infection occurred in two patients and successfully treated with acyclovir. One patient lost his graft because of uncontrolled delayed hyperacute rejection, while the actuarial 1-year, 5-year and 10-year graft survival rates are 96% 91%, and 91%, respectively.

Conclusion. Infectious complications in ABO-incompatible kidney transplantation in children are few and, if any, not severe. Pneumococcal vaccine and the use of long-term penicillin prophylaxis may be effective to prevent pneumococcal infection for young recipients with ABO-incompatible kidney allografts.

Abstract# 1522

HERPESVIRUS DNA IN RENAL ALLOGRAFTS IS ASSOCIATED WITH CHRONIC ALLOGRAFT NEPHROPATHY. Jodi Smith,[1] Connie Davis,[2] Patrick Healey,[3] Meei-Li Huang,[4] Lawrence Corey,[4] Ruth McDonald.[1] *[1]Pediatric Nephrology, Children's Hospital, University of Washington, Seattle, WA; [2]Nephrology, University of Washington, Seattle, WA; [3]Surgery, University of Washington, Seattle, WA; [4]Virology, University of Washington, Seattle, WA.*

Background: Chronic allograft nephropathy (CAN) is the major barrier to long-term graft survival. Increased potency of immunosuppressive therapy has successfully reduced the incidence of acute rejection but has resulted in a higher incidence of viral infections. Viral *disease*, particularly CMV, has been associated with chronic allograft nephropathy and recent evidence suggests a potential role of subclinical viral infection.

Methods: We performed a retrospective case-control study to evaluate for the presence of herpesvirus DNA in renal allografts with CAN. Cases were pediatric renal transplant recipients who had been diagnosed with CAN from 1999 to 2002 based on both clinical and histologic criteria. Controls were patients without CAN who were matched by age and time post-transplant. Real-time quantitative PCR was used to evaluate the viral load of EBV, CMV, HHV-6, and HHV-7.

Results: Five cases and 10 controls were available for analysis. The patient characteristics of cases and controls were similar with respect to age (mean: 14 yrs vs. 16 yrs), time post-transplant (mean: 16 months vs. 20 months), immunosuppression (CSA/MMF/pred: 4/5 vs. 6/10), and CMV and EBV donor and recipient status (CMV D+/R-: 3/5 vs. 6/10 and EBV D+/R-: 4/5 vs. 7/10). The mean creatinine was 2.6 mg/dl in cases and 1.6 mg/dl in controls. None of the patients had clinical evidence of herpesvirus disease. Herpesvirus DNA was detected in 4/5 cases compared to 1/10 controls ($p<0.001$). EBV was the most common virus detected 4/5 cases. More than one herpesvirus was detected in 3/5 cases of CAN.

Conclusion: The presence of herpesvirus DNA in the allograft was associated with CAN in this group of pediatric renal transplant recipients, even in the absence of clinical viral disease. Further evaluation of the potential role of subclinical herpesvirus infection in chronic allograft nephropathy may be important in evaluating the utility of viral surveillance and pre-emptive antiviral therapy.

Herpesvirus DNA in renal allograft biopsy specimens

CASE	CMV*	EBV*	HHV-6 *	HHV-7*
1	-	40250	-	22244
2	-	4885	414722	-
3	481	18083	1776	-
4	-	85622	1650	-
5	-	-	-	-
CONTROL				
1	-	-	1731	63014
2-10	-	-	-	-

*DNA in copies/10^6cells

Abstract# 1523

THE WAXING AND WANING OF CHRONIC ALLOGRAFT NEPHROPATHY GRADES IN PEDIATRIC PROTOCOL BIOPSIES. Patricia E. Birk,[1] Tom D. Blydt-Hansen,[1] Malcolm R. Ogborn,[1] John G. Gartner,[2] Ian W. Gibson.[2] *[1]Pediatrics, University of Manitoba, Winnipeg, MB, Canada; [2]Pathology, University of Manitoba, Winnipeg, MB, Canada.*

The presence of CAN on protocol biopsy (bx) has been used as a primary end-point for clinical trials in renal transplantation. However, the accuracy of chronic histological scoring is confounded by the heterogeneity of CAN development. In pediatric renal allograft recipients undergoing protocol bxs, the variability of Banff chronic scores is unknown. **Purpose:** Using a natural history cohort of pediatric protocol bx patients (pts), we sought to: 1. Define the incidence of bx regression error during CAN surveillance; 2. Determine if 2 sequential bxs can reliably predict a pattern of CAN development. **Methods:** Percutaneous bxs were performed using ultrasound and a 16-gauge needle (2 cores). Tissues were stained with Masson's trichrome. Bxs were evaluated (Banff criteria) by a renal pathologist. Only bxs conforming to Banff specimen adequacy standards were included. Chronic scores and CAN grades were recorded longitudinally and sequential bxs were classified as having no progression, progression or regression. Results were expressed as mean ± SD. **Results:** We studied 149 bxs (15 donor, 92 protocol, 25 acute graft dysfunction and 17 follow-up rejection therapy) from 31 pts on P/FK/MMF (0-95 mo. post-transplant). Pts had 4.8 ± 2.1 bxs. Bxs had 19.0 ± 9.2 glomeruli and 3.0 ± 1.7 arteries. 7/31 (23%) pts with 5.7 ± 5.3 mo. follow-up did not progress, while 24/31 (77%) pts had histological progression (CAN O-I: 4.8 ± 3.5 mo.; CAN I-II: 18.0 ± 11.4 mo.; CAN O-II: 7.0 ± 4.6 mo.). However, 3/7 (43%) pts with no progression and 13/24 (54%) pts with progression had at least 1 bx in which the CAN grade transiently regressed. Because these pts received no specific therapeutic interventions for CAN, their histological regression was attributed to either sampling error or scoring error. Regression errors occurred in a total of 23/149 (15%) bxs, including 15/52 (29%) bxs formerly graded as CAN I and 8/26 (31%) bxs formerly graded as CAN II (I vs. II, p=NS). Among the bxs with sufficient follow-up, 5/7 (71%) with CAN I-O regression and 3/5 (60%) with CAN II-I regression reverted to their previous grades at the next sampling point. The percentages of individually misclassified lesions were: ci (13%), cv (14%), ct (12%), cg (2%) and mm (7%). **Conclusions:** More than 2 protocol bxs may be needed to establish a pattern of CAN development. We recommend that a 15% incidence of bx regression error be applied to power calculations for pediatric clinical trials using CAN as the primary end-point.

STEROID AND IMMUNOSUPPRESSION REDUCTION/WITHDRAWAL

Abstract# 1524

LONG-TERM EFFICACY AND SAFETY OF STEROID-WITHDRAWAL IN TACROLIMUS-TREATED RENAL TRANSPLANT RECIPIENTS: RESULTS OF A 3-YEAR FOLLOW-UP. Julio Pascual,[1] Johannes P. van Hooff,[2] Kaija Salmela,[3] Budde Klemens,[4] Rigotti Paolo,[5] Lang Philippe.[6] *[1]Servicio Nefrologia, Hospital Ramon Y Cajal, Madrid, Spain; [2]Department of Internal Medicine, University Hospital Maastricht, Maastricht, Netherlands; [3]Department of Surgery, University Hospital Helsinki, Helsinki, Finland; [4]Department of Nephrology, Charite Hospital Campus Mitte, Berlin, Germany; [5]Department of Medical and Surgical Sciences, University of Padua, Padua, Italy; [6]Service de Nephrologie et de Transplantation, Hopital Henri Mondor, Paris, France.*

The original 6-month study investigated the withdrawal of steroids and MMF from a tacrolimus triple regimen (Tac/MMF/steroids). In total 833 patients were randomised at month 3 to either the control group (Tac/MMF/steroids, n=277), the steroid-withdrawal group (Tac/MMF, n=279), or the MMF-withdrawal group (Tac/steroids, n=277). The study showed that the withdrawal of steroids at month 3 was associated with a significant reduction in total cholesterol at month 6 (-0.49 mmol/L, $p<0.001$), while the overall incidence of acute biopsy-proven rejection was not different between the groups (17.0%, 15.1%, and 14.8%, p=0.744).

Methods: An investigator-driven follow-up of all patients whether withdrawn from the original study or not was initiated. The centres have been providing data at 1, 2, and 3 years post transplantation. The data have been entered electronically into a case record form via an internet-based system.

Results: By end of 2003 the 3-year data of 538 patients were available (control n=183, steroid stop n=173, MMF stop n=182). Patient survival estimate ranged between 94% and 96%; graft survival estimate ranged between 86% and 90%. The 3-year incidence of late (after month 6) biopsy-proven acute rejection was 0.6% (control), 1.7% (steroid stop) and 1.1% (MMF stop). Biopsy-proven chronic rejection was reported in 3.3% (control), 2.3% (steroid stop), and 3.3% (MMF stop). Renal function assessed as calculated creatinine clearance (CG formula) was similar in all groups with median values of 62.3 ml/min (control), 62.6 ml/min steroid stop, and 63.1 ml/min (MMF stop). Total median serum cholesterol was 4.9 mmol/L (control), 4.5 mmol/L (steroid stop), and 5.0 mmol/L MMF stop. The incidence of new onset insulin-dependent diabetes mellitus was 4.5% (control), 2.8% (steroid stop) and 5.2% (MMF stop). Median diastolic blood pressure was 80 mmHg in all groups; median systolic blood pressure was 132 mmHg in the steroid stop group, and 140 mmHg in the control group and the MMF stop group. **Conclusion:** Reduction of concomitant immunosuppression from a tacrolimus-based regimen is efficacious and safe in the long-term. The incidence of chronic rejection was low and kidney function was well preserved in all treatment groups. The steroid-free regimen showed advantages in terms of the cardiovascular risk factors serum cholesterol, blood pressure and diabetes.

Abstract# 1525

PREDNISONE (P)-FREE MAINTENANCE IMMUNOSUPPRESSION (IS) AFTER KIDNEY TRANSPLANTATION: 92% GRAFT SURVIVAL AT 4 YEARS.

Abhinav Humar,[1] Raja Kandaswamy,[1] James Harmon,[1] J. Keith Melancon,[1] David L. Dunn,[1] Miguel Tan,[1] Arthur J. Matas.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

Recent improvements in IS protocols have resulted in decreased acute rejection (AR). The goals of P-minimization protocols have been to eliminate P-related side effects without increasing AR. However, the concern is that late steroid withdrawal has led to increased AR, chronic rejection (CR), and graft loss. We studied outcome of 412 recipients treated with P discontinuation on POD 6 (296 LD of which 112 were LURD, 116 CAD; 61% male; 91% Caucasian; mean age (±SD)=47±14 yrs; 379 primary, 33 retx). Of CAD, 34% had peak PRA >10, 21% >50; 16% had DGF. IS consisted of polyclonal Ab (Thymoglobulin) for 5 days (1st dose intraop); P (Solu-Medrol 500 mg in OR, 1 mg/kg on POD 1, 0.25 mg/kg on POD 2 & 3; 0.25 mg/kg on POD 4 & 5) calcineurin inhibitor; and either MMF or sirolimus. Actuarial 4-yr patient survival was 95%; graft survival, 92% (Table 1); at 4 yrs, 89% are free of biopsy-proven AR, 95% free of biopsy-proven CR. Serum creatinine level (±SD) at 1 yr was 1.5 (.4); 2 yrs, 1.7 (.8); 3 yrs, 1.6 (.5); 4 yrs, 1.7 (.8). There were no differences between LD and CAD. Of the 412, 27 (6.6%) have had AR (% LD; % CAD) (19 mild, 6 mod, 1 severe, 1 C4d). The most common cause of graft loss (n=7) has been death with function; there have been 2 losses to CR, 0 to AR. Average weight, cholesterol, and triglycerides are unchanged from pretx (Table 2) but an increased number are taking lipid lowering meds.; 6.5% have developed CMV, 3% nonmelanotic skin cancer, 1.5% PTDM, 1% fractures, 0.5% cataracts, 0.25% PTLD, 0% osteonecrosis. Of the kidney recips, 80% remain P free. Common reasons for restarting P are rejection and side effects of other IS. We conclude that P-related side effects can be minimized in a protocol without maintence P; AR and CR rates are low, and 4-year outcome is excellent.

Table 1 - Survival - ALL (n=412)/LD (n=276)/CAD (n=116)

	1 Year	2 Years	3 Years	4 Years
% Patient Survival	97/97/98	96/97/98	95/95/98	95/94/98
% Graft Survival	96/96/97	96/95/97	93/92/96	92/91/96
% Death-censored Graft Survival	98/98/98	97/98/98	97/97/96	97/97/96
% AR-free Graft Survival	92/93/92	90/91/92	89/91/88	89/91/84
% CR-Free Graft Survival	97/98/97	95/98/97	95/95/95	95/95/95

Table 2

	Pre	1 Year	2 Year	3 Year
Weight (±SD)	76(19)	83(20)	83(22)	80(21)
Cholesterol (±SD)	177(95)	186(57)	175(37)	162(42)
Triglycerides (±SD)	183(75)	212(158)	167(93)	201(115)
% requiring lipid lowering meds	42%	47%	53%	52%

Abstract# 1526

THYMOGLOBULIN PRECONDITIONING AND TACROLIMUS MONOTHERAPY WITH SUBSEQUENT WEANING IN RENAL TRANSPLANT RECIPIENTS: AN UPDATE.

Ron Shapiro,[1] Amit Basu,[1] Henkie P. Tan,[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] Velma P. Scantlebury,[1] Mark L. Jordan,[1] Santosh Potdar,[1] Carlos A. Vivas,[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.*

We have suggested that the heavy conventional immunosuppression used for organ transplantation subverts the seminal tolerogenic mechanism of clonal exhaustion-deletion and thereby contributes to long-term drug-dependence. This self-defeating effect was avoided in 175 adult kidney recipients (mean 49.9 ± 14.3 years) treated between July 2001 and September 2003; 43 (24.6%) had adjunct donor bone marrow cell infusion. Two therapeutic principles were applied: (1) recipient pretreatment (with 5mg/kg Thymoglobulin) (2) minimal post-transplant immunosuppression (with tacrolimus monotherapy, initial target trough 10ng/ml). 25 (14.2%) patients were undergoing retransplantation, and 34 (19.4 %) had a PRA >20%. Mean HLA mismatches were 3.1 ± 1.7. 100 kidneys (57 %) were cadaveric (mean cold ischemia 25.6 ± .7 hours) and 95 (43 %) were from living donors. Mean donor age was 39.9 ± 6.3 years (range 1 - 66). Post-transplant doses of tacrolimus were spaced to every other day or longer at various times after 4 months. With a mean follow up of 16.9 ± 7.1 months, the actuarial one and 2-year patient survival is 97% and 95%, with graft survival of 91% and 84%. Mean current serum creatinine is 1.95 ± 1.14 mg/dl. The incidence of preweaning acute clinical rejection was 34.9% (6.3% steroid resistant). Weaning was attempted in 149 cases (85.1%) and interrupted by rejection in 45 (30.2%). Currently, 111 (63.4 %) patients are at various stages of weaning: 25 (14.2%) every other day, 44 (25.1%) 3 x/week, 28 (16%) 2 x/week, and 14 (8%) once/week. 10% of patients had asymptomatic CMV infection and there was one BK infection, and one case of transient post-transplant diabetes, but the incidence of CMV disease and PTLD was 0%. With significant numbers of highly weaned patients beyond the two-year mark, we continue to apply this strategy of immunosuppression, which is neither drug nor organ specific.

Abstract# 1527

CYCLOSPORINE WITHDRAWAL FROM A MYCOPHENOLATE MOFETIL-CONTAINING IMMUNOSUPPRESSIVE REGIMEN: RESULTS OF A FIVE-YEAR, PROSPECTIVE, RANDOMIZED STUDY.

Daniel Abramowicz,[1] Nesrin Gafner,[2] Peter Wijngaard.[2] *[1]Hopital Erasme, Bruxelles, Belgium; [2]F. Hoffman-La Roche, Basel, Switzerland.*

BACKGROUND: Long-term maintenance immunosuppression with cyclosporine (CsA) is associated with nephrotoxicity, hyperlipidaemia, and hypertension. In 1999, a 12-month, randomized, multicentre study involving 187 patients, indicated that CsA withdrawal from a mycophenolate mofetil (MMF)-based regimen resulted in improved graft function at the cost of a slight increase in the incidence of rejection episodes. This 4-year, follow-up (FU) study investigates the long-term efficacy and safety of the CsA-free regimen.

METHODS: 151 stable renal transplant recipients entered follow-up. 77 patients were maintained on CsA (mean dose 217 mg/day), MMF (mean dose 1.9 g/day), and steroids (mean dose 6.5 mg/day) [CsA-MMF GROUP], whilst the remaining 74 were administered with a CsA-free regimen of MMF (mean dose 2.0 g/day) and steroids (mean dose 9.5 mg/day) [MMF GROUP]. Baseline characteristics were similar for both groups. Renal function, measured via serum creatinine and creatinine clearance, was compared after 48 months. Patient and graft survival, acute rejection episodes, malignancies, blood pressure, and lipid profile were also recorded. All analyses were performed on an intention-to-treat (ITT) basis.

RESULTS: At 5 years, patient survival was similar in both groups (93% for MMF and 95% for CsA-MMF). Graft survival was 88% in the MMF cohort and 92% for CsA-MMF. Seven MMF patients experienced acute rejection episodes compared to 1 CsA-MMF patient during the 4-year FU (p=0.0263). Nine grafts were lost to chronic rejection in the MMF group versus 3 in the CsA-MMF cohort. In the MMF arm, there were no differences between rejection-free patients (n=60) and patients with acute or chronic rejection episodes (n=14) with regards to MMF or steroid doses. Over the 5-year total study period, ANCOVA, using least-square mean values, found that the MMF group showed a trend towards improved serum creatinine (127.3 vs 135.5 µmol/L) and creatinine clearance (73.3 vs 65.7 mL/min). CsA withdrawal was associated with numerically but not significantly lower total cholesterol, lower systolic and diastolic blood pressure, and fewer malignancies.

CONCLUSIONS: Withdrawal of CsA from an MMF-containing immunosuppressive regimen resulted in a sustained but modest increased risk of acute rejection episodes. However, 5-year graft and patient survival rates were similar, and CsA withdrawal had beneficial effects on renal function, lipid profile, blood pressure, and tumour rate.

Abstract# 1528

A PROSPECTIVE, RANDOMIZED, DOUBLE BLIND MULTICENTER STUDY OF EARLY (7 DAY) CORTICOSTEROID CESSATION VS. LONG TERM LOW DOSE CORTICOSTEROID THERAPY UNDER TACROLIMUS AND MYCOPHENOLATE MOFETIL THERAPY WITH ANTIBODY INDUCTION IN RENAL TRANSPLANT RECIPIENTS. E. S. Woodle,[1] for the Fujisawa Steroid Withdrawal Study Group. *[1]Division of Transplantation, University of Cincinnati, Cincinnati, OH; [2]Fujisawa Healthcare, Inc., Deerfield, IL.*

The three randomized double blind trials of corticosteroid (CS) withdrawal (WD) conducted to date evaluated CSWD after 3 months posttransplant. The current trial presents the first randomized, double blind trial of CSWD within 7 days posttransplant. **METHODS:** Inclusion criteria: primary cadaveric and live donor transplant (Tx) patients (pts) aged 18-70 yrs; between postTx day (PTD) 3-7 with at least 30% decrease in serum creatinine from baseline (pre-op) not requiring dialysis. Exclusion criteria: peak PRA>50%, current PRA>25%, repeat or multiple organ Tx. Primary endpoint was treatment failure (death, graft loss, moderate acute rejection-by Banff criteria and treatment) at 6mo/yr 1-5.Secondary endpoints were: Framingham cardiac risk estimates at 6 mo/yr 1-5, acute rejection, study drug discontinuation, PTDM, weight, infection and malignancy. Pts received tacrolimus (target 10-20 ng/ml PTD 1-90, 5-15 mg/ml PTD >90) and MMF (2 gm/d). Induction therapy was per center with IL-2R Ab or thymoglobulin (1.5 mg/kg x 4 doses). Pts were randomized to stop CS on PTD 7 or long-term CS. Long-term CS dose was 5 mg/d after PTD 180. To assess long-term graft survival and cardiac risk, blinding is planned for 5 yrs. **RESULTS:** Enrollment ceased in Oct 2002 at 397 pts. Data presented is blinded data from 386 pts. Follow-up included 36 months in 3.4% of pts, 24 months in 28%, 12 months in 57%, 6 months in 97% and 3 months in 100%. Mean follow-up was 13 months. Study withdrawal occurred in 9%. Mean age was 46.4 ± 12.4 yrs; 66% of pts were male, 67% Caucasian, 20% African American, 43% cadaveric recipients and 28% had diabetes preTx. PTDM incidence at 6 months was 5.8%. Mean # of HLA mismatches/pt was 3.5; 93% of pts had current PRA<10%. Mean cold ischemic time was 17.8 ± 6.5 hrs. 68 % received thymoglobulin induction; 32% received IL2R Ab. Over study duration, biopsy proven acute rejection occurred in 8.8% of pts, and 8.3% were treated. Pt and graft survival were 97.7% and 97.2 %. Death occurred in 9 pts (infection 3 pts, cardiac 3 pts, and unknown 2 pts, street drug overdose 1 pt). Graft loss occurred in 4 pts. (technical 1 pt., rejection 2 pts., unknown 1 pt.). **CONCLUSIONS:** Interim analysis of the first randomized double blind study of early CSWD reveals: 1) overall acute rejection rates remain very low, 2) pt and graft survivals remain excellent, and 3) incidence of PTDM is very low.

Abstract# 1529

STEROID WITHDRAWAL AT THREE DAYS AFTER RENAL TRANSPLANTATION WITH ANTI IL-2RECEPTOR THERAPY: A PROSPECTIVE RANDOMIZED MULTICENTER TRIAL. Teun van Gelder,[1] Cornelis G. ter Meulen,[2] Ronald J. Hene,[3] Maarten H. L. Christiaans,[4] George F. Borm,[2] Iza C. van Riemsdijk,[1] Luuk B. Hilbrands,[2] Willem Weimar,[1] Andries J. Hoitsma.[2] *[1]Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands; [2]Nephrology, University Medical Center "St Radboud", Nijmegen, Netherlands; [3]Internal Medicine, University Medical Center, Utrecht, Netherlands; [4]Nephrology, University Hospital, Maastricht, Netherlands.*

Introduction. Steroids have been included in most immunosuppressive regimens after renal transplantation, but are feared for their side effects. In a large prospective randomized multi-center study we investigated whether it is feasible to withdraw steroids at three days after transplantation in renal transplant recipients treated with a monoclonal interleukin-2 receptor antagonist containing immunosuppressive regimen. In the control group prednisone was tapered to zero in four months after renal transplantation as the best available alternative to limit the side effects of corticosteroids.

Methods. A total of 364 patients were randomized for two treatment regimens. All patients received tacrolimus and MMF. The daclizumab group received two doses of daclizumab (1 mg/kg) and for the first three days 100 mg prednisolone (daclizumab group n=186), with steroid-free treatment thereafter. In the control group patients were treated with prednisone for 16 weeks.(controls n=178).

Results. The incidence of biopsy-confirmed acute rejection at twelve months was not different between the daclizumab group (15%) and the controls (14%) (95% confidence interval of difference: -6 to +8%, NS). Graft survival at twelve months was comparable in the two groups (daclizumab group: 91%; controls: 90 %). Mean arterial blood pressure and serum lipids were temporary lower in the daclizumab group compared with controls, but the differences were no longer statistically significant at 12 months. During the first 12 months new-onset diabetes mellitus, defined as the institution of treatment with glucose lowering medication, developed in 12 of 186 patients (7%) in the daclizumab group, and in 21 of 178 patients in the control group (NS). Diabetes persisted until the end of follow-up in 11 and 17 patients respectively. The immunosuppressive regimen of the daclizumab group was associated with increased costs.

In conclusion, with the use of anti-IL-2Rα induction and daily therapy with tacrolimus and MMF it is feasible to withdraw steroids at three days after renal transplantation.

Abstract# 1530

COMPARISON OF EFFICACY AND SAFETY OF SIROLIMUS(SLR) AND MYCOPHENOLATE MOFETIL(MMF) AS ADJUNCT TO CALCINEURIN INHIBITOR(CNI) BASED STEROID FREE IMMUNOSUPPRESSION IN KIDNEY TRANSPLANTATION. Mysore S. Anil Kumar,[1] Billie Fyfe,[2] Debra Sierka,[3] Michael Heifets,[4] Muhammed I. Saeed,[1] Miten H. Parikh.[1] *[1]Surgery/Transplant, Drexel University College of Medicine, Philadelphia, PA; [2]Pathology, Drexel University College of Medicine, Philadelphia, PA; [3]Pharmacy/Transplant, Hahnemann University Hospital, Philadelphia, PA; [4]Nephrology/Medicine, Drexel University College of Medicine, Philadelphia, PA.*

CNI based immunosuppression with steroids and MMF or SLR has been the mainstay in kidney transplantation. We carried out a randomized prospective study to compare the efficacy of MMF and SLR in CNI based steroid free immunosuppression with regards to delayed graft function(DGF), acute rejection(AR), serum creatinine(SC), creatinine clearance(CCl), side effects and one year patient and graft survival. Protocol biopsies were completed at 1, 6 and 12 months to study the incidence of subclinical acute rejection(SCAR) and chronic allograft nephropathy(CAN). 84 recipients were treated with MMF and 71 with SLR. All recipients were given basiliximab induction. Intravenous steroid was given for 2 days, 250 mg on day 0 and 125 mg on day 1 and then totally discontinued. Tacrolimus or cyclosporine was used with equal frequency in both the groups. Recipient demography was comparable in both groups. Marginal donors were significantly higher in SLR group. Table1 shows marginal donors, DGF, AR, SCAR, CAN, SC and CCl at 1 year in the 2 groups.

Table 1

	Marginal donors	DGF	AR	SCAR	CAN	1 year mean SC(mg/dl)	1 year mean CCl(mls/minute)
MMF group (n=84)	9(10%)	28 (33%)	13 (15%)	24 (28%)	13 (15.4%)	1.9±0.8	66±29
SLR group (n=69)	14(20%)	22 (31%)	4 (5%)	16 (23%)	8 (11%)	2.0±1.6	62±29

The incidence of complications due to SLR and MMF are shown in Table 2

Table 2

Complications	Delayed wound healing and/or lymphocele	Mean hemoglobin(g/dl)	Mean white cell count/cmm	Thrombocyte count/cmm	Cholesterol level (mg/dl)	Triglceride levels(mg/dl)	Severe gastrointestinal side effects
SLR group(N=69)	9(12.5%)	11.7±1.8	6800±2500	235000±126000	177±45	252±168	10(15%)
MMF group(N=84)	8(9.5%)	12.4±1.8	5700±2100	234000±71000	166±39	185±100	13(15.5%)

This data shows that in CNI based steroid free immunosuppression, adjunct therapy with SLR results in a trend for lower incidence of AR, SCAR and CAN in protocol biopsies compared to MMF treated recipients. SLR treated patients tend to have lower hemoglobin levels and higher lipid levels at 1 year. Longer term follow up is required to assess the effect of lower AR, SCAR and CAN in SLR group on the outcome of 5 year patient and graft survival compared to MMF group.

Abstract# 1531

OUTCOME OF PREDNISONE (P) MINIMIZATION IN AN IMMUNOLOGICALLY HIGHER RISK COHORT - WHO HAS ACUTE REJECTION (AR)? J. Keith Melancon,[1] James V. Harmon,[1] Ty B. Dunn,[1] Massimo Asolati,[1] Abhinav Humar,[1] Arthur J. Matas.[1] *[1]Surgery, University of Minnesota, Minneapolis, MN.*

We studied outcome of a P minimization protocol (P stopped on POD 6) in 113 higher risk recips (retx, PRA>10, DGF, Afr Am, or Native Am). A 2nd analysis was done substituting PRA >50 (n = 89). All recips received polyclonal Ab (Thymoglobulin) for 5 days (1st dose in OR), P until POD 5, CNI, and either MMF of sirolimus. **Study 1** – Outcome for the 2 subgroups is shown (Table 1) and is similar to outcome of our total P-free group (n=413).

Table 1 - Outcome of immunologically higher risk subgroups

	All 113	Subgroup of 89
Patient survival	95%	93%
Graft survival	91%	89%
Death-censored graft survival	95%	93%
AR-free graft survival	86%	83%
CR-free graft survival	95%	95%

Study 2 – We then did univariate analyses of AR risk in subgroups (recipient race; peak PRA (<10 vs 10-100; 0-50 vs >50); DGF (Y vs N); primary tx vs retx; LD vs CAD. For these subgroups thought to be at increased risk, 3 yr AR-free GS was: Afr Am, 92%; Native Am, 80%; peak PRA >10, 83%; peak PRA > 50, 73%; DGF, 92%; retx, 70%; CAD, 88%. Significant differences were primary vs retx (p< .0001), peak PRA<10 (vs ≥10) (p<. 03), and peak PRA≥50 (vs <50) (p< .0003). Multivariate analysis (Table 2) similarly showed retx and high peak PRA to be significant risk factors for AR.

Table 2. Multivariate Analysis of AR risk

Group	Hazard Ratio	p
DGF (Y vs. N)	.45	.44
CAD vs. LD	1.3	.48
Afr or Native AM vs. others	1.3	.64
Primary tx, peak PRA >50	2.1	.3
Retx, peak PRA<≤50	4.8	.006
Retx, peak PRA >50	7.5	.0001

Study 3 – We then asked if we were harming the retx subgroup by minimizing P. We compared AR risk in these patients with a historical cohort (n = 152) transplanted from 1996- 1999 and receiving polyclonal Ab, CNI, MMF, and long-term P. There was no increased risk of AR in the current cohort (vs historical controls) (AR-free@ 3 years, 71% vs 70%; p=. 6), and there was a trend toward increased GS (90% vs 81%; p=0.1) and PS (100% vs 89%; p=. 08) in the minimization group. **Conclusion:** For patients immunosuppressed with a P minimization protocol, retx and high PRA were risk factors for AR. However, AR was not increased compared to historical controls receiving P-based immunosuppression and there was a trend toward increased graft and patient survival with P minimization.

Abstract# 1532

THE CLINICAL BENEFITS OF EARLY STEROID WITHDRAWAL (7 DAYS) AND UTILITY OF PROTOCOL BIOPSIES AT 1, 6 AND 12 MONTHS IN GUIDING STEROID-FREE IMMUNOSUPPRESSIVE THERAPY AFTER RENAL TRANSPLANTATION.

Oleh G. Pankewycz,[1] Rabie Stephan,[2] Barbara Stefanick,[3] Fadi Dagher,[2] Mary Applegate,[2] Romesh Kohli,[1] Mark R. Laftavi.[2] *[1]Medicine; [2]Surgery; [3]Pathology, SUNY, University at Buffalo, Buffalo, NY.*

In this prospective, randomized clinical trial, we examined the clinical benefits of steroid avoidance following renal transplantation. In order to assess therapeutic efficacy and improve outcomes, we performed protocol biopsies at 1, 6 and 12 months to assess intra-allograft events and guide immunosuppressive therapy. Patients were randomized into two groups: early steroid withdrawal (SW, n=27) or steroid control (SC, n=26). Demographic characteristics were equivalent in both groups except that SC patients had fewer HLA mismatches (SC 2.7 vs. SW 3.9, p=0.02). Both groups received Thymoglobulin (Total dose: SW=3.4±1.2, SC=3.3±1.1 mg/kg), tacrolimus (TAC), mycophenolate mofetil (MMF) and steroids (250 mg/iv day 0 and 125 mg/iv day 1). The SC group received prednisone starting at 30 mg/d on day 2, tapered to 5 mg/d by day 30 and continued indefinitely. The SW group received prednisone 30 mg/d on day 2 that was tapered by 5 mg/d and discontinued on day 7. Clinical rejections occurred in 3 SW patients (11%) (Grade 1A, 1B and Borderline) and 3 SC patients (12%) (3 Borderline) all of which responded to steroids (p=NS). At one year, renal function was well preserved in both groups (SC 75.1 ± 25.4 vs. SW 76.7 ± 30.7 ml/min p=NS). In the SC group, 7 of 45 protocol biopsies showed borderline rejection with the remaining being normal. One patient was treated with steroids. In the SW group, 4 of 46 protocol biopsies showed rejection (3 borderline, 1 Grade 2A) one of which required a change in therapy. Five biopsies revealed unsuspected tacrolimus toxicity and 1 showed cholesterol emboli necessitating a change in immunosuppression in 3 patients. At one year, 82%of SC and 86% of SW biopsies showed absent or minimal fibrosis (p=NS). Fewer patients in the SW arm had significant bone loss at 1 mo. (SC 61% vs. SW 14% p=0.007) or required lipid lowering therapy (SC 89% vs. SW 22%, p=0.004). Thus, a short course of Thymoglobulin induction combined with TAC and MMF prevented clinical and subclinical rejection allowing for safe steroid withdrawal without increasing the risk of graft fibrosis. SW patients enjoyed equivalent graft function with less bone loss and hyperlipidemia. Only 5% of protocol biopsies identified patients requiring a change in therapy. When conducting clinical trials of minimizing immunosuppression, protocol biopsies provide an additional assessment of safety and efficacy.

TRANSPLANT TOLERANCE II

Abstract# 1533

LONG TERM ALLOGRAFT SURVIVAL FOLLOWING ATTENUATION OF THE EARLY INFLAMMATORY RESPONSE PLUS SHORT TERM CO-STIMULATORY MOLECULE BLOCKADE.

Tarek El-Sawy,[1,2] John A. Belperio,[3] Robert M. Strieter,[3] Robert L. Fairchild.[1,2] *[1]Pathology, Case Western Reserve University School of Medicine, Cleveland, OH; [2]Immunology, The Cleveland Clinic Foundation, Cleveland, OH; [3]Medicine, The University of California at Los Angeles School of Medicine, Los Angeles, CA.*

Introduction: Polymorphonuclear cells (PMNs) are the principle cellular mediator of inflammation in cardiac allografts following reperfusion. Results from this laboratory have demonstrated a significant attenuation in PMN infiltration into allografts by antagonizing CXCR2, the receptor for KC and MIP-2. The goal of the current study was to test the role of early neutrophil mediated inflammation on the establishment of allograft acceptance via a co-stimulatory blockade strategy.

Methods: C57BL/6 (H-2b) mice, BALB/c (H-2d), and BALB/c (H-2d) CXCR2-/- mice received heterotopic A/J (H-2a) cardiac allografts. C57BL/6 mice were treated with CXCR2 antiserum or a control goat serum on days 0, 2, 5, and 8 post-transplant. Wildtype animals were treated with anti-Ly6G (RB6-8C5) on days -1, 0, and 1 and anti-CD40L (MR1) on days 0, 1, and 3 post-transplant.

Results: Treatment with CXCR2 antiserum significantly delayed cardiac allograft rejection to 15-22 days compared to 7-8 days in control treated animals. This result was also observed in CXCR2-/- recipients. T cell priming in CXCR2-/- animals, measured with IFN-g ELISPOT at days 4, 5 and 7, was identical in magnitude and tempo to wildtype animals. However, immunohistology at day 5 post-transplant demonstrated a significant delay in T cell infiltration. PMN infiltration peaks at 24 hours post-reperfusion in control animals, however there was a near absence of PMN infiltration and graft parenchymal tissue architecture was preserved in both experimental groups. At this early time intra-graft cytokine and chemokine expression was significantly reduced in both experimental groups. Administration of anti-CD40L to cardiac allograft recipients delayed rejection to day 25-30. Short term attenuation of PMN mediated effects with anti-Ly6G treatment at the time of MR1 administration prolonged cardiac allograft survival beyond 100 days.

Conclusions: The early inflammatory response to cardiac allografts recruits PMNs to the graft site within hours following reperfusion. Antagonism of early PMN mediated effects delays the infiltration of T cells and delays rejection. The establishment of a window period of attenuated inflammation prior to administration of co-stimulatory blockade prolonged allograft survival indefinitely. Such combination strategies may suggest novel methods for protecting transplanted organs and prolonging function.

Abstract# 1534

BLOCKING CD134 COSTIMULATION IS CRITICALLY IMPORTANT IN THE INDUCTION OF DONOR SPECIFIC ISLET ALLOGRAFT TOLERANCE.

Yon Su Kim,[1] Minh Diem Vu,[2] Mohamed H. Sayegh,[3] Terry B. Strom,[2] Xian C. Li.[2] *[1]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [3]Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

CD134 is a recently identified T cell costimulatory molecule, and like CD154, CD134 is a member of TNF receptor superfamily. Both CD134 and CD154 are not constitutively expressed on resting T cells but they are rapidly induced upon T cell activation. Although individual CD134/CD134L pathway and CD40/CD154 pathway have been shown to play an important role in T cell activation, littler is known about their potential interactions in regulating T cell activation, especially in islet allograft rejection. In the present study, we investigated the individual and collective role of CD134 and CD154 costimulatory pathways in allogeneic immune responses using a murine islet graft model.

The expressions of CD154 and CD134 on T cells after stimulation with anti-CD3 and anti-CD28 showed different kinetics between two molecules. CD154 was expressed after 1 day of stimulation and was more dominant in CD4 T cells. Also, CD154 was down-regulated after 3 days of in-vitro stimulation. However, expression of CD134 persisted even after 5 days of stimulation and was comparable in both CD4 and CD8 T cells. Analysis of CFSE-labeled cells proliferating in the allogeneic hosts demonstrated that while blocking either CD40/CD154 or CD134/CD134L pathway had minimal effect in blocking T cell proliferation in-vivo, blocking the both costimulatory pathways markedly inhibited the in-vivo proliferation of allogeneic T cells. In pancreas islet transplantation model in which the DBA/2 islets were transplanted into C57BL/6 mice rendered diabetic by streptozotocin, islet allograft survival was only prolonged by blocking either the CD134/CD134L pathway (16, 17, 18, 21, 31, 116 days) or the CD40/CD154 pathway (16, 17, 18, 18, >120, >120 days) as compared to control group (MST=13.5 days, n=5). However, consistent with findings in the in-vivo T cell response model, combined blockade of CD40/CD154 and CD134/CD134L pathways produced indefinite islet allograft survival (MST>120 days, n=6). Indeed, C56BL/6 recipients accepted a second donor specific islet allograft but promptly rejected a third party one, demonstrating that a state of donor specific tolerance is induced. Our data demonstrate the CD134 costimulation is critically important in supporting the islet allograft rejection, and blocking CD134 along with CD154 blockade can induce donor-specific islet allograft tolerance.

Abstract# 1535

NK CELL ALLOREACTIVITY IS AN IMPORTANT MEDIATOR OF COSTIMULATION-BLOCKADE-RESISTANT REJECTION DURING ALLOGENEIC TRANSPLANTATION. Leslie Kean,[1] Shana Coley,[1] Andrew Adams,[1] Kelly Hamby,[1] Eun Lee,[1] Eric Heiss,[1] Thomas Pearson,[1] Christian Larsen.[1] *[1]Center for Transplantation, Dept. of Surgery, Emory University School of Medicine, Atlanta, GA.*

Immune tolerance to allogeneic solid organ or bone marrow allografts remains an elusive goal of clinical transplantation. T cell tolerance can be accomplished in murine systems by simultaneously providing T cells with "signal 1" (alloantigen) while blocking "signal 2" through costimulation blockade. We have reliably used the costimulation blockade reagents CTLA4-Ig and anti-CD40L in conjunction with a nonmyeloablative bone marrow transplant to produce allogeneic transplantation tolerance in a fully MHC-mismatched murine system. However, relatively large cells doses (>$10x10^6$) of bone marrow must be transplanted for this regimen to be successful. Costimulation-blockade-resistant rejection occurs consistently when smaller cell doses of bone marrow are transplanted. In this study, we investigated the contribution of NK cell alloreactivity to this costimulation-blockade-resistant rejection response.

After a nonmyeloablative preparative regimen of 20mg/kg busulfan, increasing doses of Balb/C ($NK1.1^-$) bone marrow (0.5-, 2-, 5-, 10-, and $20x10^6$ cells) were transplanted into C57BL/6 ($NK1.1^+$) mice. Costimulation-blockade with CTLA4-Ig and anti-CD40L was given in the peritransplant period. In addition, in one-half of the transplanted animals, NK cells were depleted by IP injection of the depleting antibody, PK136. Animals with unmanipulated NK cells exhibited costimulation-blockade resistant rejection when transplanted with ≤ $5x10^6$ donor bone marrow cells. However, animals with NK cell depletion became stably chimeric with donor marrow even when very low doses (as little as $0.5x10^6$) were infused. The chimeric animals in both the NK replete and NK-depleted groups were tolerant to their allografts: They exhibited deletion of donor-reactive $vb5^+$ and $vb8^+$ $CD4^+$ T cells and accepted Balb/C skin grafts. In contrast, animals that rejected their bone marrow grafts failed to delete the vb5/8 T cells and rejected Balb/C skin grafts.

These data demonstrate the central role that NK cells play in rejection of allogeneic bone marrow and underscore the need to control NK alloreactivity during clinical transplantation.

Abstract# 1536

PREVENT TYPE I DIABETES IN NOD MICE BY GENE THERAPY. Chaorui Tian,[1] Jessamyn Bagley,[1] Daron Forman,[1] David H. Sachs,[1] John Iacomini.[1] *[1]Transplantation Biology Research Center, Mass. General Hospital, Boston, MA.*

Introduction: Multiple genes contribute to Type I diabetes, however, the principle determining genetic component in NOD mice and humans is the inheritance of mutant MHC class II β chains. Here we test the hypothesis that expression of retrovirally transduced MHC class II β chains from wild-type MHC haplotypes in bone marrow derived hematopoietic cells can be used to prevent diabetes in NOD mice. **Methods**: NOD mice (4-6 weeks) were conditioned with 10.25 Gy TBI, and followed within 24hrs by reconstitution with 5-Fu treated NOD bone marrow transduced with retrovirus encoding a fusion protein consisting of either I-$A\beta^d$ or I-$A\beta^k$ chain fused to GFP or GFP protein as a control. The blood glucose level and the frequency of GFP expressing cells in peripheral blood were monitored weekly. Mice were sacrificed for immunohistochemistry and Elispot at different time points after reconstitution. **Results**: FACS revealed that relatively stable expression of GFP is ready to detect in PBMC of mice reconstituted with I-$A\beta^d$-GFP or I-$A\beta^k$-GFP and control GFP transduced bone marrow cells during the 36 week follow-up period. Confocal microscopy showed that in PBMC from mice transduced with GFP, GFP expression was in the cytoplasm. In contrast, in MHC class II positive PBMC from mice transduced with I-$A\beta^d$-GFP, GFP expression appeared solely at the cell membrane and co-localized with expression of I-$A\beta^d$. While 79% (11/14) of mice reconstituted with hematopoietic cells transduced with GFP became diabetic, none (0/24) of the mice reconstitution with either I-$A\beta^d$-GFP or I-$A\beta^k$-GFP virus became diabetic by week 48 ($P<0.001$). Imunohistochemistry showed that islets from mice transduced with GFP showed massive T cell infiltrate and decreased production of insulin, while islets from mice transduced with either I-$A\beta^d$-GFP or I-$A\beta^k$-GFP virus had no visible cell infiltrate and normal levels of insulin production. ELISPOT analysis showed cells from diabetic mice transduced with GFP had vigorous production of IL-2, INF-γ and IL-4 in response to either GAD65 peptide or insulin protein. In contrast, cells from mice transduced with either I-$A\beta^d$-GFP or I-$A\beta^k$-GFP had very low levels of IL-2, INF-γ and IL-4 ($P<0.001$). **Conclusion**: Expression of non-susceptible MHC alleles in the hematopoietic cells is sufficient to prevent the development of functional auto-reactive T cells, insulitis and type I diabetes in NOD mice. Somatic transfer of MHC genes into autologous bone marrow using retroviral transduction represents a major new approach to re-establishing tolerance to self in Type I diabetes.

Abstract# 1537

PRE-PLASMACYTOID DENDRITIC CELLS PROPAGATED FROM BONE MARROW INDUCE ALLOGENEIC T CELL HYPORESPONSIVENESS IN VIVO. Masanori Abe,[1,2] Zhiliang Wang,[1,2] F. Jason Duncan,[1,2] Angus W. Thomson.[1,2] *[1]Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA; [2]Department of Surgery, University of Pittsburgh, Pittsburgh, PA.*

Background: Plasmacytoid dendritic cells (pDC) represent a distinct subset of DC implicated in resistance to viral infection and in immune regulation. Tolerogenic properties of human and mouse pDC have been reported in vitro. Little is known regarding the in vivo properties of pDC. In this study, we have evaluated the influence of in vitro-propagated mouse bone marrow (BM)-derived pDC on T cell responses to alloantigens in vitro and in vivo. **Methods:**DC were propagated from normal C57BL/10J (B10: $H2^b$) mouse BM cells in media containing 200 ng/ml human recombinant fms-like tyrosine kinase 3 ligand for 10 days. pDC were flow-sorted to > 98% purity on the basis of their surface phenotype ($CD11c^+B220^+CD11b^-$). Their costimulatory molecule expression and stimulatory capacity for allogeneic T cells (C3H; $H2^k$) in mixed leukocyte reactions were compared to those of classic BM-derived myeloid DC (mDC: $CD11c^+B220^-CD11b^+$). To assess their influence on T cell responses in vivo, C3H ($H2^k$) were received $2x10^6$ flow-sorted pDC or mDC intravenously from B10 ($H2^b$) donors and 7 days later, splenic T cells were restimulated with B10 (donor) alloantigen. **Results:** Unstimulated BM-derived pDC expressed little or no surface MHC class II or classic costimulatory molecules (CD40, CD80 and CD86). On the other hand, they expressed programmed death ligand 1(PD-L1), an alternative costimulatory molecule implicated in regulation of T cell responses. These cells induced poor proliferative responses and low secretion of IFN-γ and IL-10 by allogeneic T cells. Stimulation with the Toll-like receptor 9 ligand CpG-oligodeoxynucleotide upregulated MHC class II and costimulatory molecules on pDC and markedly enhanced their stimulatory capacity for naive allogeneic T cells. However, the level of proliferation was lower than that induced by mDC. T cells from mice primed with unstimulated allogeneic pDC exhibited significantly lower proliferative response to donor alloantigens compared with those from mDC-primed mice. This hyporesponsiveness was restored by addition of exogenous recombinant IL-2. **Conclusions:** BM-derived pDC are immature antigen-presenting cells that can induce T cell hyporesponsiveness to alloantigen in vitro and in vivo. This finding suggests that pDC have potential for regulation of alloimmunity and promotion of tolerance induction.

Abstract# 1538

COMPARATIVE ANALYSIS OF TOLERANCE INDUCTION IN NOD AND NOR MICE REVEALS A ROLE FOR APC FUNCTION IN ANTI-CD45RB INDUCED TOLERANCE. Daniel Moore,[1] Xiaolun Huang,[1] Muhammad M. Mohiuddin,[1] Major K. Lee,[1] Meredith Chiaccio,[1] Haiying Chen,[1] Moh-Moh Lian,[1] Robert Zhong,[2] James F. Markmann,[1] Shaoping Deng.[1] *[1]Surgery, University of Pennsylvania, Philadelphia, PA; [2]Surgery, University of Western Ontario, London, ON, Canada.*

Background: While tolerance to islet allotransplants has been achieved in several murine systems, success of standard protocols has not followed in the autoimmune NOD strain, the model for human IDDM. We have investigated tolerance induced by anti-CD45RB in a cardiac allotransplant model in the NOD background to focus on aspects of tolerance resistance in the absence of recurrent autoimmunity. Comparison to the NOR strain (88% genetic identity to NOD) permits us to elucidate the mechanisms underlying tolerance resistance in the autoimmune background. **Methods:** Cardiac allografts from C3H mice were transplanted to either pre-diabetic (<12 wks) NOD mice or diabetes resistant NOR mice. Recipients were untreated or treated with anti-CD45RB mAb (100ug ip days 0, 1, 3, 5, and 7). To investigate the effect of anti-CD45RB, extensive immunophenotyping of T cells, B Cells, and professional APC subsets was performed following exposure of animals to alloantigen in the presence or absence of anti-CD45RB. Proliferation of mitogen stimulated T cells from both strains was performed utilizing CFSE by standard protocols. **Results:** Cardiac allografts were rejected acutely with a mean survival time (MST) of 9.2 days in untreated NOD mice (n=6). In NOD mice treated with anti-CD45RB, only a minimal prolongation of graft survival was noted (MST=24.3d, n=6). However, NOR mice demonstrated a significant prolongation in graft survival (MST>63.1d, n=8). In vitro studies showed that the NOR strain shares the similar hypoproliferative phenotype associated with decreased AICD, suggesting that clearance of alloreactive specificities may contribute to tolerance induction. Immunophenotyping of the NOD strain has revealed a number of alterations in B cell surface markers following contact with CD45RB and alloantigen including downregulation in CD19 and upregulation in CD54. **Conclusions:** Short-term administration of anti-CD45RB mAb significantly prolongs allograft survival in diabetes resistant NOR mice as compared to the autoimmune prone NOD strain. However, donor specific tolerance could not be induced in either strain suggesting that graft prolongation is separable from tolerance induction. The proliferative dysfunction of T cells may account for the resistance to long-term tolerance induction as this feature is shared with both NOD and NOR.

Abstract# 1539

AN ESSENTIAL REQUIREMENT FOR AN INTACT HOST MHC CLASS I PATHWAY IN ISLET ALLOGRAFT SURVIVAL. Joshua N. Beilke,[1] Nathan R. Kuhl,[1] Zachary A. Johnson,[1] Ronald G. Gill.[1] [1]*Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO.*

Current consensus is that regulatory allograft tolerance occurs primarily through MHC class II-restricted CD4 T cell reactivity. However, little is known about MHC class I-restricted reactivity in tolerance induction. Thus, the purpose of this study was to determine the role for CD8 T cells versus general MHC class I-restricted antigen presentation in islet allograft tolerance.

Methods: Streptozotocin-induced diabetic C57Bl/6 (B6, H-2^b) controls, C57Bl/6 CD8 deficient (CD8 KO), or MHC class I-deficient C57Bl/6 β2m deficient (β2m KO) recipients were grafted with 450 allogeneic BALB/c (H-2^d) islets beneath kidney capsule. The efficacy of costimulation blockade (anti-CD154; MR-1 therapy) or adhesion molecule blockade (anti-LFA-1; KBA therapy) was assessed in these models. Some recipients also received anti-NK1.1(HB191) or anti-CD8 (2.43) antibody treatment.

Results: Either anti-CD154 or anti-LFA-1 therapy prolonged allograft survival in wild-type B6 recipients, CD8KO recipients, or in anti-CD8 treated B6 recipients treated. However, in β2m KO mice there was a striking abrogation of allograft survival; all treated animals rejected allografts in a tempo similar to untreated controls. These results indicated a requirement for host MHC class I-dependent reactivity that was not due to a classical CD8 T cell. NK cells, NKT cells, or both would fulfill this role. Thus, wild-type B6 mice were depleted NK1.1+ cells with a single dose of anti-NK1.1 one day prior to islet transplantation. Depletion of NK1.1+ cells abrogated graft survival in both anti-CD154 and anti-LFA-1 groups ($p<.05$)

Table 1. Graft survival in CD8- versus MHC class I deficient hosts

Therapy	B6	CD8 KO	B6 + anti-CD8	β2m KO	B6 + anti-NK1.1
Anti-CD154	15, 23, > 100x8	> 100 x 6	> 100 x 6	9/9 reject; MST 12.8	17/ 20 reject; MST 13.6
Anti-LFA-1	7, 19, 20, 25, 33, 49, >100 x 14	18, 40, 54, 61, 68, 93, >100 x 3	35, 41, 45, 49, 61, 63, 69, >100 x 2	7/7 reject; MST 17.0	10/11 reject; MST 29.4
Untreated	8/8 reject; MST 11.3	6/6 reject; MST 14.3	nd	7/7 reject; MST 10.3	nd

nd = not done. MST = mean survival time (days)

Conclusion: Taken together, results show a dramatic distinction between two forms of MHC class I-restricted pathways in allograft prolongation. While both therapies are CD8 T cell independent, an intact host MHC class I-restricted (β2m-dependent) pathway is nevertheless necessary for allograft survival. This pathway required NK1.1+ cells, implicating NK/NKT cells in allograft prolongation in vivo.

Abstract# 1540

ANTI-CD40 LIGAND ANTIBODY AND ANTI-CD40 ISOTYPE IgG_{2B} ANTIBODY INDUCE EQUIVALENT MURINE CHIMERISM AND SKIN GRAFT TOLERANCE UNLIKE ANTI-CD40 ISOTYPE IgG_1 ANTIBODY. Zvonimir L. Milas,[1] Andrew B. Adams,[1] Thomas Jones,[1] Meghan Durham,[1] Thomas C. Pearson,[1] Christian P. Larsen.[1] [1]*Department of Surgery, Emory University, Atlanta, GA.*

Therapeutic hematopoietic chimerism has the potential to correct many genetic hematological diseases and to eliminate the need for chronic immunosuppressive therapy following organ transplantation. To date, protocols establishing hematopoietic chimerism have required either recipient conditioning or administration of large doses of non-T cell depleted donor bone marrow to facilitate reliable engraftment of donor cells. While encouraging, there are issues that may restrict or possibly prevent the clinical application of these strategies, such as concerns of over-immunosuppression, the increased risk of malignancies from whole body irradiation, or unfeasible donor bone marrow requirements. Our lab has devised an alternative strategy utilizing a single, non-myelosuppressive dose of busulfan (a stem cell selective conditioning agent), costimulation blockade (anti-CD40L and CTLA4-Ig), and readily attainable numbers of T-cell depleted donor bone marrow (as low as $2x10^6$ cells), and thus promoting a high-level chimerism and tolerance of allogeneic skin grafts. However, CD40L is found on platelets, and the use of anti-CD40L in human and primate was significantly associated with thromboembolic events. Consequently, we compared the efficacy of inducing chimerism and allogeneic skin graft tolerance between anti-CD40 IgG_1, anti-CD40 IgG_{2b}, and anti-CD40L. We found the chimerism and skin graft tolerance was equivalent between anti-CD40 IgG_{2b} and anti-CD40L (donor chimerism 46.6% and 35.5% at 60 days, respectively). However, the anti-CD40 IgG_1 isotype failed to produce chimerism (1.03% at 60 days) or skin graft tolerance. This was significant in comparison to both anti-CD40L and anti- CD40 IgG_{2b}, $p<0.05$. Furthermore, we found that the anti-CD40 IgG_{2b} isotype and anti-CD40L inhibited or depleted memory B cell colony proliferation at day 21 using a LCMV based memory B cell model (frequency of LCMV memory B cells are less than 1 per 500,000 B cells for each antibody), while anti-CD40 IgG_1 isotype did not deplete memory B cell colony proliferation (frequency of LCMV memory B cell 1 per 39,400 B cells, $p<0.05$). We conclude that anti-CD40 IgG_{2b} isotype is as effective as anti-CD40L, but may not have adverse effects associated with binding to platelets.

Abstract# 1541

LONG-TERM ACCEPTANCE OF HETEROTOPIC CARDIAC ALLOGRAFTS BY CO-TRANSPLANTATION OF VASCULARIZED THYMIC LOBES IN FULLY MHC-MISMATCHED MINIATURE SWINE. Parsia A. Vagefi,[1] Chisako Kamano,[1] Shin Yamamoto,[1] Katsuhito Teranishi,[1] Stuart L. Houser,[1] Joren C. Madsen,[1] Emma Samelson-Jones,[1] Akira Shimizu,[1] David H. Sachs, Kazuhiko Yamada. [1]*Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA.*

Background: We have recently established the technique of Vascularized Thymic Lobe (VTL) transplantation (Tx), and demonstrated thymopoiesis and tolerance induction across fully MHC-mismatched barriers in miniature swine. In the latter study donor renal Tx was performed 3 months following the initial VTL Tx. In pursuit of a more clinically applicable allogeneic Tx protocol, and given the increased difficulty with achieving tolerance to cardiac allografts, the present study in fully MHC-mismatched miniature swine investigated cardiac allograft survival in recipients receiving same-day, co-transplanted, VTL allografts from a single donor. **Methods:** Recipient swine (n=12) underwent heterotopic cardiac allograft Tx across fully MHC-mismatched barriers using a 12-day course of FK506 (Day 0 starting dose of 0.15mg/kg/day). Animals in Group A were euthymic. All other animals were thymectomized 3 weeks prior to Tx. Group A and B recipients received a cardiac allograft alone; Group C animals received 5-10 billion donor thymocytes intravenously on the day of cardiac Tx; and Group D animals underwent simultaneous cardiac and VTL co-Tx from single donors. Cardiac allograft rejection was determined by a flat EKG, and confirmed as a non-beating graft. In vitro CML and MLR assays were performed. **Results:** Group A recipients (euthymic hosts) maintained cardiac allografts until PODs 55, 55, and 134. Group B recipients (thymectomized hosts) rejected their cardiac allografts on PODs 17, 23, and 35. Group C recipients (donor thymocyte infusion) rejected their cardiac allografts on PODs 28, 49 and 64. One Group D recipient rejected the cardiac allograft on POD69; however, the remaining recipients maintained the allografts for >125 days and >305 days. The >305 day survivor demonstrated donor-specific hyporesponsiveness post-transplantation, and histologically no rejection was noted. **Conclusions:** This is the first demonstration of tolerance induction to fully MHC-mismatched cardiac allografts in our miniature swine model. These results indicate that the presence of thymic stroma, especially donor-type, is essential for a long-term cardiac allograft survival. Current studies utilizing a 28 day-course of FK506 are underway, and may more uniformly induce tolerance in recipients of co-transplanted cardiac and VTL allografts.

Abstract# 1542

HUMAN PLATELET-DERIVED CD154 PLAYS AN IMPORTANT ROLE IN INITIATING ALLOGRAFT REJETION. He Xu,[1] Xiao-Jie Zhang,[1] Frank Leopradi,[1] Roslyn B. Mannon,[1] Allan D. Kirk.[1] [1]*Transplantation Branch, NIDDK/NIH, Bethesda, MD.*

CD154 is critical for the initiation of naive allo-immune responses. Although activated T-cells have been presumed to be the relevant source of CD154, human platelets also express CD154 upon activation, and the role of platelet-derived CD154 in allograft rejection has not been evaluated. We hypothesized that activated platelets provide ample CD154 to initiate rejection. To investigate this, mixed lymphocyte reactions (MLRs) were performed using wild-type (WT) C57BL/6 or CD154 knock-out (KO; B6.129S2-tnfsf5) responder and Balb/c stimulator lymphocytes with or without human CD154 trimers and/or anti-human CD154 (5c8). Balb/c cardiac allografts were transplanted into C57BL/6 WT or CD154 KO mice with or without adoptively transfered human platelets, and/or 5 doses (200 µg/dose at day –1, 0, 3, 5, and 7) of 5c8 or anti-murine CD154 (MR-1). CD154 KO lymphocytes proliferated poorly compared to WT lymphocytes. Proliferation was restored by the addition of human CD154 trimers, and trimer-induced proliferation was blocked by 5c8. Similarly, CD154 KO animals failed to reject cardiac allografts but rejected equal to WT animals when CD154 trimers were given. Human platelets verified to express CD154 upon activation also induced cardiac allograft rejection in CD154 KO animals that was also prevented by administration of 5c8. Histologically, rejection was acute T-cell mediated rejection. These data show that recombinant human CD154 can cross-react with murine CD40 and induce lymphocyte proliferation in vitro and cardiac allograft rejection in vivo. Furthermore, adoptive transfusion of human CD154-expressing platelets into CD154 KO mice precipiates allograft rejection, and this rejection can be prevented by anti-human CD154 blockade with 5c8. These data suggest that CD154 sufficient to induce allograft rejection can be derived from non-T-cells, and that human platelets can serve as the sole source of CD154 sufficient to induce allograft rejection. Thus, non-T-cell derived CD154 must be considered a relevant source of this critical costimulatory molecule, and inhibition of platelet-derived CD154 will be required for adequate therapeutic targeting of the CD40/CD154 pathway.

Recipient	Adaptive Transfusion	CD154 Blockade	Allograft Survival
WT (n=10)	-	-	9, 9, 7, 7, 8, 8, 7, 9, 9, 9
WT (n=6)	-	MR1	82, 97, 112, 113, >157
CD154 KO (n=7)	-	-	12, 21, 28, >291, >171, >171, >171
CD154 KO (n=5)	Human platelet	-	11, 18, 12, 12, 137
CD154 KO (n=6)	Human platelet	5c8	88, >114, >114, >95, >95, >95
CD154 KO (n=7)	Human CD154 trimer	-	8, 8, 13, 13, 14, >14, >14

Abstract# 1543

ENGAGEMENT OF THE CHEMOKINE RECEPTOR CXCR3 INDUCES TYROSINE PHOSPHORYLATION OF THE CRITICAL T-CELL SIGNALING MOLECULES LCK, ZAP-70, AND LAT. Wasim A. Dar,[1] Sandra Gould,[2] Julie DeMartino,[2] Majed Hamawy,[1] Stuart J. Knechtle.[1] *[1]Department of Surgery, Division of Transplantation, University of Wisconsin, Madison, WI; [2]Merck, Rahway, NJ.*

Introduction: The chemokine receptor CXCR3 plays an important role in recruiting T-cells into allografts. Accordingly, blocking the interaction between CXCR3 and its ligands prolongs graft survival. We examined the mechanisms of CXCR3 mediated T-cell activation

Methods: T-cell lines stably transfected with CXCR3 were stimulated with its cognate ligands Mig, IP-10, and I-TAC. After cell stimulation, cells were lysed, with whole cell lysates subjected to Western blot analysis with phosphotyrosine specific antibodies.

Results: Stimulation of CXCR3 transfected T-cells induced strong tyrosine phosphorylation of the critical T-cell signaling molecules Lck, ZAP-70, and LAT. All molecules were tyrosine-phosphorylated within minutes after cell stimulation. Phosphorylation occurred at tyrosine residues 171 and 191 in LAT and tyrosine residue 319 in ZAP-70, residues which are also phosphorylated in TCR mediated signaling. Similar results were seen whether cells were stimulated with Mig, IP-10, or I-TAC. In addition, treatment of cells with the Syk/ZAP-70 inhibitor piceatannol impaired CXCR3 mediated tyrosine phosphorylation of ZAP-70 and LAT in a dose dependent fashion. In cross talk experiments, stimulating cells first through the TCR inhibited CXCR3 mediated tyrosine phosphorylation of Lck, ZAP-70, and LAT; similarly, stimulation of cells first through CXCR3 attenuated TCR mediated protein tyrosine phosphorylation.

Conclusion: The increased tyrosine phosphorylation of Lck, ZAP-70, and LAT after CXCR3-ligand interactions strongly implicates these molecules as having an important role in CXCR3 mediated signaling. The crosstalk results indicate that these receptors share common signaling pathways and suggest that in vivo prior stimulation of one receptor may attenuate the function of the other receptor. This could be important in designing therapeutic strategies to prevent graft rejection that target these receptors on T-cells; for example, because CXCR3 and TCR share signaling pathways, one drug could be used to block the function of both receptors in turn reducing drug toxicity associated with using more than one immunosuppressant to block each receptor individually.

Abstract# 1544

GRAFT-SPECIFIC CD4 T CELL HELP INFLUENCES GRAFT SPECIFIC CD8 T CELL MIGRATION, CYTOTOXICITY AND THE GENERATION OF MEMORY BY CD154 DEPENDENT AND INDEPENDENT MECHANISMS. Dawn Taylor,[1] Elizabeth Ingulli.[1] *[1]Center for Immunology, University of Minnesota, Minneapolis, MN.*

Although it is well established that both CD4 and CD8 T cells are involved in the rejection of grafts mismatched for minor histocompatibility antigens, the specific mechanisms of cooperation between the two populations are not well defined. This is due in part to technical limitations that prevent the direct monitoring of graft-specific T cells during the rejection process. To address this, we have used a mouse model system in which we track, in vivo, the behavior of T cell receptor (TCR)-transgenic chicken ovalbumin (OVA)-specific CD4 and CD8 T cells in response to skin grafts mismatched for a single foreign "minor" antigen, OVA. Donor tail skin is obtained from transgenic mice that express OVA under the control of chicken beta-actin promoter on the surface of most cells (Act-mOVA). **RESULTS**: Naive OT-I TCR transgenic CD8 T cells were intravenously injected alone or together with naïve OT-II TCR-transgenic CD4 T cells into B6 recipients. A group of recipients were left uninjected as controls. Maximal rejection of Act-mOVA tail skin grafts was dependent on the presence of both OT-I and OT-II cells. Control B6 isografts were accepted indefinitely. OT-I and OT-II cells were activated in draining lymph nodes (DLN) and not spleens as evidenced by CD69 expression. The migration and accumulation of OT-I cells in spleens and grafts occurred subsequent to their activation and proliferation in DLN. OT-II cells had several measurable effects on OT-I cells. First, they enhanced the accumulation of OT-I cells within skin grafts and migration of OT-I cells specifically to the skin as measured by enhanced expression of fructosylated form of PSGL-1. This was completely blocked in the absence of CD154 expression on OT-II cells. Second, they enhanced the cytotoxicity of OT-I as measured by in vitro chromium release assay and in vivo killing of OVA expressing cells. This effect was independent of CD154 expression on OT-II cells but may be related to IL-12 production by an activated DC. Thirdly, they enhance the generation of memory cells as evidenced by persistence and effector function in vivo. This effect was also independent of CD154 expression on OT-II cells. **CONCLUSION**: Using a CD154-dependent mechanism, graft-specific CD4 T cells facilitate graft-specific CD8 T cell migration and graft infiltration. Using a CD154-independent mechanism, graft-specific CD4 T cells enhance CD8 T cell cytotoxicity and generation of memory cells.

Abstract# 1545

EFFECTS OF IMMUNOSUPPRESSIVE DRUGS ON REGULATORY T CELL FUNCTION. Hee Gyung Kang,[1] Yon Su Kim,[1] Sophoclis Alexopoulos,[1] Terry B. Strom,[1] Xin Xiao Zheng.[1] *[1]Medicine, Division of Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

Allograft outcome, rejection or tolerance, often depends on the balance between cytopathic and regulatory T cells. We previously reported that treatment with rapamycin and costimulation blockade resulted in massive apoptosis of alloreactive T cells and produced stable skin allograft tolerance. In contrast, treatment with cyclosporine plus costimulation blockade abolished T cell apoptosis, as well as tolerance induction. In this study we further investigated the effects of these two immunosuppressive drugs on the immunosuppressive function of regulatory T cells.

We used an adoptive transfer skin allograft model and combined donor specific transfusion (DST) plus anti-CD154 treatment, a tolerizing protocol known to enhance the immunosuppressive effect of CD4+CD25+ regulatory T cells in an alloantigen-specific manner. Sorted CD4+CD25+ T cells from nave or DST plus anti-CD154 with or without rapamycin or cyclosporine treated C57BL/6 hosts were adoptively transferred into syngeneic RAG1 (-/-) hosts together with the same number of nave CD25- T cells before transplantation with DBA/2 skin allografts. For *in vitro* study of regulatory T cells, T cell proliferation in mixed lymphocyte reaction (MLR) was assayed.

In accord with our previous studies, CD4+CD25+ T cells from DST plus anti-CD154 treated, but not from nave, hosts were capable of delaying skin allografts rejection in this model. Interestingly, the addition of cyclosporine abolished the DST plus anti-CD154 effect, since CD4+CD25+ T cells harvested from the hosts treated with adjunctive cyclosporine could not significantly delay skin allograft rejection. In contrast, the addition of rapamycin to the basic regimen further enhanced the immunoregulatory function of CD4+CD25+ T cells, as all skin allograft survived more than 40 days. The addition of cyclosporine to the basic regimen also abrogated the suppressive effect of CD4+CD25+ T cells as compared to CD4+CD25+ T cells from hosts treated with DST plus anti-CD154 as assessed in a mixed lymphocyte reaction.

In this study, we found that the addition of cyclosporine abrogated, while addition of rapamycin enhanced, the tolerizing effect of DST plus anti-CD154 treatment to enhance the immunosuppressive effect of CD4+CD25+ regulatory T cells, which suggests an important difference in mode of action of these two immunosuppressive drugs. Moreover, these data indicate that regimens that promote tolerance must block alloaggressive, but spare or strengthen, immunoregulatory components of the allograft response.

CLINICAL PLEANRY II

Abstract# 1546

INCREASED PATIENT SURVIVAL AFTER SOLITARY PANCREAS TRANSPLANTS. Rainer W. G. Gruessner,[1] David E. R. Sutherland,[1] Angelika C. Gruessner.[1] *[1]Department of Surgery, University of Minnesota, Minneapolis, MN.*

Introduction: According to UNOS/IPTR data, since 1999 proportionally more transplants (txs) have been done in solitary pancreas (pa) tx categories (pa after kidney [kd] tx [PAK], pa tx alone [PTA]) than in the simult. pa and kd tx (SPK) category. For SPK, both multi-and single center studies have shown increased patient (pt) survival rates as compared with deceased kd txs alone (KTA) in diabetic pts. For PAK and PTA, we determined impact of solitary pa tx on pt survival rates by comparing tx mortality vs. waiting list mortality.

Methods: Between Jan 1, 1995 and May 20, 2003, 3094 diabetic patients were listed for PAK and 1149 for PTA. For data analysis, several criteria were used: (1) pts listed for or who previously underwent non-renal tx were excluded; (2) pts with multiple listings at different centers were counted only once (with waiting times added from date of first listing); (3) pts whose recipient category was changed were censored. We identified all pts who died on, or who were removed from, the waiting list. The Social Security Death Master File (SSDMF) supplemented follow-up in the UNOS/IPTR data base. Uni- and multivariate analyses for mortality assessment on the waiting list versus after tx were performed. For univariate analysis, unadjusted survival rates were calculated using Kaplan Meier. Mortality on the waiting list was calculated from time of listing; after pa tx, from time of tx, disregarding waiting time. Time-dependent causes of death were categorized as tx-, diabetes-, or non-tx/non-diabetes-related. For multivariate analysis, only risk factors for both wait-listed and transplanted pts were included (pt age, sex, diabetes duration, race).

Results: SSDMF-corrected 1- and 4-year pt survival rates on the PAK waiting list were 98% and 85%; on the PTA waiting list, 96% and 90%. Pt survival after pa tx at 1 and 4 years in the PAK category was 95% and 85%; in the PTA category, 97% and 86%. Only 45% of pt deaths > 90 days posttx were tx-related. Multivariate mortality analysis showed that risk of death after PAK and PTA was significantly lower than risk of death while waiting for pa tx. In the PAK category, relative mortality risk after tx (vs. staying on waiting list) was 0.5 (p=0.0006); in the PTA category, relative mortality risk was 0.5 (p=0.02).

Conclusions: The relative risk of death after solitary pa tx (PAK, PTA) is significantly lower than the risk of death while waiting for solitary pa tx. This UNOS/IPTR data analysis shows that survival for nonuremic and posturemic diabetic pts is increased with pa tx.

Abstract# 1547

FAVORABLE EFFECT OF EVEROLIMUS ON CARDIAC ALLOGRAFT VASCULOPATHY IS MAINTAINED THROUGH 24 MONTHS. E. M. Tuzcu,[1] J. A. Kobashigawa,[2] H. J. Eisen,[3] R. C. Starling,[1] T. Crowe,[1] K. Abeywickrama,[4] J. Jaffe.[4] *[1]Cleveland Clinic Foundation, Cleveland, OH; [2]UCLA, Los Angeles, CA; [3]Temple University, Philadelphia, PA; [4]Novartis Pharmaceuticals, E. Hanover, NJ.*

Everolimus (EV, RAD) treatment leads to lower indices of intimal thickness (IT) compared to azathioprine (AZA) at 12 months. Detailed analysis of IVUS parameters of allograft vasculopathy (AV))through 24 months is reported.

Methods: Patients (N=634) were randomized to EV (1.5 or 3 mg/day) or AZA(1.0 -3.0 mg/kg/d) in combination with cyclosporine (CsA), and steroids. Of these, 211 had baseline and 1 year IVUS images matched for a minimum of 11 slices 1mm apart, and 149 had matched baseline and 2 year IVUS; 117 had matched baseline, 1 and 2 year IVUS images. Changes from baseline were calculated.

Results: The 2 y IVUS population was balanced for demography. EV groups demonstrated significantly reduced markers for allograft vasculopathy relative to AZA through 24 months:The change in mean Maximal Intimal Thickness(MIT) (mm) was significantly smaller in EV 1.5 (0.07, p= 0.014) and EV 3 (0.06, p= 0.004) than AZA (0.15), as was change in Mean Intimal Area (IA) in mm2 for EV 1.5 (0.79, p=0.011) and EV 3 (0.83, p=0.012) compared to AZA (1.52). There was less mean % cross sectional area stenosis for EV 1.5 (4.32%, p=0.007) and EV 3 (4.22%, p=0.003) vs AZA (8.62%). The change in Intimal Volume (mm3) of a 10mm segment around the most severely diseased site in a given patient (the site of maximum IA) was also less for EV 1.5 (13.21, p=0.018) and EV 3 (12.54 p=0.011) vs AZA (20.25).

The findings were similar in 117 patients in whom IVUS segments at baseline could be matched at both 1 and 2 y, demonstrating EV treated patients had consistently lower changes in allograft vasculopathy throughout the course of therapy.

Conclusions: Consistent with the 12 month report, EV with CsA reduced indices for AV through 24 months. In patients who were examined by IVUS at 12 and 24 months, increases in all indices of IT were less in Everolimus treated patients in comparison to those receiving conventional treatment.

Abstract# 1548

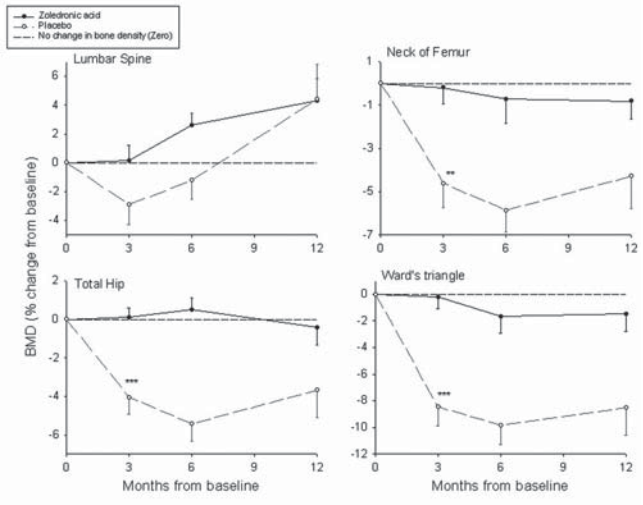
IVIG MODULATION OF ANTI-HLA CLASS I AND II ANTIBODY PRODUCTION IN HIGHLY SENSITIZED PATIENTS IS SELECTIVE, BUT NOT RANDOM: AN ANALYSIS OF THE NIH IG02 STUDY. Mieko Toyoda,[1] Anna Petrosyan,[1] Lara Hilo,[1] Andy Pao,[1] Gail Rodich,[1] Matthew McIntosh,[2] Stanley C. Jordan.[1] *[1]Transplant Immunology Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Statistical Coodinating Center, The EMMMES Corporation, Rockville, MD.*

Backgound: IVIG reduces anti-HLA antibody (Ab) levels and increases rates of transplantation (Tx) in highly sensitized patients (HS pts). This IVIG action may be due to neutralization of anti-HLA Ab, non-specific suppression of Ig production, development of non-responsiveness and/or deletion of anti-HLA specific B cells. Here we examined the effect of IVIG on modulation of Ab production in HS pts treated with IVIG to determine if the IVIG effect is selective by measuring the levels of anti-HLA class I, class II, anti-endothelial cell Ab (AECA) known to be associated with humoral rejection, anti-CMV Ab, total IgG and IgM. **Methods**: 80 HS ESRD pts were treated with IVIG (42) or placebo (38) at 0, 1, 2, 3, 12 and 24 months (NIH IG02 Study). Sera collected before each Rx and additionally at 4, 6 and 18 months were used for quantitation of the Ab levels. Anti-class I, class II (One Lambda) and anti-CMV (BioCheck) ELISA kits, and a cell-based AECA and IgG/IgM ELISAs developed in our lab were used. The results were expressed as ratios of post-Rx to pre-Rx levels. **Results**: Anti-class I, II and anti-CMV levels were significantly reduced in the IVIG group by average of 20, 40 and 25%, respectively during the first 4 months. No reductions were seen in the placebo group. AECA and total IgG levels increased in the IVIG group by 40% during the Rx with no change in the placebo group. IgM levels did not change in either group. The increase in AECA is likely an artifact caused by non-specific binding of IVIG contained in sera in the AECA ELISA since IgG, but not IgM levels increased during the Rx. The levels of anti-class I, II and anti-CMV remained lower than the pre-Rx (by15, 30 and 25 %, respectively) for up to 30 months in 70 % of IVIG treated-pts. Concordance of suppressed Ab levels were observed in most pts. The levels of AECA, total IgG and IgM returned to the pre-Rx levels by 2 months after the IVIG-Rx in most pts. **Conclusions**: 1) IVIG showed non-specific inhibition of Ab production during Rx. 2) The reduced levels of anti-class I, class II and anti-CMV persisted after the IVIG-Rx while AECA and total IgG returned to the pre-Rx levels, suggesting that IVIG selectively, suppressed or deleted B-cells resulting in reducing Ab production. 3) In IVIG responsive HS pts, the persistence of low levels of anti-class I and II Abs contributes to improved chances of receiving a crossmatch (-) kidney transplant.

Abstract# 1549

A DOUBLE BLINDED RANDOMISED CONTROL TRIAL OF ZOLEDRONATE TO PREVENT BONE LOSS IN LIVER TRANSPLANT PATIENTS. G. McCaughan,[2] S. Duke,[1] C. Kam,[2] A. Donaghy,[2] J. Pavlovic,[3] P. Angus,[3] S. Strasser,[2] B. Crawford.[1] *[1]Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW, Australia; [2]Australian National Liver Transplant Unit, Royal Prince Alfred Hospital/University of Sydney, Sydney, NSW, Australia; [3]Victorian Liver Transplant Unit, Austin Hospital, Melbourne, VIC, Australia.*

Earlier studies have shown that significant bone loss occurs within 3 months following liver transplantation. Despite this, there is no data to suggest this bone loss can be prevented with therapeutic intervention.**METHODS:** The aim of the study was to conduct a 12 month, double blinded randomised placebo-controlled trial of zoledronic acid 4 mg, given within 7 days after liver transplantation, and again at months 1, 3, 6 and 9 post-transplant. All patients received calcium carbonate 600 mg and ergocalciferol 1000U daily. We present here the result of the interim analysis of the primary end-point, the 3 month BMD of hip and lumbar spine (LS). BMD was measured on a Lunar (Prodigy) densitometer and the data (g/cm²) was analysed by repeated measures ANOVA. Data is mean ± SEM. **RESULTS**: The 2 treatment groups (n=24, 21) were well balanced with respect to age, sex, underlying disease, baseline BMD and baseline 25 vit D levels. Baseline BMD levels (and mean T scores) in males and females, respectively, were: LS 1.12 ± 0.03 g/cm² (-0.9 SD); 1.01 ± 0.04 g/cm² (-1.6 SD); neck of femur (NOF) 0.95 ± 0.03 g/cm² (-0.8 SD); 0.81 ± 0.04 g/cm² (-1.3 SD); total hip 1.00 ± 0.02 g/cm² (-0.6 SD); 0.85 ± 0.04 g/cm² (-1.2 SD). At all sites the placebo group lost bone density at 3 months, ranging from 2.8% in the LS to 8.0% in the trochanter (Fig 1). This loss was not seen at any site in patients treated with zoledronic acid. The difference between the 2 groups was highly significant for all hip parameters (total hip and Ward's p<0.0001, NOF p=0.002; trochanter p=0.02) and close to significance in LS (p=0.09). In addition, Zolendronate prevented an increase in the urinary bone resorption marker DPD/Cr at the one month time point. **CONCLUSION**: Intravenous zoledronic acid at the time of transplant and 1 month post-transplant prevents the acute bone loss associated with liver transplantation.



Abstract# 1550

RENAL DENDRITIC CELL-MEDIATED ANTIGEN TRAFFICKING. Xiangyang Dong,[1] Lori A. Bachman,[1] Matthew D. Griffin.[1] [1]*Department of Medicine, Division of Nephrology, Mayo Clinic and Foundation, Rochester, MN.*

Background: Genetic variability, infection, injury, or transplantation, may modify DC-mediated antigen trafficking to induce maladaptive immunity. The normal kidney is rich in DCs but the degree to which DC-mediated antigen trafficking occurs between the kidney and its draining lymph nodes during health and disease has not been closely studied. **Methods:** Experiments were carried out in healthy adult mice to examine antigen uptake, trafficking, and presentation by renal DCs (rDCs). DCs from kidney, spleen, renal lymph nodes (RLN), and subcutaneous lymph nodes (SLN) were identified, using antibodies against MHC II and/or CD11c, by flow cytometry of collagenase-digested kidneys, and by immunoperoxidase/immunofluorescence microscopy. Antigen uptake was determined using i.v. injection of filtered (Mr 40,000) and unfiltered (Mr 500,000) FITC-dextran. Staining of RLNs for the kidney-specific protein Tamm Horsfall Protein (THP) was used to detect DC-mediated antigen trafficking from the kidney. Antigen presentation was assayed using adoptive transfer of CFSE-labeled ovalbumin-specific T-cells (DO11.10) and injection of ovalbumin beneath the left renal capsule. Systemic inflammation was induced by i.v. injection of LPS. **Results:** MHC II+ve/CD11c+ve cells with dendritic morphology were observed as an extensive network throughout the cortical interstitium of the healthy adult kidney and within the T-cell zone of the RLNs. rDCs ingested both filtered and unfiltered FITC-dextran particles with higher capacity than splenic DCs. LPS injection resulted in clearance of DCs from the kidney between 24 and 48 hrs later and in a large increase in DC numbers within the RLNs. CD11c+ve cells with intracellular THP+ve material were detected within RLNs (but not SLNs) at 48 hrs and 7 days following LPS injection. These THP-containing DCs were not detected in the absence of LPS. Even in the absence of LPS, injection of ovalbumin beneath the left renal capsule resulted in proliferation of DO11.10 T-cells within the left RLN but not within the right RLN, spleen, or SLN. The extent of this localized antigen-specific T-cell proliferation was significantly augmented by concomitant LPS injection. **Conclusions:** Renal DCs ingest both filtered and unfiltered material in the interstitium. Following a proinflammatory stimulus, rDCs undergo a large-scale migration to RLNs which is associated with transport of kidney-specific proteins and enhancement of localized antigen presentation. Antigen trafficking by rDCs may be a modifiable contributing factor to progressive injury of native and transplanted kidneys.

Abstract# 1551

A TWO STEP MODEL OF ACUTE CD4-MEDIATED CARDIAC ALLOGRAFT REJECTION. Todd J. Grazia,[1] Biagio A. Pietra,[2] Zachary A. Johnson,[3] Robert J. Plenter,[3] Ronald G. Gill.[3] [1]*Department of Pulmonary and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, CO;* [2]*Department of Pediatric Cardiology, The Childrens Hospital, Denver, CO;* [3]*Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO.*

Purpose: To test the hypothesis that graft hematopoietic cells function as primary APCs in acute cardiac rejection, we determined the ability of CD4 T-cells to reject cardiac allografts expressing MHC class II exclusively on hematopoietic or somatic cells respectively.

Methods: B6 MHC class II-deficient (C2D) mice were lethally irradiated followed by grafting with MHC class II+ B6 bone marrow tagged with a transgenic green fluorescent protein (GFP) marker. Conversely, B6 mice were lethally conditioned followed by rescue with C2D (MHC class II -) GFP tagged bone marrow. Complete donor chimerism (B6—>C2D and C2D—>B6) heterotopic cardiac allografts were transplanted into BALB/c Rag1-/- recipients. Purified BALB/c CD4 T-cells were then transferred into heart-grafted BALB/c Rag1-/- hosts and graft function was monitored by daily palpation.

Results: FACS of peripheral blood demonstrated complete (>99%) donor chimerism in B6—>C2D and C2D—>B6 hosts with GFP+ CD45+ cells. FACS analysis of whole heart digests confirmed complete chimerism. CD4 T-cells triggered acute rejection in B6 control allografts (n=5) in <15 days and in B6—>B6 chimeric control allografts in <16 days (n=3). In contrast, MHC class II- (C2D) allografts (n=3) survived >60 days after CD4 T-cell reconstitution. Chimeric (B6—>C2D) hearts, in which MHC class II was expressed only on hematopoietic cells, did not vigorously restore acute allograft rejection with 5 of 6 allografts surviving >60 days post reconstitution. Chimeric (C2D—>B6) hearts, in which MHC class II was expressed only on donor somatic cells survived >60 days post CD4 T-cell reconstitution (n=5). Immunization of C2D—>B6 chimeric allografts on day #3 post CD4 reconstitution with B6Rag1-/- splenocytes (a source of MHC class II+ APCs) resulted in acute rejection (n=4) in <16 days.

Conclusions: Results of these experiments demonstrated that *both* graft hematopoietic and graft somatic MHC class II expression were individually *rate limiting* for CD4-mediated acute cardiac rejection. Importantly, MHC class II+ somatic cells were *not* acting as primary APCs, but in fact were *targets* of activated effector CD4 T-cells. These studies support a straightforward two-step model of CD4-mediated acute cardiac rejection in which the donor graft hematopoietic cells *must* directly stimulate host CD4 T-cells (step 1) which in turn activate graft destructive CD4 T-cells that *must* directly engage MHC class II on the parenchymal compartment of the graft to mediate acute rejection (step 2).

Abstract# 1552

PRETREATMENT WITH FIXED IMMATURE DENDRITIC CELLS OF DONOR STRAIN POWERFULLY PROMOTES ALLOGRAFT ACCEPTANCE. Yon Su Kim,[1] James Kenny,[1] Hee Gyung Kang,[1] Wenda Gao,[1] Terry B. Strom.[1] [1]*Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

The propensity of the immature dendritic cells (DC) to mature phenotype in an inflamed environment may limit the utility of DCs as a means to promote graft acceptance. We hypothesized that peritransplant administration of donor immature DCs may fail to optimally enhance engraftment because of the inability to maintain the immature phenotype of DCs. In order to lock DCs into a stable phenotype, we fixed immature and mature DCs with paraformaldehyde. In this study we have compared the ability of untreated and fixed donor DCs to promote graft acceptance. Cultured bone marrow derived DCs from CB6F1 mice ($H\text{-}2^{b,d}$) were predominantly immature as defined by the expression of MHC and costimulatory molecules and could be fully maturated by stimulation with LPS. Both mature and immature untreated DCs were able to prime naïve allogeneic C3H ($H\text{-}2^{k}$) T cells in mixed culture. These in-vitro allogeneic T cell responses were markedly attenuated when fixed-immature DCs were used as stimulating cells. In contrast, fixed-mature DCs stimulated allogeneic T cell proliferation. In a pancreas islet transplantation model in which the fully MHC mismatched CB6F1 islets were transplanted into C3H mice rendered diabetic by streptozotocin, mean survival time of islets was 14 days in control recipients (n=4). The administration of fresh immature DCs prior to transplantation did not lead to prolonged engraftment (MST; 12 days, n=4). However, consistent with findings in the in-vitro T cell response model, treatment with bone marrow derived fixed-immature CB6F1 DCs 2 weeks before operation, markedly prolonged islet survival (58, >70, >70, >70, >70 days). Interestingly, pretreatment with fixed-mature DCs did not prolong islet graft survival (MST; 16.5 days, n=4). Studies to determine whether true donor specific tolerance is present in the long term C3H recipients are undergoing. Furthermore, T cells from TCR transgenic mice whose TCR recognizes $I\text{-}E^{d}$ presented by $I\text{-}A^{b}$, maintain naïve phenotype as $CD62L^{high}$ after mixed culture with fixed-immature CB6F1 DCs. These findings demonstrated the maintenance of immaturity of DCs is a key factor in modulating alloimmune responses, and suggest therapeutic implications for allograft transplantation.

Abstract# 1553

ACQUISITION OF HLA-DR AND COSTIMULATORY MOLECULES BY T CELLS FROM ANTIGEN PRESENTING CELLS AMPLIFIES HUMAN ALLORESPONSES. David S. Game,[1] Nicola J. Rogers,[1] Robert I. Lechler.[1] [1]*Department of Immunology, Imperial College Faculty of Medicine, Hammersmith Hospital, London, United Kingdom.*

There is accumulating evidence that cell surface molecules may be transferred between cells during an encounter. The aim of these experiments was to determine whether this phenomenon could be relevant to human alloresponses. CD4+ cells were co-cultured with M1 cell (human fibroblasts) transfectants expressing HLA-DR1, CD80 and CD86 alone or in combination. Up to 95% of the allogeneic T cells became positive for HLA-DR and the appropriate costimulatory molecules after only 4 hours of co-culture. This was also observed if the CD4+ cells were pretreated with cyclohexamide implying acquisition rather than up-regulation. Acquisition was confirmed by demonstrating high expression of porcine-CD80 on human T cells after co-culture with M1 transfectants expressing HLA-DR and porcine CD80. The phenomenon required cell contact because transfer was abolished by paraformaldehyde pre-treatment of the M1s and by transwell separation. The presence of cyclosporin, RAD, basiliximab or CD4+CD25+ cells in the initial co-culture did not influence acquisition. Flow cytometric sorting of T cells after co-culture and subsequent mixed lymphocyte assays demonstrated that the T cells that had acquired both HLA-DR and costimulatory molecules could act as potent antigen presenting cells. APC function was demonstrated by enhanced autoproliferation, stimulation of resting autologous CD4+ cells and stimulation of resting allogeneic third party CD4+ cells; equivalent data were obtained for cell proliferation and IL2 secretion. Finally, matured human DCs were also shown to transfer HLA-DR and B7 molecules to CD4+ cells, which could then act as APCs, including stimulation of a CD4+ cell line specific for the acquired HLA-peptide complex. Taken together, these data suggest a novel pathway for the amplification of human alloresponses that is resistant to suppression.

Abstract# 1554

CD40-DEPENDENT SIGNALING IN ENDOTHELIAL CELLS (EC) RESULTS IN THE ASSOCIATION OF THE ADAPTOR PROTEINS TRAF2, TRAF3 AND TRAF6 WITH THE RAS SIGNALING PATHWAY. Jesse A. Flaxenburg,[1,2] David M. Briscoe,[1,2] Soumitro Pal.[1,2] *[1]Transplantation Research Center, Children's Hospital and Brigham and Women's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA.*

Interactions between the cell surface receptor CD40 and its cognate ligand, CD40 ligand (CD40L), are functional in many inflammatory processes including atherosclerosis, autoimmunity and allograft rejection. The activation of CD40 on vascular endothelial cells results in the expression of adhesion molecules, chemokines and vascular endothelial growth factor (VEGF) all of which are functional in cell-mediated immune inflammation. Despite the central role of CD40 signaling in endothelial cells, little is known of the intracellular signaling mechanisms by which CD40-CD40L interactions exert their influence in EC. In previous studies performed in B cells, the signaling molecule Ras has been shown to be a key mediator of CD40 signaling. Here, we have examined the importance of Ras and Ras-associated signaling molecules in CD40-dependent activation of EC. We cotransfected EC with a full-length (2.6kb) VEGF promoter reporter construct and either an active mutant of Ras (12V) or a dominant negative mutant of Ras (DN). Ras 12V resulted in VEGF transcriptional activation similar to that found with ligation of CD40 (using sCD40L); and CD40-dependent overexpression of VEGF was reduced by Ras DN (up to 90%). This demonstrates that CD40 signaling in EC is mediated through Ras. By immunoprecipitation, we further identified that CD40 activation results in a time-dependent association between Ras and its downstream effector molecules, Raf, Rho and PI-3 Kinase. Because the cytoplasmic tail of CD40 lacks intrinsic kinase activity, we next wished to identify the adaptor protein(s) that link CD40 ligation with activation of Ras. Our initial results through immunoprecipitation studies demonstrated that CD40-CD40L interactions in EC promote the association of Ras with the adaptor molecules TRAF2, TRAF3 and TRAF6 in a time-dependent manner. Together, these studies are the first, to our knowledge, to identify the TRAF molecules as key mediators of CD40 signaling in EC. Because CD40 is expressed on human but not mouse EC, we suggest that this pathway may represent an important proinflammatory signaling cascade unique to humans. Thus, this pathway in humans may be an important consideration in designing novel therapies to prevent graft rejection and vasculopathy.

Abstract# 1555

TARGETING OF ADHESION MOLECULES THAT REGULATE CC CHEMOKINE-INDUCED TRANSENDOTHELIAL MIGRATION OF DC SUBSETS. B. L. Colvin,[1] A. H. Lau,[1] A. M. Schell,[1] A. W. Thomson.[1] *[1]T.E. Starzl Transplantation Inst, U of Pittsburgh, Pittsburgh, PA.*

Immature dendritic cells (DC) are induced to migrate to sites of inflammation by so-called "inducible" chemokines. Upon uptake of antigen (Ag) and maturation, DC are directed through endothelial cell (EC)-lined lymphatic or blood vessel walls by "constitutive" chemokines to draining secondary lymphoid tissues. Therein they present Ag to naïve and memory T cells, initiating the immune response. We have reported that mature (m) murine CD8α$^+$ DC are able to migrate, albeit poorly compared to CD8α$^-$ mDC, in response to CCL19 (macrophage inflammatory protein-3β) and CCL21 (secondary lymphoid chemokine) that are constitutively expressed in T cell areas and high endothelial venules, respectively, by quantifying their *in vitro* chemotaxis through unmodified Transwell® filters. We hypothesized that the inferior migratory ability of CD8α$^+$ mDC might be due to their low surface expression of the adhesion molecule CD11b, which may hamper their ability to adhere to filter pores *in vitro* and/or endothelial cells *in vitro/in vivo*. To test whether expression of this and/or other adhesion molecules plays a role in regulating CD8α$^+$ DC migration, we analyzed whether incubating CD8α$^-$ mDC with blocking mAbs against CD11b would reduce their migration to the level of CD8α$^+$ DC. We further tested whether blocking adhesion molecules expressed on both subsets of DC (e.g. CD31, CD54, CD62L) would affect their transendothelial migration. Resting or TNFα-activated mouse EC were layered on 5 μm, Transwell® filters, and overnight-matured splenic, magnetic bead-sorted DC were placed into the upper wells of Transwell® chambers for 4 h (for resting EC) or 2 h (for activated EC). Migrated DC then were removed from the lower chamber and enumerated using a Coulter counter. Both DC subsets migrated to CCL19 through resting EC (albeit less than in the absence of EC), and migration through TNFα-activated EC was enhanced. Contrary to reports for human monocyte-derived DC, transendothelial migration of the murine mDC was markedly impaired when CD54 was blocked by specific mAb, but migration was not impaired by blocking CD31 or CD11b. Interestingly, mDC showed decreased migration to CCL19 but not CCL21 when CD62L was blocked. This is the first report evaluating adhesion molecules involved in directing murine DC subset transendothelial migration *in vitro*, and the data implicate specific adhesion molecules in facilitating this migration *in vivo*. These molecules may serve as targets for interrupting trafficking of immunostimulatory DC to secondary lymphoid organs.

Abstract# 1556

INFLUENCE OF DIRECT AND INDIRECT ALLORECOGNITION PATHWAYS ON CD4+CD25+ REGULATORY T CELL FUNCTION IN TRANSPLANTATION. Alberto Sanchez-Fueyo,[1] Christoph Domenig,[1] Christoph Mariat,[1] Sophoclis Alexopoulos,[1] Terry B. Strom,[1] Xin Xiao Zheng.[1] *[1]Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

Alloantigens are recognized through direct and indirect pathways, both of which are involved in allograft rejection. In contrast, tolerance may be a phenomenon exclusively dependent on indirectly-triggered immunoregulation. Hence, it has been postulated that CD4+CD25+ regulatory T cells (Tregs), required for tolerance induction in multiple models, may also have indirectly-biased allospecificity. In the current study, we have sought to dissect the influence of direct and indirect allorecognition on Treg function by using an adoptive transfer model in a variety of different strain combinations. To assess how the loss of the direct pathway influences Treg suppressive function, we used 3 different skin allograft models: (1) Balb/c Rag$^{-/-}$ (H-2^d) hosts grafted with MHC-II$^{-/-}$ B6 (H-2^b) grafts, (2) Balb/c Rag$^{-/-}$ mice transplanted with B10D2 minor histocompatibility mismatched grafts, and (3) B6 Rag$^{-/-}$ (H-2^b) hosts grafted with DBA/2 (H-2^d) chimeric skin bearing B6 antigen presenting cells only. Allograft recipients were then injected with titrated numbers of syngeneic CD4+CD25- and/or Tregs. As compared with the corresponding controls, performed using wild type MHC mismatched allografts, in the 3 experimental settings in which direct allopresentation was unavailable, CD4+CD25- T cells were less capable of inducing allograft rejection. In contrast, Tregs were more effective on a cell-per-cell basis in protecting the graft. This indicated that the absence of direct allorecognition favored regulatory over effector immune responses. We then tested whether indirect allorecognition was actually indispensable for Treg function, and repeated the transfer experiments after grafting DBA/2 skin allografts onto either B6 Rag$^{-/-}$/MHC-II$^{-/-}$ or B6 Rag$^{-/-}$/MHC-II$^{+/+}$ hosts. CD4$^+$CD25$^-$ T cells alone rapidly rejected allografts placed onto MHC$^{-/-}$ recipients. In contrast, Tregs transferred into MHC-II$^{-/-}$ hosts exhibited a much weaker capacity to suppress CD4$^+$CD25$^-$ T cells than when injeted into MHC-II$^{+/+}$recipients. Although Tregs could indeed prevent effector T cells from rejecting the grafts in the MHC-II$^{-/-}$ hosts, this required administration of a very high Treg to CD4$^+$CD25$^-$ T cell ratio (4:1). In short, CD4$^+$CD25$^+$ T cells can suppress both directly and indirectly generated alloimmune responses *in vivo*. However, in the absence of indirect presentation, Tregs exert weak suppressive effects. This is consistent with the Treg repertoire being biased towards self-MHC recognition.

Abstract# 1557

A NEW MHC CLASS II-RESTRICTED TCR TRANSGENIC MOUSE MODEL FOR THE STUDY OF ALLOIMMUNITY. Todd V. Brennan,[1] Kym R. Garrod,[1] Catherine K. Chang,[1] Sang-Mo Kang.[1] *[1]Surgery, Division of Transplantation, University of California San Francisco, San Francisco, CA.*

T cell receptor (TCR) transgenic (tg) mice have revolutionized the study of T cell responses *in vivo*. Adoptive transfer of TCR-tg T cells facilitates precise tracking, as well as phenotypic and functional analysis of antigen-specific T cells *in vivo* under both stimulatory and tolerogenic conditions. The well-established 2C TCR-tg model has revealed important insights into the role of CD8+ alloreactive T cells in graft rejection. However, the lack of a complementary CD4+ allospecific TCR-tg model has limited detailed mechanistic studies of the role of CD4+ T cells in allograft rejection. We therefore sought to develop a CD4+ restricted TCR-tg mouse with direct specificity against donor MHCII and with a strain specificity identical to the 2C TCR-tg mouse (B6 → Balb/c). B6 mice were immunized with mature Balb/c dendritic cells (DCs). Sorted CD4+ T cells from immunized mice were restimulated with Balb/c DCs *ex vivo* and then fused with the BW5147a-b- murine thymoma cell line. The resulting hybridomas were screened by their ability to produce IL-2 in response to Balb/c DCs. From reactive clones, cDNA was produced and the TCR alpha and TCR beta subtypes were identified by sequencing PCR products obtained using consensus primers spanning the TCR variable (V) and constant (C) regions. V and C specific primers were then used to isolated full-length TCR chains, which were cloned into transgenic expression vectors with the TCR alpha (Va8) and TCR beta (Vb13) chain under the control of the human CD2 and the murine CD4 promoters, respectively. Both transgenes were injected together into DBA2/B6 embryos. A founder mouse (4C) expressing Vb13 on >99% of peripheral CD4+ T cells was identified and back-bred onto the B6/Ly5.1 background. RT-PCR was used to confirm Va8 expression. We find the 4C TCR-tg CD4+ T cells to be specific to H-2d antigen *in vitro* by MLR and *in vivo* by CFSE dilution assays of adoptively transferred 4C CD4+ T cells into Balb/c hosts. In addition, we find the 4C TCR-tg to be able to reject Balb/c skin transplants in 10-13d. We have thus developed a CD4+ TCR-tg mouse with direct alloreactivity to Balb/c. Adoptive transfer of TCR-tg T cells from the 4C mouse and the 2C mouse will provide a novel means of observing the cooperativity between the CD4+ and CD8+ T cell compartments during transplant rejection as well as the induction of allospecific tolerance.

Abstract# 1558

INDIVIDUAL ROLES OF DONOR AND HOST APC IN THE PRESENTATION TO CD8+ T CELLS OF DONOR-DERIVED CLASS I-RESTRICTED MHAG. Stacy L. Dalheimer,[1] David M. Richards,[1] Daniel L. Mueller.[1] *[1]Center for Immunology and Department of Medicine, University of Minnesota, Minneapolis, MN.*

Indirect recognition of donor-derived mHAg has been shown to play an important role in the targeting of grafts for destruction by CD8+ T cells. Naive T cell activation is thought to depend on the recognition of Ag/MHC complexes on professional APC such as DC within the draining lymph nodes. Recipient APC that pick up donor mHAg may then present the Ag to alloreactive CD8+ T cells; however, Ag may also be presented to the T cells by donor-derived APC carried within the graft if there is sharing of class I alleles between the donor and host. Investigations of CD8+ alloreactivity against mHAg have been complicated because lymphoid populations are heterogeneous in specificity and the important graft target Ag are poorly understood. We developed a model of skin allograft rejection that relies on the adoptive transfer of OT-I TCR-transgenic CD8+ T cells (specific for OVAp SIINFEKL in the context of Kb) into recipient mice and the transplantation of skin from either MHC identical or MHC mismatched mice that carry a transgene for a transmembrane form of OVA protein. Using wild-type B6 (Kb+) or mutant bm8 (Kb-) graft recipient mice, and either B6-OVA (Kb+) or BALB/c-OVA (Kb-) donors, it was determined that both donor and recipient APC were competent to induce CFSE dye-dilution and a 5 fold clonal expansion of the OVA-reactive OT-I CD8+ T cells in the graft tissue. In contrast, OT-1 CD8+ T cell enrichment within allograft tissue proved to be highly dependant on the presence of Kb on both donor and recipient cells. OT-I T cells reached 80% of total infiltrating CD8+ T cells when mHAg could be presented by both donor and recipient cells, whereas enrichment decreased to only 17% when only recipient APC could present the mHAg and 7% when only donor-derived Kb was available. To determine whether the decrease in OT-I enrichment observed in MHC-mismatched BALB/c-OVA allografts was due to competition with other endogenous H-2d reactive CD8+ T cells or the lack of a Kb molecule on the donor cells, we used (BALB/c x B6) F1 OVA donors. The OT-I T cells found in the F1 OVA graft tissue represented 55% of the total CD8+ T cells, a frequency more than twice that observed in the mismatched BALB/c-OVA grafts. Thus, the cross-presentation of mHAg by host APC can be an important stimulus for the clonal expansion of mHAg-specific CD8+ T cells within the draining lymph nodes. Nevertheless, class I MHC matching between donor and host can promote graft infiltration and the retention of mHAg-reactive CD8+ T cells. Work supported by NIH PO1 AI50162.

BK VIRUS

Abstract# 1559

NATIONAL SURVEY OF POLYOMA (BK) VIRUS IN RENAL TRANSPLANT RECIPIENTS (RTR). P. Kadambi,[1] R. Baliga,[1] B. Javaid,[1] H. Robert,[2] W. James,[2] J. Thistlethwaite,[2] M. Josephson.[1] *[1]Department of Medicine, Univ of Chicago, Chicago, IL; [2]Department of Surgery, Univ of Chicago, Chicago, IL.*

BK virus associated nephropathy (BKVN) is a growing concern in RTR. There are no standardized practice guidelines for this condition. We conducted a national survey to study current practices in the transplant community.

Methods: We mailed a survey about current management of BKVN to 280 UNOS certified kidney and kidney pancreas txp ctr. 25% (68) have responded to date. The survey was filled out by 28 surgeons (41%), 36 nephrologists (53%), 1 RN (1%), 2 transplant physicians (3%) and 1administrator (1%).

Immunosuppression (IS): Most ctr used a combination of Tacrolimus (tac), MMF, Sirolimus, Cyclosporine A, Azathioprine and steroids. 8, 6, and 5 did not use steroids, tac and MMF respectively. Induction therapy was used routinely at 82% (56) ctr of which 66% used thymoglobulin (37), 39% basiliximab (22), 21%daclizumab (12), OKT3 and Campath were each used by 2% (1).

Results: 95% (65/68) ctr have seen BKVN accounting for 320 cases. Male: female ≈ 2: 1, Whites: Blacks: Hispanics ≈ 50%:35%:10%. 47% (32) perform BK surveillance, 18% (12) in all pts, 26% (18) for increased serum creatinine (Scr) and 3% (2) for other reasons. Blood BKV PCR (BBKV) was performed at 31% (21), Urine BKV PCR (UBKV) at 30% (20), Scr at 12% (8), and Urine decoy cells (DC) at 16% (11). Diagnosis was established by kidney biopsy (bx) at 16% (11), bx with immunohistochemistry (IHC) at 78% (53). Graft loss occured in 50% (159). Of the remaining allografts, 50% had an increased Scr. Interestingly, 8 ctr that accounted for 149 cases (46%), lost only 22 grafts (14%), of which 6 performed routine surveillance (UDC, BBKV and UBKV) and 2 performed surveillence in patients with increased Scr (UDC and bx with IHC). Of the 4 centers that did not see BKVN, 3 did not perform surveillance, 2 did not use steroids and 3 did not use tac.

Management: Of the 65 ctr, IS was reduced at 40% (26), reduced IS+ cidofovir at 51% (33), reduced IS+ leflunomide at 9% (6) and reduced IS+ IVIG at 14% (9). Post diagnosis monitoring was done primarily by UBKV at 66% (43) and BBKV at 75% (49), while 40% (26) performed follow-up bx. Reason to stop treatment: Negative PCR at 51% (33), stable Scr at 20% (13) and negative IHC stain on bx at 27%(18).

Conclusions: 1. Many centers now see BKVN. 2. Graft loss due to BKVN is high. 3. Centers vary in their approach toward surveillance and management. 4. Centers with more cases may have better outcomes. 5. These results suggest that increased surveillance might lead to early diagnosis and intervention with improved long-term graft survival.

Abstract# 1560

HISTOLOGIC GRADING OF PVN BIOPSIES: CORRELATION WITH VIRAL LOAD MONITORING AND RENAL FUNCTION. J. Trofe,[1] E. Woodle,[1] J. Gordon,[2] G. Wadih,[1] P. Succop,[1] R. R. Alloway,[1] G. Mutema,[1] T. Cavallo,[1] S. Heffelfinger,[1] M. A. Cardi,[3] J. Austin,[3] S. Goel,[3] S. Safdar,[3] S. Huang,[3] S. Raabe,[3] R. Munda,[1] J. Buell,[1] M. Hanaway,[1] J. W. Alexander,[1] R. Boardman,[1] C. Rogers,[1] M. Kidd,[1] A. Jain,[1] E. Berilla,[3] A. Demmey,[3] K. Khalili,[2] P. Roy-Chaudhury.[1] *[1]Div of Transplantation, Univ of Cincinnati, OH; [2]Ctr for Neurovirology/Cancer Biology, Temple Univ, Phila, PA; [3]The Christ Hospital, Cincinnati, OH.*

Correlation of BKV molecular parameters with PVN biopsy (bx) findings have not been defined. **Purpose:** Establish correlations between histologic injury patterns (acute or chronic), molecular parameters (BKV load monitoring, BKVantibody titer) and creatinine (SCr). **Methods:** 26 bx from 11 pts diagnosed w/ PVN and w/o concurrent rejection were reviewed. Bx were assigned an overall "acute injury" score based on: degree of in-situ hybridization for BKV, acute cytopathic changes, and presence of acute interstitial infiltrates. A "chronic injury" score was assigned based on: chronic interstitial fibrosis/tubular atrophy and glomerulosclerosis. Acute and chronic pathology were graded on a semi-quantitative scale 0= no PVN, 1=mild, 2= moderate, 3= severe. Bx data was correlated with BKV urine and serum loads (Taqman PCR), BKV antibody titer (Hemagglutination assay), and SCr at time of bx. Acute and chronic bx scores were also divided into 2 groups: low (score 0-1) and high (score 2-3). Statistics: Point-biserial correlations were used for BKV serum and urine loads, BKV antibody titers, SCr and bx scores while t tests assuming unequal variance were used to compare low and high acute and chronic scores. **Results:** BKV urine loads correlated well with acute bx scores ($p=0.004$, $r=0.57$) but BKV serum loads, BKVantibody titers or SCr did not correlate with overall acute bx scores. BKV urine loads were significantly lower in pts with a lower acute score (1.52×10^7 vs 9.98×10^8 cop/mL, $p = 0.02$). SCr correlated well with overall chronic bx scores ($p=0.001$, $r = 0.62$), but BKV urine, serum loads or BKV antibody titers did not correlate with overall chronic bx scores. **Conclusions:** 1) BKV urine load, but not BKV serum load may provide a good marker of acute histologic injury in PVN. 2) In contrast, chronic injury score correlated well with renal function. 3) These results indicate that BKV urine may provide a reliable non-invasive marker of BKV induced injury and provide a useful therapeutic monitoring tool for PVN.

Abstract# 1561

THE ADVERSE IMPACT OF PRETRANSPLANT BK POLYOMA VIRUS INFECTION ON RENAL ALLOGRAFT FUNCTION. Vivekanand Jha,[1] Ashiq Hayat,[1] Srinivasan Radhika,[2] Manupdesh S. Sachdeva,[3] Ritambhara Nada,[3] Harbir S. Kohli,[1] Kamal Sud,[1] Krishan L. Gupta,[1] Kusum Joshi,[3] Vinay Sakhuja.[1] *[1]Nephrology, Postgraduate Medical Institute, Chandigarh, India; [2]Cytology, Postgraduate Medical Institute, Chandigarh, India; [3]Histopathology, Postgraduate Medical Institute, Chandigarh, India.*

The frequency of BK polyoma virus (BKV) infection before renal transplantation and its impact on graft function has not been studied. This prospective study was undertaken over an 18-month period with a view to 1. estimate the prevalence of BKV excretion in urine in CRF patients awaiting transplantation and their donors, and 2. assess the impact of BKV infection on graft function in living donor transplants. Urine was screened for the presence of "decoy cells" on Papanicoloau smear, immunohistochemistry using antibodies against SV-40 T antigen and electron microscopy before transplant, at the end of 3 months after transplantation and during episodes of graft dysfunction. BK virus infection was looked for in graft biopsies on liight microscopy and immunoperoxidase stains. All patients received cyclosporine-based immunosuppression. Immunosuppression was reduced in those with biopsy-proved BKV infection. A total of 68 patients (age 34.1+11.3 years, 59 males) were included in the study. Urinary decoy cell excretion were documented in 22 (32.4%) patients and 4 (5.9%) donors ($p<0.0001$). Post-transplant urine cytology was positive in 24 of 64 (37.5%) cases. Acute graft dysfunction developed in 25 patients, all of whom underwent a biopsy. BK viruria was significantly more frequent in those with acute graft dysfunction (63% v 28%, $p=0.006$). Anti-rejection therapy was given to 24 cases; 18 responded completely, 3 had a partial response, and 4 were non-responders. A total of 5 patients showed evidence of BKV infection on immunohistochemistry of renal biopsy. All 5 patients showed decoy cells on Pap smear. When compared to patients without BKV infection on biopsy, these patients had a higher follow-up serum creatinine (3.42+1.59 v 1.8+1.77 mg/dl; $p=0.032$). Also, the mean follow-up serum creatinine was higher in those with BK viruria compared to BKV-negative patients (1.42+0.55 v 2.01+1.39 mg/dl; $p=0.029$). To conclude, 1. the prevalence of BKV infection is increased in CRF patients, 2. BK viruria is associated with increased incidence of acute graft dysfunction and poorer long-term graft function, 3. absence of decoy cells in the urine virtually rules out the possibility of BKV infection of the allograft, and 4. the outcome of patients with biopsy-documented BKV infection of the allograft is poor despite reduction of immunosuppressive therapy.

Abstract# 1562

FACTORS ASSOCIATED WITH DEVELOPMENT OF BK NEPHROPATHY AMONG PATIENTS RECEIVING KIDNEY TRANSPLANTS AT A SINGLE CENTER DURING 2001. Hasan A. Khamash,[1] Adit Mahale,[1] Christopher K. Buehrig,[1] Fernando C. Cosio,[1] Donna J. Lager,[2] Mary E. Fidler,[2] Thomas R. Schwab,[1] James M. Gloor,[1] Sandra J. Taler,[1] Stephen C. Textor,[1] Mark D. Stegall,[3] Timothy S. Larson,[1] Matthew D. Griffin.[1] *[1]Dept. Medicine, Divisions of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Dept. Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [3]Dept. Surgery, Division of Transplantation Surgery, Mayo Clinic, Rochester, MN.*

Background and Aims: Use of highly potent immunosuppression has resulted in substantial reduction of acute rejection rates among kidney transplant (KTx) recipients. Counteracting this success has been the emergence, since 1999, of BK nephropathy (BKN) as a major cause of KTx injury. At our center, the occurrence of BKN (diagnosed by either surveillance "protocol" biopsy or by clinically-indicated biopsy) peaked among patients transplanted in 2001. We sought to determine the clinical factors associated with development of biopsy-proven BKN within this cohort. **Methods:** Clinical and laboratory data was reviewed for 238 patients receiving KTx between 1/1/01 and 12/31/01. Ten recipients with graft failure or death within 4 months of transplantation were excluded. Of the remaining 228, 24 have been diagnosed with BKN at a mean of 11.8± 6.3 months post-transplant. These 24 recipients were compared with the 204 who have not developed BKN. **Results:** See table:

	BKN (n = 24)	No BKN (n = 204)	P
Age at Transplantation (yrs)*	54.8 ± 11.6	47.8 ± 15.5	0.03
Gender (M:F)	1:1.6	1:0.8 0.23	
Deceased Donor (n , %)	3 (12%)	36 (18%)	0.45
Donor-neg / Recipient-neg for CMV (n , %)	3 (12%)	55 (27%)	0.06
Number of HLA Mismatches*	2.92 ± 1.63	2.83 ± 1.66	0.76
Acute Rejection (clinical + subclinical) During First Year (n , %)	4 (17%)	22 (11%)	0.43
Anti-Thymocyte Globulin Induction (n , %)	23 (96%)	179 (88%)	0.82
Tacrolimus-based Immunosuppression (n , %)	21 (88%)	153 (75%)	0.62
GFR (Iothalamate) 1 Month Post-Transplant (mL/min/SA)*	56.1 ± 17.6	56.1 ± 19.1	0.99
GFR (Iothalamate) 1 Year Post-Transplant (mL/min/SA)*	41.1 ± 13.8	55.0 ± 20.5	0.002

* Results expressed as mean ± SD

Conclusions: (a) Development of BKN in this cohort was associated with older recipient age but not with greater degree of MHC mismatching, higher rate of acute rejection, or early graft dysfunction. (b) There was a trend towards lower frequency of D-/R- serologic status for CMV among recipients who developed BKN. (c) Loss of GFR between 1 and 12 months post-transplant was predominantly accounted for by BKN among these 228 recipients.

Abstract# 1563

POLYOMA NEPHROPATHY: LEFLUNOMIDE BLOOD LEVELS NEEDED FOR CONTROL OF THIS INFECTION. Michelle Josephson,[1] Basit Javaid,[1] Harland Robert,[1] Rajivv Poduval,[1] Pradeep Kadambi,[1] Preston Foster,[2] Garfinkel Marc,[1] Walter Atwood,[3] Shane Meehan,[1] Michael Millis,[1] James Williams, Dick Thistlethwaite. *[1]Nephrology, University of Chicago, Chicago, IL; [2]Surgery, Texas Transplant Institute, San Antonio, TX; [3]Molecular Biology and Immunology, Brown University, Providence, RI.*

OBJECTIVE: Polyoma BK virus (BKV) is a significant cause of renal allograft loss and no specific treatment is known. Leflunomide (Arava, Aventis) is a potent immune suppressant and recent *in vitro* data showing BKV inhibition has prompted our evaluation of leflunomide as BKV therapy.

METHODS: From Nov., 2001 to Aug., 2003, 21 patients with biopsy documented BKV nephropathy and 3 to 26 months of study, were treated with lef alone (n=11) or lef+Cidofovir (Cidof 4-14 doses) n=10. CellCept was stopped, Prograff and pred essentially unchanged, and the targeted lef blood level, measured by HPLC, was 50 ug/ml to100 ug/ml. The patients were followed with frequent assessment of bone marrow and liver functions and graft biopsy was repeated as indicated. The % changes in viral load (PCR measured) and serum creatinine were analyzed for significance using the Wilcoxan test.

RESULTS: There was a significant decrease in blood and urine viral loads in both groups ($p<0.05$ at 2 and 3 months compared to baseline), with falling titers in 20/21 patients. One patient (lef alone) did not initially experience a decrease in viral load or improvment on biopsy; however, he was sub-therapeutic for lef (lef level 30ug/ml) and responded with additional therapy. Repeat biopsy in 16 others showed resolution or substantial improvement of BK virus stainining (SV40). Although renal function remained stable, blood viral titers rose in two additional patients when blood levels of lef fell below 35 ug/ml. Another patient (lef + Cidof) with pre-existing advanced renal damage (Cr 4.5) required dialysis despite reduced viral load. The inclusion of Cidofovir did not significantly improve viral load or renal function compared to lef alone. ($p>0.05$) One patient died of unrelated causes. No significant changes in liver or bone marrow function have appeared in the 19, all of whom are still receiving leflunomide.

CONCLUSION: Leflunomide blood levels above 35 ug/ml provide effective BKV therapy and adding Cidofovir does not appear to improve viral clearance nor renal function. Leflunomide may have the singular advantage of providing both effective viral treatment and immunosuppression. An understanding of the molecular basis for this activity could open a fresh approach to both anti viral and immune suppressant drug discovery.

Abstract# 1564

USE OF FLUOROQUINOLONES TO TREAT BK INFECTION IN RENAL TRANSPLANT RECIPIENTS. Anil Chandraker,[1] Syed Ali,[2] Cynthia B. Drachenberg,[3] Ravinder Wali,[3] Hans Hirsch,[4] James DeCaprio,[2] Emilio Ramos.[3] *[1]Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital, Boston, MA; [2]Dana Faber Cancer Institute, Boston, MA; [3]University of Maryland, Baltimore, MD; [4]Department of Internal Medicine, University of Basel, Basel, Switzerland.*

We have previously demonstrated the in-vitro effectiveness of a variety of quinolones, namely ciprofloxacin, ofloxacin, levofloxacin and trovofloxacin against the BK related polyoma virus SV40. All four quinolones were shown to inhibit the formation of viral plaques in a viral plaque inhibition study. They also inhibited viral DNA replication. Mechanistic studies indicated that the quinolones were able to inhibit the ability of the SV40 large T antigen (T Ag) to function as a helicase (separating double stranded DNA in preparation for replication). In this study we tested the in vitro and in vivo effectiveness of Gatifloxacin against polyoma virus. In vitro in a T Ag assay, Gatifloxacin at a 0.5 mM concentration was shown to inhibit SV40 T Ag function by > 50%. We next studied the ability of Gatifloxacin to treat renal transplant patients with evidence of active BK viral replication. Ten patients who had evidence of active BK polyoma virus infection were treated with a 10 day course of Gatifloxacin, 500mg po qd. Evidence of BK viral replication was checked by testing blood for viral load of BK virus and urine for the presence of decoy cells immediately prior to treatment. Immunosuppression was not decreased at the time of treatment with the quinolone. Mean time prior to treatment that immunosuppression was decreased was 6 months (range 0.75-11 months). Blood and urine were checked again 2 months after completion of treatment. All patients tolerated the antibiotic with no reported serious side effects. Seven of the 10 patients responded with a significant reduction in viremia (> 80%) or disappearance/reduction of decoy cells in the urine.

Patient	Gender	Pre Cytology	Post Cytology	Pre Blood PCR	Post Blood PCR
1	Male	+	-	509,000	92,000
2	Female	+	-	121,000	17,000
3	Female	+	-	119,000	15,000
4	Female	+	-	236,000	awaited
5	Female	+	-	0	0
6	Male	+	±	0	0
7	Female	+	±	0	0
8	Male	+	+	0	0
9	Male	+	+	80,400	256,000
10	Female	+	+	96,000	118,000*

* Viral load subsequently dropped to 56,000

One further patient had a 50% decrease in viremia at a later time point. These data indicate that Gatifloxacin is effective against active BK viral infection in renal transplant recipients.

Abstract# 1565

URINE DECOY CELL SHEDDING IS LESS SENSITIVE THAN QUANTITATIVE PCR FOR SCREENING POLYOMAVIRUS INFECTION IN KIDNEY TRANSPLANT RECIPIENTS. Parmjeet S. Randhawa,[1] Andrew Ho,[1] Abhay Vats,[2] Ron Shapiro,[3] Karen Weck.[1] *[1]Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA; [3]Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA.*

Background: Urine cytology has been suggested as a simple technique to screen kidney transplant patients for BK virus (BKV) activation. Virus infected 'decoy cells' can be always found in all patients with histologic nephropathy, and in up to 29% of stable transplant recipients. We have sought to determine whether using PCR as the primary screening technique would offer significant advantages over urine cytology

Design: 100 urine samples from 62 kidney transplant patients were examined for decoy cells by light microscopy. BK and JC virus load was assessed in aliquots of the same samples by quantitative real time PCR using the Roche LightCycler.

Results: Definite decoy cells were seen in 8% and possible decoy cells in 5% of samples. Quantitative PCR showed BKV DNA in all the preceding samples, and in additional 18% of samples in which no decoy cells could be detected. BKV genomic copy number in the positive samples varied from <200 to 1.49E+09 copies/ml. Thus, the use of decoy cells as a marker of polyomavirus viruria has a sensitivity of 41.9% and negative predictive value of 82.8%. The specificity and positive predictive value are 100%. False negative results occurred in samples with suboptimal cellularity, vaginal contamination, and a low viral load. One patient with a false negative urine cytology developed BKV nephropathy on follow up. Two samples from a single patient had a mixed BK and JC virus infection. Four samples from 3 patients with JC viruria were negative for decoy cells.

Conclusion: Compared to PCR, urine cytology has lower sensitivity. It cannot detect free viral particles or distinguish BK virus from JC virus. Finally, it may not be informative if the viral load is below the critical mass needed for the formation of intranuclear inclusions detectable by light microscopy.

Abstract# 1566

BK NEPHROPATHY MANAGED BY REDUCED IMMUNOSUPPRESSION: PROGRESSIVE LOSS OF GRAFT FUNCTION IS DICTATED BY TIMING OF DIAGNOSIS AND BY EXTENT OF THE INITIAL FIBROTIC INJURY. Hasan A. Khamash,[1] Adit Mahale,[1] Christopher K. Buehrig,[1] Fernando C. Coisio,[1] Donna J. Lager,[2] Mary E. Fidler,[2] Thomas R. Schwab,[1] James M. Gloor,[1] Sandra J. Taler,[1] Stephen C. Textor,[1] Mark D. Stegall,[3] Timothy S. Larson,[1] Matthew D. Griffin.[1] *[1]Dept Medicine, Divisions of Nephrology and Hypertension, Mayo Clinic and Foundation, Rochester, MN; [2]Dept. Laboratory Medicine and Pathology, Mayo Clinic and Foundation, Rochester, MN; [3]Dept. Surgery, Division of Transplantation Surgery, Mayo Clinic and Foundation, Rochester, MN.*

Background and Aims: Diagnosis of BK nephropathy (BKN) in renal transplants is frequently followed by loss of function (LOF). Reduction of immunosuppression (IS) remains the central treatment and repeated graft biopsy is considered the "gold standard" for determining persistence of BKN. We examined the factors contributing to progressive LOF in biopsy-proven BKN managed by reduced IS alone. **Methods:** Clinical, laboratory and histologic data was reviewed for 37 renal allograft recipients with biopsy-proven BKN, managed by reduced IS (without antiviral therapy) with at least 1 year of follow-up. Follow-up biopsies were carried out on 32 of 37 cases and 6 grafts were lost during the first year post-diagnosis. GFR at diagnosis of BKN and 1 year later was estimated by modified MDRD formula. The cases were subdivided into those with stable GFR (< 10% loss from diagnosis to 1 year) and those with progressive LOF (≥ 10% loss). **Results:** Results (see table) are shown as mean ± SD.

Conclusions: (a) Almost 50% of the BKN cases managed by reduced IS alone had progressive LOF during the first year following diagnosis. (b) Diagnosis later post-transplantation and degree of chronic tubulointerstitial injury at the time of diagnosis were associated with progressive LOF. (c) Increased tubulointerstitial injury during the first year following BKN diagnosis was also observed in grafts with stable GFR. (d) Absence of BKV on follow-up biopsy did not guarantee stable graft function.

	All Patients (n = 37)	GFR Loss < 10% (n = 19)	GFR Loss ≥ 10% (n = 18)	p
Age at Transplantation (yrs)	53.7±13.2	55.5±14.5	51.8±11.6	0.28
Time of BKN Diagnosis (mths post Tx.)	11.8±10.3	9.2±9.9	14.5±10.3	0.02
GFR (ml/min) at BKN diagnosis	33.4±12.5	35.4±10.4	31.2±14.4	0.31
GFR (ml/min) at i year post-diagnosis	34.0±12.8	38.8±11.1	27.2±12.3	0.01
Banff i + t on diagnostic biopsy	3.14±1.60	3.37±1.42	2.89±1.78	0.37
Banff ci + ct on diagnostic biopsy	2.49±1.73	1.79±1.44	3.22±1.73	0.01
Banff i + t on follow-up biopsy	1.78±1.70	1.53±1.65	2.15±1.77	0.36
Banff ci + ct on follow-up biopsy	3.36±1.88	3.47±2.09	3.85±1.57	0.60
Follow-up biopsy negative for BKV	21 of 32	13 of 19	8 of 13	NS

Abstract# 1567

BKV-SPECIFIC CELLULAR IMMUNITY IN PEDIATRIC PATIENTS WITH ACTIVE BKV INFECTION AFTER KIDNEY TRANSPLANTATION. Patrizia Comoli,[1] Alberta Azzi,[2] Sabrina Basso,[1] Gerardo Botti,[3] Giancarlo Basile,[3] Iris Fontana,[4] Angela Cometa,[1] Francesco Perfumo,[3] Umberto Valente,[4] Franco Locatelli,[1] Rita Maccario,[1] Fabrizio Ginevri.[3] *[1]Transplant Immunology and Pediatric Hematology/Oncology, IRCCS Policlinico San Matteo, Pavia, Italy; [2]Department of Public Health, University of Firenze, Firenze, Italy; [3]Pediatric Nephrology Unit, Istituto G. Gaslini, Genova, Italy; [4]Department of Transplantation, S. Martino Hospital, Genova, Italy.*

Polyoma BK virus-associated nephritis (BKV-N) is a relevant cause of reduced renal allograft survival. As demonstrated for other viral infections relevant to transplantation, failure to mount a protective immune response may be a central event in the development of BKV-active infection and the related pathology. In order to assess BKV-specific cellular immunity, we retrospectively analysed by ELISPOT assay the frequency of IFNγ-secreting cells after stimulation with dendritic cells pulsed with inactivated BKV in pediatric kidney recipients with or without BKV infection/reactivation, as compared with healthy controls. While healthy BKV-seronegative individuals (n=2) did not show IFNγ secretion in response to the virus, the mean frequency of cytokine-producing cells in seropositive controls (n=7) was $151/10^6$ cells (range 30-430). Patients with BKV-active infection and good renal function (n=6; viruria+/viremia-) had a mean BKV-specific lymphocyte frequency 2-log lower than healthy controls ($5.5/10^6$ cells; range: 0-9), and in the same range as KTx recipients seropositive for BKV in the absence of active infection (n=6; viruria-/viremia-; frequency: $4/10^6$ cells; range: 0-9). Conversely, patients with BKV-N (n=4; viruria+/viremia+) revealed undetectable levels of BKV-specific cells. However, upon repeated stimulations in vitro with the antigen, in all 4 patients a measurable frequency of specific T cells was obtained, which resulted in the expansion of BKV-specific cytotoxic T cell lines. Indeed, one patient from the latter cohort treated with immunosuppression reduction, and evaluated prospectively, showed emergence of specific immunity, with IFNγ production in the same range as healthy seropositive controls. In conclusion, our preliminary data suggest that: i) in the presence of immunosuppressive drugs, the capacity to develop a primary immune response or expand memory BKV-specific lymphocytes upon virus infection/reactivation is hampered; ii) the degree of immune deficiency, likely coupled with the extent of viral load, may determine the progression to graft damage; iii) immunosuppression reduction allows the emergence of protective BKV-specific immunity also in patients with deeply defective immune response.

GROWTH FACTORS, TISSUE REPAIR AND METABOLIC PATHWAYS

Abstract# 1568

CARBON MONOXIDE EXPOSURE INDUCES UPREGULATION OF HIF/VEGF IN LONG TERM PRESERVED KIDNEY GRAFTS, AMELIORATES I/R INJURY, AND IMPROVES SURVIVAL. Joao Seda Neto,[1] Anna J. Romanosky,[1] Kei Kimizuka,[1] Atsunori Nakao,[1] Donna B. Stolz,[1] Noriko Murase.[1] *[1]Surgery, Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.*

Background: Hypoxia and accumulation of metabolic wastes during preservation result in vascular endothelial cell (VEC) injury and impairment of tissue microcirculation, followed by parenchymal cell damage during ischemia/reperfusion (I/R) injury. We have shown that carbon monoxide (CO) provides protection against cold I/R injury by inhibiting apoptosis, reducing proinflammatory mediators, and maintaining graft blood flow. This study examined roles of inhaled CO during I/R injury in activating hypoxia inducible factor (HIF-1α) and promoting repair mechanisms, including VEGF, VEGF receptors, HO-1 and iNOS. **Methods**: A Lewis rat kidney graft, preserved in UW at 4°C for 24 hrs, was orthotopically transplanted into binephrectomized syngeneic rats. Recipients were maintained in room air or exposed to CO (250 ppm) for 1 hr before and 24 hrs after transplant and sacrificed 1, 3, 6, and 24 hrs. Separate groups of animals were followed to assess long term kidney function and survival. **Results**: At 1 hr after reperfusion, F-Actin and CD31 (PECAM) expression on podocytes, tubular epithelial cells (TEC), and VEC was lost in AIR-exposed animals, indicating significant damages to kidney grafts. Lack of nephrin stain on podocytes or cadherin on TEC further confirmed the significant I/R injury in control group. In contrast, CO-treated animals showed nearly normal stain patterns, indicating well-preserved kidney graft structure. HIF-1α was upregulated on glomeruli of CO-exposed, but not AIR-treated animals as early as 1 hr after reperfusion. VEGF and VEGF receptors, FLK1 and neuropilin, were upregulated on VEC and TEC of CO-exposed, but not AIR-exposed kidney grafts at 1 hr and continuously for 24 hrs after reperfusion. CO exposure also resulted in lower HO-1, iNOS, and endotelin 1 expression during 24 hrs after transplant compared to controls. These early protection and repair promotion by CO associated with improved long-term kidney function. CO-treated animals had significantly lower creatinine levels (2.2±0.2 vs 6.2±2.8 mg/dl) and higher creatinine clearance (0.18±0.05 vs 0.04±0.03 ml/min) than controls at 24d after transplantation and improved survival. **Conclusions:** The study demonstrates that the protective effect of CO involves HIF/VEGF and VEGF receptor upregulation early after transplant. The impact of these early protective changes reflect on the better long term function and survival of CO-exposed animals.

Abstract# 1569

CONNECTIVE TISSUE GROWTH FACTOR (CTGF): A POTENTIAL EFFECTOR OF ISCHEMIA/REPERFUSION INJURY (IRI). Xiaojie Zhang,[1] Orlena H. Cheng,[1] Roslyn B. Mannon.[1] *[1]Transplantation Branch, NIDDK/NIH/DHHS, Bethesda, MD.*

IRI is a known risk factor for graft loss following kidney transplantation due to chronic allograft nephropathy (CAN). CTGF is a CCN protein and downstream effector of TGFβ. It is produced by a variety of cells, and mediates angiogenesis, cell proliferation and fibrosis. We have recently demonstrated that CTGF is upregulated in kidneys with CAN. We hypothesize that CTGF may be a link between ischemic injury and the development of CAN. To this end, we evaluated renal cell lines mIMCD-3 (inner medullary collecting duct), Mes-13 (SV40-transformed mesangial cells), and MCT (proximal tubule epithelial cells) for the expression of CTGF mRNA using real-time PCR. Cells were cultured at ambient conditions or subjected to 60 minutes of cold hypoxia, followed by rewarming. In the absence of hypoxia, CTGF mRNA was increased in all cell lines after 24h treatment with 10ng/mL TGFβ (2.1-4.6 fold), compared to cells cultured without TGFβ. While hypoxia had little effect on CTGF mRNA levels in Mes-13 and MCT cells, CTGF mRNA increased by 2.3±0.6-fold in mIMCD cells (p=0.02). Furthermore, CTGF mRNA levels in these cells remained substantially elevated at 60, 120, and 240 minutes of rewarming (18.7±7.5, 8.9±3.1, and 3.5±0.5-fold, respectively) and CTGF protein levels are also increased by 87%, 149% and 125%, respectively, compared to cells cultured in ambient conditions. We next evaluated CTGF mRNA responses in vivo following 30 min unilateral warm ischemia and reperfusion for 2h. CTGF mRNA was significantly elevated in the medulla and cortex of the ischemic kidney (13.5±4.5 and 4.2±3.0-fold, respectively; p<0.001) compared to normal kidney and contralateral, nonischemic kidney. Similarly, in human kidney grafts following implantation and 30min reperfusion, CTGF mRNA expression is elevated by 4.4±1.1-fold compared to normal kidneys (p=0.01). When the donor origin and graft outcome are assessed, the highest levels of CTGF mRNA are in cadaveric grafts in recipients that went on to develop CAN (6.3±1.4-fold; p=0.03), compared to the levels in cadaveric grafts in recipients that have not developed CAN (1.7±0.5-fold) or in living donor grafts (0.6±0.2-fold). Thus, brief hypoxia and rewarming increase steady state levels of CTGF mRNA and protein production in a mouse medullary cell line. Moreover, CTGF mRNA is induced following IRI in the mouse, as well as post-reperfusion in man. These data suggest that CTGF may be a novel regulator of ischemic injury. Elucidating this mechanism may lead to new strategies to ameliorate ischemic injury and ultimately CAN in man.

Abstract# 1570

MIGRATION OF EXOGENOUS ENDOTHELIAL CELLS AND BONE MARROW CELLS TO DAMAGED ENDOTHELIUM. Kathryn M. Verbanac,[1] H. Keith Pittman,[1] Bill T. Storey,[1] Carl E. Haisch.[1] *[1]Surgery, East Carolina University, Greenville, NC.*

We have shown that exogenous endothelial cells (EC) have an affinity for vasculogenic and angiogenic sites *in vivo*. Bone marrow-derived EC precursors (ECp) also migrate to an injured organ and induce vasculogenesis and angiogenesis in certain models. These cells thus have the potential in transplantation to focus therapy to damaged endothelium and promote endothelialization, restore normal EC function and enhance graft acceptance. AIM: To investigate the *in vivo* localization of EC and ECp to sites of vessel damage. METHODS: We used transgenic mice expressing enhanced green fluorescent protein (EGFP) as cell donors to enable *in vivo* tracking. Microvascular EC were isolated from neonatal GFP mouse hearts by collagenase digestion, density gradient centrifugation and differential adherence. Bone marrow cells (BM) containing ECp were flushed from the long bones of adult GFP mice and injected immediately or after removal of lineage-committed cells. The left renal veins of haploidentical C57Bl/6 mice were compressed with microvascular clamps for 30 min. Mice were injected i.v. with 2 x 10^6 EC (n=3), 2 x 10^6 intact BM (n=4) or 5 x 10^5 lin^- BM (n=2) 72 hrs after vessel damage and sacrificed 48 hrs after injection. To assess vessel damage, mice were injected i.v. 30 min before sacrifice with Evans Blue dye. Tissue damage (i.e. vessel permeability to dye) and GFP-cell biodistribution were determined by fluorescence microscopy of 10 micron frozen tissue sections (n=40/ tissue). RESULTS: Vessel clamping resulted in consistent and localized damage, as visualized by Evans Blue. GFP-cells were detected only in the damaged renal veins of all mice injected with exogenous cells (n=9). No fluorescent cells were detected in the contralateral undamaged control vessels (n=5; p<0.002) or in any other normal tissues. Localization was greatest in the luminal regions of damaged vessels and in the areas directly clamped. Mean cells per section were 106 GFP-VEC, 97 intact GFP-BM and 37 lin^- GFP-BM. Studies are underway to test the ability of localized cells to survive and proliferate, to investigate the mechanism of homing and to define GFP-cell surface markers. CONCLUSIONS: Exogenous EC and BM containing ECp home to sites of damaged endothelium. Others have observed that migration of transplanted progenitor cells to damaged brain and heart tissue is associated with regenerative effects. These data support the hypothesis that native or genetically modified BM-derived EC may repair localized damage, improve graft function and modify anti-graft immune responses.

Abstract# 1571

EXPRESSION OF KIDNEY INJURY AND FUNCTIONAL GENES CORRELATE WITH REDUCED NEPHRON MASS IN CYCLOSPORINE-INDUCED NEPHROTOXICITY. Ida Fernandes,[1] Maria Hong,[1] Mouer Wang,[1] Ye Zhang,[1] Hemangshu Podder,[1] Richard Knight,[1] Stanislaw M. Stepkowski,[1] Barry D. Kahan.[1] *[1]Surgery, UT-Medical School, Houston, TX.*

Objective: Cyclosporine (CsA)-induced nephrotoxicity is exacerbated by sirolimus (SRL) especially in patients with reduced nephron mass. We examined the mechanism of nephrotoxicity by measuring expression of kidney functional genes: kidney injury molecule-1 (KIM-1), aquaporin (AQP)2 and AQP3, paracellin-1 (PCLN-1), sodium hydrogen exchanger-3 (NHE-3), and sodium/phosphate cotransporter-2 (NaPi-2). Methods: Renal function was tested in salt-depleted rats bearing two kidneys (2K) or only one-half kidney (½K). These rats (5-6/group) were untreated or treated (p.o.) for 7 or 28 days with CsA (5 mg/kg) and/or SRL (0.8 mg/kg). Serum creatinine clearance (CrCl) and other biochemistries were compared using ANOVA test. PCR-based method was used to measure KIM-1, AQP2, AQP3, PCLN-1, NHE-3, and NaPi-2 mRNA expression. Results: At 7 days in 2K, CrCl was similar in untreated (2.7± 0.7 ml/min), as well as in CsA- (2.3± 0.9 ml/min; NS) and SRL- (2.5± 0.3 ml/min; NS) treated groups, but decreased in CsA/SRL-treated group (1.2± 0.1 ml/min; p<0.05). Although reduced nephron mass to ½K significantly decreased CrCl values in all groups [control, 0.9 ±0.3 ml/min; CsA, 0.6± 0.3 ml/min; and SRL, 0.8 ±0.4 ml/min], it most dramatically affected CsA/SRL group (0.4± 0.1 ml/min; p<0.05). The extended treatment to 28 days was again significantly worse for ½K compared to 2K. These results were reflected by PCR results: KIM-1 mRNA expression was low in normal 2K as well as 2K treated with CsA and SRL alone, but increased in two-drug combination (p=0.01; n=3). In contrast, KIM-1 mRNA level was elevated in ½K in all groups (p<0.05; n=3). Furthermore, in comparison to 2K, AQP2, AQP3, PCLN-1 and NaPi-2 mRNA levels were decreased in ½K (p<0.05; n=3), especially in CsA/SRL group (p<0.01; n=3). Conclusion: Impaired kidney function is accentuated by reduced nephron mass in CsA- and CsA/SRL-induced nephrotoxicity. These changes correlate with elevated expression of kidney injury gene (KIM-1) as a possible protective mechanism of kidney function in response to significant decrease in expression of multiple kidney functional genes (AQP2, AQP3, PCLN-1and NaPi-2). We propose that elevated expression of KIM-1 may be established as a marker for kidney injury.

Abstract# 1572

POTENTIAL OF REPAIRING ISCHEMICALLY DAMAGED KIDNEYS EX VIVO. Lauren Brasile,[1] Bart M. Stubenisky,[1] Carl E. Haisch,[2] Gauke Kootstra.[3] *[1]Reconstructive Surgery, UMCU University Hospital, Utrecht, Netherlands; [2]Surgery, East Carolina University, Greenville, NC; [3]Surgery, AZM University Hosptial, Maastricht, Netherlands.*

Introduction- Treatments following warm ischemia (WI) accelerating cellular recovery could have major impact on increasing kidneys procured from non-heartbeating donors. Warm perfusion (32°C) was employed to deliver growth factors (fibroblast growth factor (FGF)-1 and 2) to kidneys damaged by 2hrs WI. Sequelae determining pro- or anti-apoptotic pathways were assessed using a TUNEL assay. Junctional integrity protein, ZO-1 was used to determine recovery of cytoskeletal integrity. Upregulation of proliferating cell nuclear antigen (PCNA) was used as a marker for DNA repair and recovery of synthetic functions. Ability to repair the injury following 2-hrs of WI was correlated with clinical outcomes. **Methods-** Using a canine autotransplant model, kidneys were subjected to 2 hrs of WI and divided into 4 groups:

Group 1 (n=2) 2 hrs WI, followed by immediate reimplantation

Group 2 (n=2) 2 hrs WI, 24 hrs hypothermic machine perfusion (4°C) and reimplantation

Group 3 (n=2) 2 hrs WI, 24 hrs warm perfusion without growth factor (32°C) and reimplantation

Group 4(n=4) 2 hrs WI, 24 hrs warm perfusion plus growth factors (32°C) and reimplantation

Results- ***TUNEL assay-*** Following reimplantation, the number of TUNEL stained cells in Group 2 and 3 were 50 and 20% respectively. In Group 4, the kidneys were TUNEL negative following 24-hrs of perfusion and reperfusion. ***ZO-1-*** Kidneys in Group 2 demonstrated intense positive staining for ZO-1 accompanied by a degree of disintegration. Kidneys that were warm perfused with growth factors (Group 4) normalized ZO-1 expression in a time dependent fashion during warm perfusion. Normalization was impaired in the absence of FGF(s). ***PCNA*** - WI damaged kidneys in Group 2 resulted in 2% PCNA staining. In contrast, in kidneys in Group 4 an up-regulation of PCNA expression was observed with >80% of the cells staining positive. Absence of FGF(s) impaired PCNA expression. ***Function-*** FGFs induced up-regulation of PCNA, accompanied by the down-regulation of ZO-1 that occurred in Group 4 kidneys was found to correlate with outcomes posttransplantation. Reimplantation of "repaired" kidneys resulted in life-sustaining function. Reimplantation in Groups 1,2 or 3 resulted in non-viable kidneys. **Conclusion**- Metabolism was sufficiently restored to support upregulation of cellular processes with potential to modulate both injury and repair proteins in WI kidneys. Results suggest that administration of FGF(s) triggers pathways for new synthesis leading to cellular recovery rather than activation of the apoptotic pathway.

Abstract# 1573

FTY720 ATTENUATES SMALL-FOR-SIZE LIVER GRAFT INJURY - THE SIGNIFICANCE OF ACTIVAION OF CELL SURVIVAL SIGNALING PATHWAY. Yi Zhao,[1] Kwan Man,[1] Chung-Mau Lo,[1] Kevin T. P. Ng,[1] Xian-Liang Li,[1] Chris K. W. Sun,[1] Xian-Wei Dai,[2] Sheung-Tat Fan.[1] *[1]Surgery, University of Hong Kong, Hong Kong, China; [2]Surgery, The Second Affiliated Hospital of China Medical University, Shen Yang, China.*

Background:
To expand the potential donor pool, marginal donors are often utilized. The damage of marginal-sized grafts is common. Development of novel pharmaceutical therapy to rescue small-for-size liver grafts is always important.

Objective:
The aim of the study is to probe into the protective mechanism of FTY720 in a rat liver transplantation model using small-for-size graft by the investigation of cell survival and apoptosis pathways.

Materials and methods:
A rat orthotopic liver transplantation model using 40% of liver grafts was applied. In the FTY720 treatment group, FTY720 was administrated by iv injection (1mg/kg) (1) at 20 minutes before graft harvesting in the donor, and (2) immediately before liver out in the recipient and (3) immediately after reperfusion in the recipient. 7-day survival rate was compared. Liver tissues were sampled at 6 and 24 hours after reperfusion for morphological examination (hepatic ultrastructure), apoptotic nuclei staining (TUNEL), and intragraft gene detection for cell survival (Akt) and apoptosis (Caspase) signaling pathways by Western blot and immunostaining. Blood samples were analyzed for liver function.

Results:
The 7-day graft survival rates in the FTY720 group were significantly improved compared to the control group (100% (6/6) vs 40% (4/10), p=0.034). FTY720 significantly reduced serum ALT and AST levels at 24 hours after liver transplantation (ALT: 1215 (722-1289) vs 1970 (709-3170) U/L, p=0.035; AST: 851 (582-1089) vs 1840 (1207-4090) U/L, p=0.011). The cell survival signaling Akt pathway was significantly activated at ser^{473} after FTY720 treatment by phosphorylation of GSK-3, Bad and FKHR in FTY720 group at 6 hours and 24 hours after liver transplantation. The cleaved Caspase 3, 7 and 9 were down regulated accompanied with less apoptotic nuclei after FTY720 treatment. The anti-apoptotic gene A20 was up-regulated and iNOS was down-regulated at 6 and 24 hours after reperfusion in FTY720 groups. Hepatic ultrastructure was well maintained in FTY720 group compared to the control group with disruption of hepatic sinusoids.

Conclusions:
FTY 720 attenuates small-for-size liver graft injury by significant activation of cell survival signaling pathway.

Abstract# 1574

METABOLIC SIGNATURES OF MILD AND SEVERE ISCHEMIA/ REPERFUSION INJURY IN RAT KIDNEY TRANSPLANT MODEL BASED ON ^{1}H-MRS METABONOMICS. Natalie J. Serkova,[1] Florian T. Fuller,[2] Jost Klarwitter,[1] Chris E. Freise,[2] Ryutaro Hirose,[2] Claus U. Niemann.[3] *[1]Department of Anesthesiology, University of Colorado Health Sciences Center, Denver, CO; [2]Department of Surgery, University of California San Francisco, San Francicso, CA; [3]Department of Anesthesia and Perioperative Care, University of California San Francisco, San Francicso, CA.*

Introduction: A precise prediction of non-immune injury, such as severe ischemia reperfusion (IR), and its differentiation from acute kidney rejection and calcineurin inhibitor toxicity remains difficult. ^{1}H-NMR quantitative metabonomics was used to establish metabolic markers of mild and severe IR injury in a rat kidney transplant model.

Methods: Male Lewis rats were used for syngeneic kidney transplantation. Donor kidneys were cold stored for various times (24 and 42 hours) (CI) followed by transplantation and organ harvest on day 1 (TX 24/24; TX 42/24); n= 6 each group). Donor kidneys with cold storage only (CI 24; CI 42; n=6 each group) as well as native kidneys served as controls. Kidney and blood samples were frozen, extracted using acid/lipid extraction procedure and analyzed by ^{1}H-MRS with a Bruker 500 Avance system.

Results: Over 50 endogenous metabolites were quantified from each kidney and blood extract. Logistic regression analysis distinguished two IR renal tissue markers, poly-unsaturated fatty acids (PUFA) and allantoin (a novel marker of oxidative stress and endproduct of the xanthine pathway). Two markers, trimethylamine-N-oxide (TMAO) and allantoin were sensitive markers for the severity of IR injury in the blood. Decreased PUFA and increased TMAO concentrations are markers of non-specific renal injury and were seen previously with cyclosporine toxicity. However, the high correlation with elevated allantoin concentrations in all reperfusion study groups allowed discrimination of the severity of renal IR injury. Allantoin was seen only after reperfusion (control 0.02±0.03 µmol/mL, TX24 1.13±0.22, and TX42 1.89±0.38). The correlation coefficient for allantoin concentration was excellent for cold ischemia time (r=0.96) and for graft function (r=0.97).

Conclusion: MRS based metabolic profiling allowed to identify and quantify the extent of IR injury (allantoin) and correlation of medullary damage (TMAO). In the future, this high correlation between different surrogate markers may distinguish pathologic processes such as early rejection and severe IR injury.

Abstract# 1575

HEPATOCYTE GROWTH FACTOR IMPROVES SURVIVAL AFTER TRANSPLANTATION OF COLD-PRESERVED STEATOTIC LIVER IN RATS. Xiangdong Zhao,[1] Michihiro Hayashi,[2] Satoshi Kaihara,[1] Toshimi Kaido,[3] Takaaki Koshiba,[4] Ying Li,[1] Tatsuaki Tsuruyama,[5] Shigeki Arii,[6] Hiroto Egawa,[1] Koichi Tanaka.[1] *[1]Department of Transplantation Immunology, Faculty of Medicine, Kyoto University, Kyoto, Japan; [2]Department of Gastroenterological Surgery, Osaka Medical College, Osaka, Japan; [3]Department of Surgery, Otsu Municipal Hospital, Otsu, Shiga, Japan; [4]Horizontal Medical research Organization, Faculty of Medicine, Kyoto University, Kyoto, Japan; [5]Department of Pathology, Faculty of Medicine, Kyoto University, Kyoto, Japan; [6]Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental College, Tokyo, Japan.*

Transplantation of liver with severe steatosis may result in primary nonfunction (PNF) of the graft. To date, protective strategy is still rare. As previously described by our group, human recombinant hepatocyte growth factor (hrHGF) has a cytoprotective effect on fatty liver during cold preservation in rats. Here we further examined the effects of hrHGF on survival and graft function in rat fatty liver transplantation.

Methods Severe steatosis was induced by a 15-day choline-deficient diet in male Lewis rats. The fatty graft was orthotopically transplanted in normal male Lewis rat after 12 hr cold preservation in UW solution. In the HGF group, donor rats were pretreated with a single bolus of systemic injection of hrHGF (100µg/rat), and 30µg of hrHGF was also added to the perfusion/preservation solution. Animal survival, graft function and morphological changes were compared with those of the untreated group. Liver cell apoptosis was detected by TUNEL stain and liver regeneration was evaluated by BrdU stain at 2 hrs after reperfusion of the liver graft.

Results One-week survival was significantly improved in the HGF group compared with the untreated group (83% vs. 33%, p<0.01, n=18/group). AST, ALT and LDH levels in the graft rinse effluents at the end of cold preservation and serum ALT and LDH at 2 hrs after reperfusion were significantly low in the HGF group. Although there was no significant difference in hyaluronic acid (HA) level in the rinse effluents at the end of preservation between the two groups, exogenous HA uptake rate (HAUR) at 2 hrs after reperfusion was improved in the HGF group (0.62±0.05 vs. 0.79±0.11, p<0.05). Morphologically, sinusoidal endothelial cells (SECs) and hepatocytes were well preserved as observed by transmission electron microscope in the HGF group compared with the control. hrHGF pretreatment also significantly reduced the (SEC) apoptotic index (1.65±0.60% vs. 4.90±1.25%, p<0.01) and increased BrdU incorporation index (2.07±0.62% vs. 0.06±0.04%, p<0.01) at 2 hrs after reperfusion.

Conclusions hrHGF prevented the death of PNF in steatotic liver transplantation by attenuating preservation–reperfusion injury and facilitating recovery of the graft, probably through its antiapoptotic properties and acceleration of liver regeneration at early stage after transplantation.

Abstract# 1576

SIROLIMUS MODULATES INFLAMMATION DURING POSTTRANSPLANT REPERFUSION INJURY IN THE KIDNEY VIA UPREGULATION OF VEGF AND VEGF-R2. Duska Dragun, Melina Nieminen-Kelhae, Denise Markmann, Anna Lizak, Jan H. Braesen, Lutz Liefeldt, Wolfgang Schneider, Harm Peters, Hans H. Neumayer, Klemens Budde. *Nephrology and Transplantation, Charite University Hospital, Berlin, Germany.*

Sirolimus is a novel immunosuppressant with proven antirejection properties. Recent studies show antiangiogenic activities of sirolimus in tumors that were linked to a decrease in VEGF production. As VEGF acts as a survival factor for tubular and endothelial cells, antiangiogenic properties of sirolimus may be detrimental for regeneration after posttransplant reperfusion injury. We tested whether or not putative antiangiogenic properties of sirolimus may make renal allografts more susceptible to posttransplant preservation/reperfusion injury. We employed F344-to-Lew rat low-responder model where renal grafts were 2-hours cold preserved in UW solution and transplanted with an average 30 min anastomosis time. Therapeutic trough level was achieved in recipients after 3 mg/kg loading and daily 1.5 mg/kg maintenance sirolimus dose administered by gavage. Grafts were harvested 2, 5, and 7 days after transplantation for functional, morphological and gene expression studies. Beginning at 2 d posstransplant, sirolimus treated animals showed significantly higher creatinin clearance and lower histological tubular damage score. Functional integrity of tubular cells was preserved in sirolimus group as demonstrated by diminished low-molecular weight proteinuria and improved urine concentrating capacity. Posttransplant reperfusion injury induced 2-fold decrease of VEGF mRNA in control animals compared to basal conditions. Sirolimus treatment up-regulated VEGF mRNA beyond the level observed in native kidneys. The expression of VEGFR-2 was influenced in the same

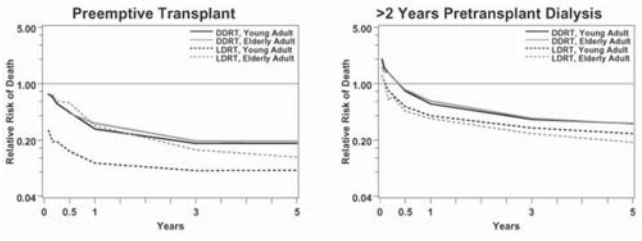
manner. The increased expression of VEGF was localized to tubular epithelial cells of the cortex and outer medulla in sirolimus group. Furthermore, sirolimus treatment resulted in reduced inflammatory response comprised as significantly decreased infiltration with effector cells: His-48+ granulocytes, ED-1+ monocytes, MHC class II positive cells and CD8+ lymphocytes. All sirolimus mediated protective effects persisted during the time course of the study. We obtained no significant differences in peritubular capillary density as measured by RECA, PECAM-1 and eNOS expression. Sirolimus does not act antiangiogenic during posttransplant reperfusion injury of the kidney. VEGF and VEGF-R2 upregulation observed in sirolimus treated animals may provide antiinflammatory signals and protect renal allografts from preservation/ reperfusion injury.

Kidney and Pancreas Transplantation: Public Policy and Economics

Abstract# 1577

THE TIMING OF LIVING DONOR AND DECEASED DONOR RENAL TRANSPLANTATION IN ELDERLY PATIENTS. Aloke K. Mandal,[1,2] Benjamin K. S. Chan.[3] *[1]Surgery, Oregon Health & Science University, Portland, OR; [2]Portland VA Research Foundation, Portland Veterans Administration Medical Center, Portland, OR; [3]Clinical Information Analysis and Decision Support Center & Evidence-Based Practice Center, Oregon Health & Science University, Portland, OR.*

Introduction: The elderly (≥60 years) comprise the fastest growing group of patients placed on the renal transplant waiting list (WL) and are at high risk of graft loss and death after transplantation. In the general population, reducing pretransplant dialysis exposure and emphasizing live donor renal transplantation (LDRT) over deceased donor renal transplantation (DDRT) improve outcomes. The purpose of the current study is to determine how these two factors affect renal transplantation in elderly recipients. *Methods:* Using data from the United States Renal Data System, 120,610 adults (≥18 years) who either remained on the WL or underwent first-time, solitary renal transplantation between 1991 and 2000 with no history of another solid organ transplant were studied. *Results:* LDRT and DDRT improved survival in both elderly and younger patients, independent of time on the WL. For younger adults with no dialysis exposure, the relative risks (RR) of death at 1 year were: 1.00 for remaining on the WL, 0.27 (95%CI: 0.23-0.33) after preemptive DDRT, and 0.10 (0.08-0.14) after preemptive LDRT. In elderly patients, preemptive LDRT (RR: 0.30) and DDRT (0.32) have similar improvements in survival.



With any amount of dialysis exposure, the survival benefits of LDRT over DDRT in elderly patients became significant. For example, in elderly patients with >2 years of dialysis, the 1 year RR of death were: 1.00 for remaining on the WL, 0.61 (0.57-0.71) after DDRT, and 0.37 (0.24-0.52) after LDRT. *Conclusions:* The elderly are at higher risk of death regardless of WL time and transplant status. LDRT and DDRT improve survival in both elderly and younger adults regardless of time on the waiting list. For the elderly with any amount of dialysis exposure, LDRT should be emphasized over DDRT.

Abstract# 1578

RISK FACTORS FOR DEATH AND GRAFT FAILURE IN RENAL TRANSPLANT RECIPIENTS OVER 65 YEARS: A MULTIVARIATE ANALYSIS OF UNOS DATABASE. V. Ram Peddi, Steven Katznelson, Vish Halukurike, Timothy J. Baker, Maureen A. McBride. *California Pacific Medical Center, San Francisco, CA; , UNOS, Richmond, VA.*

Overall incidence of ESRD continues to rise with greatest increase in pts >65y. USRDS data indicate that the median age of pts with ESRD is 64.5y. Pts >65y comprise 13% of pts on the UNOS cadaver kidney wait list & received 6.2 % of all kidney txp in 1994 vs. 16% in 2001. Despite improving results, RR for death with function in cadaver renal txp recipients >65y is 6.7(USRDS). Multivariate analysis of UNOS database was undertaken to identify risk factors for death in solitary renal txp recipients >65y. 5219 pts transplanted between Apr 1994 and Dec 2000 with graft survival of at least 1-yr were included.

Variables with an impact on patient survival

Variable	P	RR
Recipient Age: 75+ vs. 65-69	0.0294	1.377
Recip Female vs. Male	0.0037	0.817
Recip Hispanic vs. White	0.0132	0.725
Recip Asian vs. White	0.0120	0.572
Pre-txp Dialysis: Yes vs. No	0.0595	1.261
ESRD cause: Diabetes vs. Other	0.0009	1.293
History of Angina/CAD: Yes vs. No	0.0233	1.193
Peripheral Vasc Disease	0.0005	1.514
Living Donor vs. CAD	0.0113	0.772
Treated for Rejection in 1-yr	0.0302	1.163

Variables that do not have an impact on patient survival

Variable	P	RR
Recip Black vs. White	0.9327	1.008
Recip Previous Kidney TX: Yes vs. No	0.6059	1.092
Peak PRA: >=20% vs. <20%	0.0856	1.164
Donor Cause of Death: CVA vs. non-CVA	0.1659	1.119
Donor HTN	0.7662	1.026
Cold Ischemia Time: >24 vs. <=24 hrs	0.6306	1.037
HLA MM:2-6 vs. 0-1	0.1589	1.116
Induction	0.0976	1.110
Discharge S.Creat > 1.5 mg/dl vs. <=1.5	0.3534	1.067
DGF	0.1109	1.136

Multivariate analysis was also conducted to analyze risk-factors for graft failure. Of note, following variables were not significant causes for graft loss: Previous kidney txp(P=0.08;RR=1.29); Peak PRA(P=0.11;RR=1.14); Donor death due to CVA vs. non-CVA(P=0.06;RR=1.15); Donor HTN(P=0.56; RR= 1.05); CIT>24h(P=0.99;RR=1.00); HLA MM (P=0.08;RR=1.14); Induction(P=0.22;RR=1.07); DGF(P=0.13;RR=1.12). **Conclusions:** 1) Particular attention should be paid towards risk-factors identified to have an impact on Pt survival 2) Risk-factors traditionally associated with poor outcome do not appear to have the same adverse effect on long-term graft & Pt survival in older recipients. These findings will have a strong impact on how cadaver organs are utilized.

Abstract# 1579

MEDICARE KIDNEY TRANSPLANT AND ORGAN ACQUISITION REIMBURSEMENT ECONOMICS: A FIVE YEAR TREND REVIEW. E. Y. Zavala,[1] B. Reeb,[2] J. C. Rogers,[3] C. W. Pinson.[1] *[1]Vanderbilt Transplant Center, Nashville, TN; [2]The Johns Hopkins Comprehensive Transplant Center, Baltimore, MD; [3]University of California, San Diego, CA.*

Medicare pays for approximately 50% of all kidney transplants as the primary payor in the U.S. Medicare hospital reimbursement has two significant components: DRG302 and organ acquisition. The DRG is formula driven and has minor variability. Organ acquisition can be highly variable based on a hospital's capabilities to capture all allowable pre-txp costs. We reviewed the economic trends of DRG302 and organ acquisition from 1998-2002. **Methods:** We calculated a national average for DRG302 by averaging facility and area specific variables from 1998-2002. Organ acquisition data from completed cost reports was acquired utilizing the Center for Medicare and Medicaid Services Healthcare Cost Report Information System Data Set. Organ acquisition was calculated using total direct organ acquisition costs plus costs of services of teaching physicians divided by total usable organs. The average number of completed kidney acquisition cost center reports used in this analysis was 180/yr. **Results:** The 5 year trend is shown in the table.

Year	1998	1999	2000	2001	2002
DRG302	$26,094	$25,047	$24,783	$24,282	$24,163
Organ Acquisition	$37,791	$37,967	$41,897	$45,731	$49,863
TOTAL	$63,885	$63,014	$66,680	$70,013	$74,026

Conclusion: The DRG302 reimbursement decreased by 7.5% over 5 years. Organ acquisition increased by 32% over 5 years. Organ acquisition for pre-txp services is cost-based reimbursement. Unless the txp center is capturing all allowable organ acquisition pre-txp costs, the txp center could be subsidizing Medicare txps and this has been problematic in many txp centers. The DRG portion of reimbursement is fixed to a large degree and this is the area where profitability would occur if the resource utilization in the txp operative and in-patient course of care is managed well. Kidney txp programs must have defined processes in place to assure that all allowable organ acquisition costs are being captured and reported since it can account for as much as 67% of Medicare reimbursement for kidney txp. It is imperative to be clinically and economically effective in the txp operative and in-patient course of care to ensure economic viability of performing Medicare txps.

Abstract# 1580

RACIAL DISPARITIES FOR PANCREAS TRANSPLANTATION IN THE US. Ross B. Isaacs,[1] Peter I. Lobo,[1] Wida Cherikh,[2] Rasheed Balogun.[1] *[1]Department of Medicine, University of Virginia, Charlottesville, VA; [2]Department of Medicine, University of Virginia, Charlottesville, VA; [3]UNOS; [4]Department of Medicine, University of Virginia, Charlottesville, VA.*

Intro.: Despite the increase of diabetes in minority populations, access and outcomes for pancreas transplantation remains controversial. The purpose of our study was to assess the impact of ethnicity of access to transplantation and evaluate long term outcomes. Methods: All patients who were wait listed and underwent SPK, PAK or PTA in the US between 1990 and 2001 were compared by race/ethnicity. A separate Kaplan Meir and multivariate analysis was performed to evaluate 5 year graft survival rates by ethnicity. Results: Table 1 - Although improvements in outcomes were seen for all forms of pancreas transplants, no ethnic differences were seen for PAK or PTA. SPK survival rates are summarized in Table 2. Conclusion: Important disparities persist for Type 1 diabetic minority patients with or without CKD, despite the increase of Type 1 diabetes in these populations. No significant outcome differences exist between blacks and whites although outcomes for blacks decreased over time and Hispanics had the highest graft survival rates. More efforts are needed to increase pancreas transplantation in minority and underserved populations.

Table 1 - Access to Transplantation

	1990-1995			1996-2002		
	PAK	PTA	SPK	PAK	PTA	SPK
Caucasian	96	94	89	89	93	82
African American	2	1	7	6	4	10
Hispanic	1	4	3	4	2	5
Other	1	1	1	1	1	3

Table 2: SPK 5 yr Outcomes

	1990-1995	1996-2000
Caucasian	65%	70%
African American	66%	63%
Hispanic	68%	73%

Abstract# 1581

COMPLICATIONS, COST, AND RESOURCE UTILIZATION OF LIVING-DONOR ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION VERSUS ABO-COMPATIBLE TRANSPLANTATION. Jason Schwartz,[1] Lynette Fix,[1] Marco Covarrubias,[1] James Gloor,[2] Mark Stegall.[1] *[1]Department of Surgery, Division of Transplantation Surgery, Mayo Clinic, Rocester, MN; [2]Department of Medicine, Division of Nephrology, Mayo Clinic, Rochester, MN.*

Introduction. The waiting time for kidney transplantation (TX) in those without an ABO-compatible (ABOc) donor can exceed five years at an approximate cost of $60,000/yr for dialysis. ABO-incompatible (ABOi) living-donor kidney transplant (LDKT) is thus an increasingly viable option to increase access to KT, yet it requires more therapy and is considered more costly than ABOc TX. This study examines the increased cost and resource utilization associated with ABOi-KT. **Methods.** One-year graft survival, resource utilization and costs pre-op and for the first 90 days post-op were compared retrospectively in ABOi vs. ABOc transplants. ABOi patients underwent pre-TX conditioning with plasmapheresis, splenectomy on day of TX, and surveillance biopsies on days 3, 7, 14. Immunosuppression was similar in the two groups. Resource utilization (surgical procedures, hospitalizations, hospital days, complications, etc.) was compared using the Mann-Whitney rank sum test. Cost data was obtained from an institutional cost data center. **Results.** One-year graft survival was 94% for ABOi vs. 96% for ABOc transplants. During this time, the surgical morbidity rate was 65.9% for ABOi vs. 41.5% for ABOc individuals. There was no significant difference between the number of hospital admissions, medical and total complications, pre-and post-op visits, and radiologic procedures. Resource utilization was significantly higher for ABOi TX in several categories (Table).

Resource Utilization of ABOi vs. ABOc Kidney Transplants

	ABOi	ABOm	p
Hospital days per patient*	14.45±2.69	9.05 ±0.99	0.023
Total rejections	13	1	0.037
Surgical complications per patient*	1.32±0.26	0.53±0.11	0.016
Kidney biopsies per patient*	4±0.30	1.51±0.22	<0.001
Isoagglutinin titres per patient*	27.78±2.4	0.48±0.14	<0.001

*Expressed as mean±SE

The increase in cost was more than $50,000/patient of which 80% was post-TX due to increased surveillance and hospitalizations. **Conclusion.** ABOi-LDKT acheives short-term graft survival similar to ABOc KT. Despite the increased resource utilization, morbidity, and cost, ABOi TX compares well to the annual cost for dialysis and decreases wait-time associated morbidity.

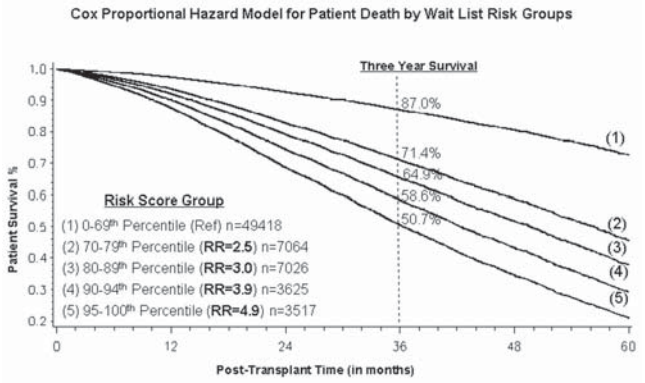
Abstract# 1582

IDENTIFICATION OF "HIGH-RISK" WAIT LIST CANDIDATES FOR KIDNEY TRANSPLANTATION. Jesse D. Schold, Bruce Kaplan, Pamela R. Patton, Titte R. Srinivas, Richard J. Howard, Herwig-Ulf Meier-Kriesche. *University of Florida, Gainesville, FL.*

Waiting list management is becoming increasingly challenging with extended waiting times and consequently elevated numbers of patients on the list. Resources to properly evaluate and follow potential recipients on the waiting list are often limited, yet tracking the progress of these patients is increasingly important. In the attempt to individualize high risk patients who might benefit from early re-evaluation while on the waiting list, we analyzed records of patients from the USRDS database who began their first wait list period from 1995-2000.

A multivariate Cox model was constructed for the primary endpoint of patient death, censored at the time of transplant. Cardiac death and death after transplant were secondary endpoints in the analysis. The combination of significant patient risk factors was used to generate a risk score applicable at the time of wait listing. The factors of highest significance included patient age, primary diagnosis, prior cardiac events, race, length of time on dialysis, and BMI. We derived the risk scores from a randomly selected sample constituting 2/3^{rds} of available records and tested results on the remaining cohort.

Outcomes were consistent in the model and test groups, and we observed a significant strong association between the risk score and patient survival. With the lowest 70% risk scores as reference, the relative risk of patients in the 70-79th percentile was 2.5, 80-89th percentile 3.0, 90-94th percentile 3.9, and 95-100th percentile 4.9.



The identification of high risk patients on the waiting list would provide valuable information to centers and allow them to discriminate follow up resources appropriately. With a growing volume of wait list candidates, the ability to understand important risk factors and manage patients accordingly is a critical component of pre-transplant care and waiting list management.

Abstract# 1583

FTY720 VS. MYCOPHENOLATE MOFETIL, ECONOMIC IMPACT IN CYCLOSPORINE BASED IMMUNOSUPRESSION FOR DE NOVO RENAL TRANSPLANTATION. Robert Balshaw,[1] Paul Keown,[2] Daniel E. Hricik,[3] Gerardo Machnicki,[4] Zoltan Kalo.[4] *[1]Statistics and Actuarial Science, Simon Fraser University, Burnaby, BC, Canada; [2]Medicine, University of British Columbia, Vancouver, BC, Canada; [3]Nephrology, Case Western Reserve University, Cleveland, OH; [4]Novartis Pharma, Basle, Switzerland.*

Body: Background: FTY720, a novel immunomodulator, has been shown to be both safe and efficacious for de novo renal transplants. Preliminary economic studies have also demonstrated important potential economic benefit for FTY720 in combination with cyclosporine (CsA) in renal transplantation.

Methods: We conducted an economic analysis based on clinical results from two phase 2 studies comparing FTY720 and mycophenolate mofetil (MMF) in combination with CsA and steroids. In these studies, patients receiving FTY720 0.25-5 mg/day in combination with full or reduced dose CsA + steroids for 3-months (n=167) or 12-months (n=222) post-transplant were compared with a comparator arm receiving MMF 2 g/day + full dose CsA + steroids. Principal clinical outcomes, i.e. acute rejection, graft loss and death were used to populate an economic decision model. Total direct medical costs during the first year post-transplant included costs and probabilities of initial hospitalization, acute rejection (AR), immunosuppressive therapy, adverse events, graft maintenance/failure, and dialysis for each treatment strategy. We compared only the 2.5 mg FTY720 + full dose CsA to the MMF + full dose CsA arms. All resources were valued in 2002 US dollars and the yet unknown cost of FTY720 was not included.

Results: Event rates in study 1 (3 month outcomes) were 9.8% vs. 17.1% (AR), 2.4% vs. 4.9% (graft loss), and 2.4% vs. 0% (death) in the FTY720 and MMF arms respectively. Corresponding rates in study 2 (12 month outcomes) were 14.5% vs. 23.1 (AR), 11.8% vs. 10.3% (graft loss), and 2.7% and 2.6% (death), respectively. The incidence of serious

CMV was 0% vs. 4.9%, and 1.3% vs. 5.1%; the incidence of bradycardia reported as a serious adverse event was 1.2% vs. 0%, and 1.8% vs. 0%, respectively. These values were used to establish plausible ranges for each outcome at 12 months. Incorporating these outcomes into the economic model yielded total costs of $61,566 for the MMF arm and an incremental savings between $7,633 and $11,093 at 12 months for the FTY720 arm. This is attributable primarily to a reduced cost of treatment of AR, dialysis following graft loss, and maintenance immunosuppression. This savings excludes the cost of FTY720.

Conclusion: These results indicate that FTY720 may provide, beyond clinical benefits, an additional valuable potential economic benefit in de novo renal transplantation resulting from improved clinical immunosuppression.

Abstract# 1584

COMPLETION OF THE RENAL TRANSPLANT EVALUATION PROCESS BY PREEMPTIVE VS. DIALYSIS-DEPENDENT PATIENTS.

Francis L. Weng,[1] Kevin C. Mange.[1] *[1]Renal-Electrolyte & Hypertension Div. and Center for Clinical Epidemiology & Biostatistics, Univ of Pennsylvania, Philadelphia, PA.*

Intro: Preemptive renal transplantation requires that patients (pts) complete the transplant (txp) evaluation process prior to dialysis initiation. We compared rates of completion of the initial txp evaluation between pts not on chronic dialysis (PREDIAL) and pts already on chronic dialysis (DIAL), and whether factors that impact rates of completion are distributed differently between these groups.

Methods: From 9/02 to 9/03, 253 pts undergoing their initial renal txp evaluation were enrolled in a prospective cohort study. Pts completed a questionnaire gathering information on demographics, views of txp and dialysis, and existence of potential living donors (LD). For the 133 pts who had ≥6 months follow-up, Cox proportional hazards models were fit to determine factors associated with time to completion of the txp evaluation.

Results: Of the 133 pts, 60 (45.1%) were PREDIAL, while 77 (54.9%) were DIAL (74.0% hemo, 12.3% peritoneal, 13.7% unknown). 60.9% were male, with 54.1% Whites and 38.4% Blacks. After 6 months, 57 pts (42.9%) had completed their txp workup, defined as (1) listing for a cadaveric renal txp (84.2%) or receipt of a txp (5.3%), whichever occurred first; or (2) determination of medical unsuitability for a renal txp (10.5%). 61 pts were awaiting further evaluations (cardiovascular 50.6%, hepatologic 11.8%, GI 15.3%, Pap smear 17.7%, mammogram 14.1%, percentages sum to >100%). Age, gender, existence of potential LDs, and being PREDIAL were not associated with time to completion of the txp evaluation (P>0.25), but race and other factors were associated (Table).

Conclusions: In this interim analysis, faster completion of the txp workup was associated with White race, expectation of a cadaveric txp, and the influence of persons other than pts' physicians, but was not associated with dialysis status.

Unadjusted and adjusted hazard ratios (HR) for completion of txp evaluation

	Unadjusted		Adjusted	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
DIAL at time of evaluation (vs. PREDIAL)	0.82 (0.49-1.38)	0.45	0.82 (0.46-1.47)	0.50
White race (vs. non-White)	3.15 (1.75-5.70)	<0.001	3.05 (1.65-5.65)	<0.001
Expect to receive a cadaveric txp (vs. expect LD txp)	1.54 (0.85-2.77)	0.15	1.72 (1.03-5.03)	0.01
Considers spouse as a potential LD (vs. no spousal LD)	1.47 (0.83-2.59)	0.19	1.89 (0.98-3.64)	0.06
Most influenced to come for txp evaluation by non-physician (vs. most influenced by physician)	1.77 (1.05-2.99)	0.03	2.14 (1.19-3.86)	0.01

Abstract# 1585

UNETHICAL OR HELPFUL?: KIDNEY DONORS' ATTITUDES ABOUT FINANCIAL COMPENSATION FOR DONOR-SWAPPING.

Amy D. Waterman,[1] Barry Hong,[2] Daniel C. Brennan,[3] Emily Schenk,[1] Tonie Covelli,[1] Anne Barrett,[4] Sarah Stanley,[4] Tracye Davis,[5] Mark A. Schnitzler.[5] *[1]General Medical Sciences, Washington University School of Medicine, Saint Louis, MO; [2]Psychiatry, Washington University School of Medicine, Saint Louis, MO; [3]Transplant Nephrology, Washington University School of Medicine, Saint Louis, MO; [4]School of Public Health, Saint Louis University, Saint Louis, MO; [5]Health Administration, Washington University School of Medicine, Saint Louis, MO.*

Purpose: Financial compensation for donor-swapping is controversial – concerns about the coercive effect of payment for donation must be considered along with the potential reduction in waiting list length and cost-savings. To include the voice of donors in this debate, we measured their attitudes about receiving financial compensation for donor-swapping.

Methods: We surveyed 54 living donors who were ruled out for their recipients due to ABO- or HLA-incompatibility to examine if they thought receiving financial compensation for donor-swapping was unethical and if they would accept money for their donation. If donors would accept money, we asked if eight amounts were either "too little," "enough," or "too much."

Results: Ruled-out donors were predominately Caucasian (82%), family members of the recipients (70%), female (54%), and willing to participate in donor-swapping (78%). Sixty-one percent thought that it was ethical for the government to pay donors for donor-swapping. However, only 43% would accept money for donor-swapping if it was offered and only 10 donors (19%) said that money would increase their willingness to donate. Donors reported that $1000-$5000 would be enough compensation, possibly considering this amount sufficient to cover unreimbursed donation expenses and time off work (Table 1). Of the 12 people not willing to consider donor-swapping, 3 individuals reported that money would increase their willingness.

Conclusions: Greed and high levels of coercion were not found in ruled-out donors; Although compensation of $1000-$5000 for unreimbursed donation expenses was appreciated by some donors, most reported that they would not take money, nor would it influence their donor-swapping willingness. Further research must be done to determine if race or socioeconomic status impacts these attitudes.

Table 1. Donor Opinions about Financial Compensation

Compensation for Donor Swapping	Too Little	Enough	Too Much
$ 100	80%	15%	5%
$ 500	70%	25%	5%
$ 1000	40%	**55%**	5%
$ 5000	10%	**70%**	20%
$ 10,000	5%	30%	65%
$ 50,000	5%	15%	80%
$100,000	0%	20%	80%
$250,000	0%	15%	85%

N=20 donors who would accept money

KIDNEY TRANSPLANTATION: LIVING DONORS

Abstract# 1586

COMPARATIVE OUTCOMES OF UNRELATED LIVE DONOR AND IDEAL DECEASED DONOR KIDNEY TRANSPLANTATION.

Francis L. Delmonico,[1] Joshua McGowan,[2] Akinlolu Ojo.[2] *[1]Transplantation Unit, Massachusetts General Hospital, Boston, MA; [2]Scientific Registry of Transplant Recipients, University Renal Research and Education Assoc, Ann Arbor, MI.*

Nearly a decade has passed since the national report of successful unrelated live donor kidney transplantation. Current data to sustain the ethical propriety of this practice are needed. If HLA matching is not a requirement for successful unrelated transplants, does HLA matching influence the outcome of ideal deceased donor kidney transplants?

Study Groups: The study cohorts consisted of living donor (LD)(n=32,316) or deceased donor (DD) kidney transplants (n=55,349) between March 6, 1995 and March 31, 2002. Recipients were followed until death, or onset of graft failure.

Methods: Allograft failure was defined as initiation of maintenance dialysis, graft nephrectomy, or occurrence of repeat renal transplantation. Patients who died with a functioning allograft or without a documented graft failure event were censored from graft survival analysis. A Cox regression model was used to assess LD graft survival rates adjusted for the following covariates: donor age, sex, race ethnicity; recipient age, sex, race, ethnicity, cause of ESRD, PRA, and year of transplant; and the effect of donor-recipient relationship and level of haplotype matching. A Cox regression model assessed the risk of graft failure for DD kidney transplant recipients by the following additional covariates: donor history of hypertension, terminal creatinine level and cold ischemia time. A separate adjustment was made for DR mismatch among unrelated LD recipients (n=6,844) to specifically test DR matching effect on graft survival and upon ideal DD recipients (n= 1452). Ideal Donor = age between 18 and 34; cold ischemia time 20 hours or less; trauma victim.

Results: Among LD recipients, there was no independent effect of DR matching on graft survival as indicated by a 5-year survival rate of 79%, 78% and 77% for 0-DR mm, 1-DR mm and 2-DR mm respectively (p=0.77 and 0.40 compared to 0-DR mm; thus, not significantly different). For DD recipients from ideal donors, a 5-year graft survival was 75% for 0-DR mm compared to 72% (p=0.30) for 1 DR mm and 71% for 2 DR mm. Therefore, DR matching had no effect upon allograft survival of LD unrelated or ideal DD kidney transplants.

Conclusions: The propriety of an increasing practice of unrelated live donor kidney transplantation is affirmed by these current data. HLA typing of a live donor who is a spouse, friend, or unknown to the recipient, is no longer necessary to achieve a successful outcome. Declining a deceased donor kidney from an ideal donor because of the degree of HLA mismatch is not indicated.

Abstract# 1587

WHO IS THE BETTER LIVING KIDNEY DONOR? A PREDICTIVE MODEL BASED ON SIMPLE DONOR-RECIPIENT DEMOGRAPHICS. Todd V. Brennan,[1] Stephen J. Tomlanovich,[1] Alan Bostrom,[2] 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.*

Living donor kidney transplantation (LDKT) is becoming increasingly common. Today, many candidates present with more than one potential donor (D) who differ in age, gender, size and relationship - characteristics which determine nephron mass and may therefore impact upon graft function and longevity. This study aims to develop a model to predict the risk posed by D demographic factors for suboptimal graft function (SubOpGF) for a given recipient (R). **Methods:** Of the 20,528 adult LDKTs performed between 1999 to 2002 within the SRTR database, our study cohort included the 8,603 LDKTs which had complete D and R demographics and 1-yr graft function information. Logistic regression analysis was used to identify D and R characteristics associated with SubOpGF, defined as a serum creatinine (Cr) >1.5 mg/dL. **Results:** 3,140 recipients (36.5%) had SubOpGF which has been strongly associated with graft loss. D and R age, D and R gender, D and R BSA, R black ethnicity, and D-R relatedness emerged as strong and independent predictors of SubOpGF (Table). Multivariate analysis enabled the derivation of a model to predict the probability of SubOpGF (area under the ROC curve = 0.75). Using the model, for a 34yo Caucasian woman who is 5'4" 140 lb (BSA 1.68), her 60yo father [6'0" 190 lb (BSA 2.08)] would, compared to her 35yo female friend [5'4" 140 lb (BSA 1.68], present a comparable risk for SubOpGF (24% vs. 21%). Alternatively, for a 60yo black man who is 5'10" 170 lb (BSA 1.95), his 60yo wife [5'4" 140 lb (BSA 1.68)] would, compared to his 35yo son [5'10" 170 lb (BSA 1.95)], present a substantially higher risk of SubOpGF (76% vs. 42%). **Conclusions:** Simple D and R demographics are potent factors in determining 1-yr graft function after LDKT. In the setting of multiple potential living Ds for a single R, our study provides a model which facilitates quantitative assessment of the risk associated with each D profile for SubOpGF at one year.

Multivariate Analysis for Predictive Model of One Year Cr > 1.5

VARIABLE	REF. GROUP	ODDS RATIO (95% CI)	P-VALUE*
R Male	R Female	2.76 (2.45 - 3.10)	<0.0001
R Age (yrs)	R Age < 35		<0.0001
35 - 45		0.73 (0.63 - 0.83)	
46 - 55		0.57 (0.50 - 0.66)	
> 55		0.38 (0.33 - 0.44)	
R BSA (m^2)	R BSA < 1.72		<0.0001
1.72 - 1.90		1.40 (1.20 - 1.64)	
1.91 - 2.06		1.93 (1.64 - 2.26)	
> 2.06		2.91 (2.46 - 3.43)	
R Black	R Caucasian	2.23 (1.94 - 2.56)	<0.0001
D Male	D Female	0.82 (0.73 - 0.92)	0.001
D Age (yrs)	D Age < 30		<0.0001
30 - 40		1.58 (1.36 - 1.84)	
41 - 50		2.34 (2.01 - 2.72)	
> 50		4.08 (3.46 - 4.80)	
D BSA (m^2)	D BSA < 1.72		<0.0001
1.72 - 1.90		0.94 (0.82 - 1.08)	
1.91 - 2.06		0.90 (0.78 - 1.05)	
> 2.06		0.68 (0.58 - 0.80)	
D/R Unrelated	D/R Related	1.23 (1.11 - 1.37)	0.0001

**Chi-squared*

Abstract# 1588

LONG TERM FOLLOW UP OF 800 LIVING RELATED KIDNEY DONORS. Adib Rizvi,[1] Anwar Naqvi,[1] Mirza Naqi Zafar.[1] *[1]Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Sindh, Pakistan.*

Purpose: To study post nephrectomy biochemical and clinical follow up of living related donors.

Methods: Eight hundred donors were closely followed in the last sixteen years 1986 – 2002. There were 2 cross sectional recalls in 1994 and 1997 and thereafter all donors have been followed in a dedicated clinic, monthly for 6 months, thereafter 6 monthly for 1 year and then yearly. All kidney donors were subjected to a detailed physical examination, a complete biochemistry and haematology analysis along with urine metabolic studies and ultrasound scan of the remaining kidney. Donor demography, rehabilitation, post nephrectomy change in weight, development of Hypertension and Diabetes Mellitus, status of renal function, lipid levels, total caloric and protein intake and any serious illness in the intervening period were noted. All donors were also questioned about their emotional feelings on donating a kidney.

Results: The mean age of donors was 37 ± 11 years with a mean follow-up 4.8 ± 3 years. The mean pre and post Nephrectomy parameters were creatinine 0.97 ± 0.1 vs 0.97 ± 0.12 mg / dl, albumin 4.2 ± 0.4 vs 4.4 ± 0.2 g/dl, creatinine clearance 92 ± 15 vs 86 ± 25 ml/min and urinary protein 102 ± 32 vs 110 ± 65 mg/24 hours. The pre-nephrectomy mean weight was 60.84 ± 12.27 kg and the mean weight on follow-up was 61.82 ± 12.87 kg. Hypertension was detected in 114 cases (14.3%) and diabetes mellitus in 26 (3.3%). The mean serum Cholesterol was 181.35 ± 40.52 and serum Triglycverides was 163.85±99.51. The mean daily caloric intake of males was 1997.131 kcals, proteins 63.03±19.76.2 gms and that of females was 1716.68 ± 354.23 kcals proteins 49.95±19.14 gms respectively. Proteinuria was found in 6% of the donors. Majority of these comprised donors older than 45 years at the time of donation. All 800 donors were rehabilitated within 40 days.

Conclusion: All kidney donors had voluntarily donated their organs and for emotional reasons. Renal function was well preserved, majority were rehabilitated and had not encountered any serious health problems in the intervening period. The results of the donor follow up can prove to be a motivating factor for prospective donors giving informed consent in a live related transplantation programme.

Abstract# 1589

'MINI NEPHRECTOMY' AN IDEAL DONOR NEPHRECTOMY PROCEDURE? Surendra Shenoy, Martin Jendrisak, Jeffrey Lowell, Niraj Desai, Anita Vijayan, Karen Hardinger, Debra Kemp, Mary Ann Laflin, William C. Chapman. *Surgery, Barnes-Jewish Hospital at Washington University, St. Louis, MO.*

Introduction: Laparoscopic donor nephrectomy (LDN), the common minimally invasive procedure reduces morbidity associated with conventional open donor nephrectomy (ODN). Despite the donor appeal, it has low but significant (transperitoneal approach bleeding) morbidity and mortality. The 'mini nephrectomy' a minimally invasive, open, retroperitoneal, live donor nephrectomy; was devised to further reduce the mortality and morbidity. We describe our results with this procedure.

Methods: 130 consecutive 'mini nephrectomies' performed between Feb, 2001 and Nov, 2003 were prospectively evaluated. Demographics, laboratory data, operative details, graft characteristics, post operative complications, length of stay, return to activity and pain medications are described. Quality of life (QOL) analysis was administered to 47 patients using SF-36 forms. **Results:** 'Mini nephrectomy'can be safely performed in all donors regardless of body weight, renal anatomy or side of retrieved organ with excellent QOL. The operative time, technique and warm ischemic time are similar to traditional ODN thereby providing excellent quality grafts. The length of the incision for this procedure is smaller than LDN incisions. The length of stay and time to resuming normal activity is similar to other minimally invasive nephrectomy procedures. The perioperative risks are minimal. With followup for >2 years, no long term complications have been encountered.

Total number of patients (n)	130
Follow up (years) (mean/SD)	1.6/0.7
Age (years) (mean/SD)	39/10
Gender (M/F)	49/81
Relationship (LRD/LURD)	95/35
Side of harvest (R/L)	21/109
BMI (kg/m2) <29/>29	94/36
Abnormal anatomy	9
EBL (mL)(mean/SD)	151/125
Transfusion requirement	0
Dissection time (mins)(mean/SD)	141/47
Length of incision (cm)(mean/SD)	6.8/1.0
Immediate graft function (%)	130 (100%)
Length of stay (days) (mean/SD)	2.7/1.0
PCA requirement (hours) (mean/SD)	23/10
Conversions (%)	2 (1.5%)
Readmission (%)	2 (1.5%)
Postoperative complications (%)	4 (3.0%)

Conclusions: The 'mini nephrectomy' procedure combines the benefits of the open retroperitoneal approach and minimally invasive surgery. In contrast to LDN, this procedure does not need expensive instrumentation and is easy to learn. The data presented in this abstract establishes the safety and feasibility of this procedure for all live kidney donors. There have been no long term complications associated with this safe and attractive procedure for live kidney donors.

Abstract# 1590

EXCELLENT RENAL FUNCTION OBTAINED BY RETROPERITONEOSCOPIC LIVE DONOR NEPHRECTOMY UNDER LOW CO_2 GAS PRESSURE. Kazunari Tanabe,[1] Naoshi Miyamoto,[1] Tadahiko Tokumoto,[1] Hirofumi Yamamoto,[1] Hideki Ishida,[1] Tsunenori Kondo,[1] Nobuo Ishikawa,[1] Hiroaki Shimmura,[1] Hiroki Shirakawa,[1] Tomokazu Shimizu,[1] Kiyoshi Setoguchi,[1] Hiroshi Toma.[1] *[1]Department of Urology, Tokyo Women's Medical university, Shinjuku, Tokyo, Japan.*

INTRODUCTION: Transperitoneal laparoscopic live donor nephrectomy is becoming a standard technique for donor kidney retrieval. However, many authors have reported that about 5% of recipients required hemodialysis after renal transplantation due to poor renal function. This poor renal function may be caused by renal artery spasm or venous congestion caused by the high pressure of pneumoperitoneum during the procedure. Also, the transperitoneal approach always carries a potential risk of bowel injury and of late onset bowel obstruction due to adhesion of the intestines. Therefore, we tried to establish the technique of retroperitoneoscopic live donor nephrectomy (RPLDN) under low CO gas pressure as an ideal method in laparoscopic live donor nephrectomy.

PATIENTS AND METHODS: Between July 2001 and June 2003, 120 live donor kidney grafts were procured by retroperitoneoscopic live donor nephrectomy. The patient was placed in a lateral decubitus position. A retroperitoneal working space was developed with a balloon dilator. Three retroperitoneoscopic ports were inserted in all cases. All procedures were performed through the retroperitoneal approach without opening the peritoneal cavity. Low (average 7mmHg) CO_2 gas pressure was employed during the procedure. Kidneys were retrieved through a 5-cm flank incision (initial 38 cases) or a 5-cm Pfannenstiel incision (recent 70 cases). The hand-assisted technique was not used in any of the cases.

RESULTS: One hundred and ten cases were of left and 10 cases of right nephrectomy. Thirty-eight patients had more than 2 renal arteries (2-4 renal arteries). Donor nephrectomy was carried out successfully in all patients. The operative time was 293±75 min. One case was changed to open donor nephrectomy because of severe adhesion around the renal vein due to previous surgery. No serious complications, such as massive bleeding or bowel injury were encountered. Ureteral complications occurred in one patient and was treated with retrograde ureteral stenting. No patients required hemodialysis after transplantation due to acute tubular necrosis. Creatinine levels returned to normal levels within one week in all patients.

CONCLUSION: RPLDN seems to be an excellent method without functional impairment of the transplanted renal graft.

Abstract# 1591

HAND-ASSISTED RETROPERITONEOSCOPIC NEPHRECTOMY. EXPERIENCE WITH THE FIRST 50 CONSECUTIVE CASES. Jonas Wadström.[1] *[1]Department of Surgery, University Hospital, Uppsala, Sweden.*

Laparoscopic live donor nephrectomy has been shown to lower the morbidity. Life threatening complications have, however, occurred. These complications have been related to severe bleedings or visceral injuries during the operation. To reduce the risk of these complications hand-assisted techniques have been introduced as well as the retroperitoneal approach. We have combined these techniques and introduced a hand-assisted retroperitoneoscopic nephrectomy (HARS) technique. This presentation describes our experience with the first 50 consecutive cases.

Materials and methods

The demographics of the donors and operative parameters are summarized in table 1 (mean, min-max, WIT=warm ischemia time).

Age	Female (%)	BMI	Op time (min)	Bleeding (ml)	WIT (sec)	2 arteries (n=)
48 (27-68)	61	26 (20-32)	143 (95-260)	228 (50-700)	177 (90-510)	7

The operation: The left kidney was harvested in all cases. The operation starts with an infraumbilical incision without entering the abdomen and a hand port is introduced into the wound. A retroperitoneal cavity is created with blunt manual dissection. Gas is insufflated and a 12 mm port is placed lateral to the hand port. A 10 mm port is placed on the sub costal margin and a 5 mm port is placed in the flank.

Results

Donors: In 4 cases it was not possible to loosen the peritoneum from the abdominal wall due to earlier operations and the operation was thus preformed intraperitonealy. In 10 cases a hole was caused in the peritoneum but this did not affect the operation or have any influence on the work space. There were no conversions. One donor was reoperated due to suspicion of a trocar hernia but with a negative finding. One donor developed pulmonary embolism 3 moths post operatively.

Recipients: One ureter was anastomosed to the recipient ureter due to lesion of the donor's ureter. There was one urinary leakage. All kidneys except one had immediate onset of function (intraoperative venous thrombosis). The over all graft survival was 96% with a mean follow up of 490 (11-969) days. One graft was lost due to rejection after 20 months and one patient died with functioning graft due to myocardial infarction 7 months after the transplantation.

Conclusions

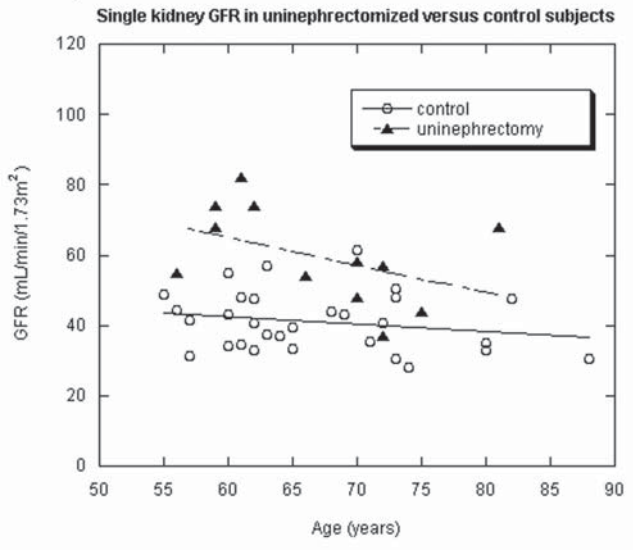
We conclude that the newly developed hand-assisted retroperitoneoscopic nephrectomy (HARS) technique that was developed to minimize the risk of life threatening complications can be preformed with low complication rates, fairly short operating times and excellent graft outcome. It is proposed that this technique could become a new standard for endoscopic live donor nephrectomy.

Abstract# 1592

ADAPTIVE RENAL HYPERFILTRATION IS PRESERVED IN OLDER LIVING KIDNEY DONORS FOLLOWING UNINEPHRECTOMY. Anjali Bhatt,[1] Bryan D. Myers,[1] Geraldine C. Derby,[1] Kristina L. Blouch,[1] Jane C. Tan.[1] *[1]Division of Nephrology, Stanford University, Stanford, CA.*

Introduction: Older donors (age ≥ 55 years) are increasingly being utilized both as deceased and living kidney donors. While kidney function is known to decline with age, little is known about the capacity of the older kidney to undergo adaptive hyperfiltration after uninephrectomy. The purpose of this study was to examine the ability of the older kidney to undergo adaptive renal hyperfiltration following uninephrectomy. Methods: 12 patients aged ≥ 55 who underwent uninephrectomy for kidney transplant donation or hypernephroma between 1 and 5 years ago were recruited for this study (UNX). These subjects were compared to a "control" group of 30 healthy volunteers (CRL), matched for age. Inclusion criteria for all subjects were: (1) lack of comorbid disease, including diabetes, hypertension, severe cardiovascular disease; (2) absence of significant proteinuria; and (3) serum creatinine < 1.5 mg/dL prior to surgery. Subjects underwent determination of glomerular filtration rate (GFR) and renal plasma flow (RPF) using the technique of inulin and PAH clearance, respectively. Results: (expressed as mean ± SEM) Age, at the time of study, did not differ between the groups (67y ± 2 in UNX vs. 67y ± 2 in CRL; p=0.9). Mean arterial blood pressure was also similar in both groups during the study (96 ± 2 mmHg in UNX vs. 96 ± 1 mmHg in CRL; p=0.9). GFR following uninephrectomy was compared to one kidney GFR (50% of the 2-kidney GFR) in controls. One-kidney GFR in the UNX group was significantly higher than the corresponding GFR in the CRL group (60 ± 4 mL/min vs. 41 ± 2 mL/min; p<0.0002) (figure 1). Similarly, RPF was higher in UNX than in CRL (289 ± 23 mL/min vs. 200 ± 12 mL/min; p<0.001). Conclusion: Subjects ≥ 55 years of age maintain the ability to undergo adaptive renal hyperfiltration and hyperperfusion following uninephrectomy. These adaptive responses are comparable in magnitude to values reported in younger subjects.

Figure 1



Abstract# 1593

LIVING KIDNEY DONOR OUTCOMES IN LAPAROSCOPIC VS. OPEN DONATION. Jane S. Benjey,[1] Leslie Trumbull,[1] Jill E. Martin.[2] *[1]Transplant Services, University Hospital, Cincinnati, OH; [2]College of Pharmacy, University of Cincinnati, Cincinnati, OH.*

Purpose: To compare outcomes for live kidney donors related to the surgical procedure, laparoscopic or open, during a two-year period.

Method: An analysis was done of 11,415 living kidney donors who donated during 2000 and 2001 bason on OPTN data as of June 9, 2003. Comparison was made of laparoscopic versus open procedure type, comparing elements related to initial hospitalization, 6-month, and 1-year follow-up periods. Initial hospitalization elements included length of stay, blood transfusions, use of antihypertensives, incidence of pulmonary embolism or infection, and any return to the operating room. At 6-month and 1-year follow-ups, elements included blood pressure, creatinine, incidence of dialysis, any institution of maintenance dialysis or listing for kidney transplant, and any readmissions. Each data element was assessed; Donors with incomplete or questionable data were deleted from that analysis. Of 6264 laparoscopic donors, a maximum of 6256 had usable data; Of 5151 open donors, a maximum of 5072 had usable data.

Results: During initial hospitalization, significant differences were seen between the two groups in length of stay, return to the OR, and infections. During the 6-month follow-up period, a significant difference was seen in incidence of dialysis. At one year, a significant difference was seen in use of antihypertensive drugs. No patient in either group required maintenance dialysis or listing for a kidney transplant. Although there were two donation-related deaths in the laparoscopic donor group, this was not found to be significant.

RESULTS

Time Period	Elements	Lap donors (n)	%	Open donors (n)	%
Initial Hospitalization	Length of stay 1-3 days	4555	60.4	4483	26.3*
	Length of stay 4-6 days		37.1		67.5*
	Return to OR	6256	0.7	5072	0.4*
	Infections	6177	0.6	5072	0.9*
6-month follow-up	Dialysis	4111	0.4	3664	1.7*
1-year follow-up	Antihypertensives	1730	2.6	1409	0.6*

*p<0.0001

Conclusion: Laparoscopic kidney donation compares favorably with open donation in regard to length of hospital stay and incidenc of infections during the initial hospitalization as well as incidence of dialysis at 6 months. Open donation compares favorably with return to operating room during initial hospital stay and use of antihypertensives at one year.

Abstract# 1594

OBESITY IN LIVING RENAL DONORS: IMPACT ON PERI-OPERATIVE AND SHORT-TERM RESULTS. Julie K. Heimbach,[1] Sandra J. Taler,[1] Mikel Prieto,[1] Fernando G. Cosio,[1] Stephen C. Textor,[1] Yogish C. Kudva,[1] George K. Chow,[1] Michael B. Ishitani,[1] Timothy S. Larson,[1] Mark D. Stegall.[1] *[1]Transplant Center, Mayo Clinic, Rochester, MN.*

The short and long-term risks of kidney donation appear to be low, however, the risk for obese donors has not been determined. Prevalence of obesity in the renal donor pool has forced consideration of potential long-term risks, including the development of diabetes mellitus or hypertension and secondary renal disease.

We examined the perioperative course and early follow-up (mean 6±0.3 months, range 0-18) of 553 consecutive living renal donors (related 64%, unrelated 36%) who completed renal donation from 1/1/00 to 4/1/03 using body mass index (BMI) as a marker of obesity. We assessed surgical (operative time, peri-operative complications, length of hospital stay (LOS)), and medical outcomes (GFR by iothalamate clearance, blood pressure (BP) and fasting glucose (FG)).

BMI group, kg/m²	< 25	25-29.9	30-34.9	≥35
Mean BMI, kg/m²	22.4±0.1	27.5±0.1	31.8±0.1	38.7±0.6
N	170	211	114	58
Gender (M:F)	53:117	109:102	59:55	25:33
Operative time, minutes	127±3	134±2	151±4 *	146±6 *
Peri-operative complications, %	5	10	11	14
Baseline clinical characteristics				
BP, mm Hg	126±1/ 74±1	132±1*/ 78±1*	137±2*+ / 80±1*	141±2*+ / 80±2 *
Fasting glucose, mg/dL	91±1	94±1 *	98±1 *+	98±1 *+
GFR uncorrected, ml/min	102±2	116±2 *	118±2 *	128±4 *+
GFR corrected, ml/min/1.73m²	100±1	103±1	100±2	98±2
Post nephrectomy characteristics				
BP, mm Hg	121±2/ 72±1	129±2 */ 75±1	141±2*+ / 80±1*+	139±4*+ / 77±3*
GFR uncorrected, ml/min	67±2	76±2 *	78±3 *	91±6 *+
GFR corrected, ml/min/1.73m²	67±1	67±1	70±2	72±4

Mean±SEM
*p< 0.05 vs BMI group 1, + p< 0.05 vs BMI group 2

Operative times were longer with BMI≥30 kg/m² (p<0.05). There were no differences in peri-operative complications or LOS (mean 2.2±0.1 days). Baseline BP (Dynamap) and FG were higher and rose incrementally with BMI. Lipid profiles were less favorable (higher total and LDL cholesterol and triglycerides, lower HDL) with higher BMI. There were no differences in age (mean 41.6±0.5 years) or serum creatinine (mean 1.1±0.01 mg/dL). Post nephrectomy BP and FG were higher with BMI group but did not differ from pre-donation values. Absolute GFR was greater with higher BMI before and after donor nephrectomy but did not differ once corrected for body surface area. There were no differences in degree of microalbuminuria (mean 8±1 mg pre, 9±1 post) across BMI groups.

Our results support the short-term safety of living kidney donation for carefully selected obese renal donors. While these results are reassuring, continued monitoring of BP levels, glycemic control and renal function over time will be vital to our efforts to quantify health risks in obese donors.

Liver Transplantation: Complications of Immunosuppression

Abstract# 1595

INTERIM ANALYSIS OF A PROSEPCTIVE RANDOMIZED TRIAL TO ASSESS REVERSAL OF EARLY STAGE CALCINEURIN INHIBITOR INDUCED NEPHROTOXICITY USING SIROLIMUS BASED IMMUNOSUPPRESSION IN STABLE LONG TERM LIVER RECIPIENTS. Surendra Shenoy, Karen L. Hardinger, Jeffrey Lowell, Niraj Desai, Thalachallour Mohanakumar, Jeffrey Crippin, Maurico Lisker-Melman, Kevin Korenblat, Mary Ann Laflin, William Chapman. *Surgery, Barnes-Jewish Hospital and Washington University, St. Louis, MO.*

The calcineurin inhibitors (CNI), which have revolutionized the field of liver transplantation, are associated with significant nephrotoxicity potentially leading to renal failure. Switching patients with early CNI induced renal dysfunction to Sirolimus (SRL), a m-TOR inhibitor with no nephrotoxicity, has shown potential in reversing this problem. We present the interim results of a randomized trial to measure the effect of CNI withdrawal on early progressive renal dysfunction after liver transplantation. **Methods:** Stable liver recipients 6 months post transplant, with evidence of early renal dysfunction [24-hr creatinine clearance (CrCl) 50 -80ml/min] were included. Patients were randomized to receive either SRL based immunosuppression (n=20, SRL arm) or to continue CNI based immunosuppression (n=20, control arm). SRL was prescribed (3-5mg/day) to achieve a target serum level of 6-10 ng/ml and CNI was discontinued within 2 weeks. Renal function was assessed by 24-hour CrCl at 1 and 3, 9, and 12 months after enrollment. **Results:** 38 (95%) patients have completed nine months of follow-up and 66% have completed the trial. The initial mean baseline CrCl was similar (64mL/min SRL; 50mL/min control). Significant improvement in renal function was seen in the SRL arm, while renal function remained unchanged in the control arm.

	Creatinine Clearance (mL/min)			
	Baseline	1 month	3 months	9 months
Sirolimus	64±19	75±24	75±24	79±32
Control	60±12	60±17	56±19	62±14
P-value	0.401	0.054	0.012	0.069

Two acute rejections occurred, one in each arm. They were successfully treated with a steroid recycle. One patient on SRL required resumption of cyclosporine for pruritis. Three SRL recipients and two control patients showed altered lipid profiles necessitating lipid lowering agents. Four patients (30%) on SRL reported mouth sores that resolved spontaneously within one month.**Conclusions:** Interim data from this prospective, randomized trial reveals SRL based immunosuppression appears to be a safe and effective alternative to CNI in liver transplant recipients. Withdrawal of CNI provides significant functional recovery of renal function in stable liver transplant recipients with early renal dysfunction.

Abstract# 1596

SUPERIOR RENAL FUNCTION FOLLOWING CONVERSION TO SIROLIMUS AFTER LIVER TRANSPLANTATION - PRELIMINARY RESULTS FROM A RANDOMISED TRIAL. Christopher J. Watson,[1] Graeme J. Alexander,[2] Michael E. Allison,[2] Paul Gibbs,[1] Jane C. Smith,[2] Christopher R. Palmer,[3] Alexander E. Gimson.[2] *[1]University Dept of Surgery, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Cambridge Transplant Unit, Addenbrooke's Hospital, Cambridge, United Kingdom; [3]Cambridge Applied Medical Statistics, Institute of Public Health, Addenbrookes hospital, Cambridge, United Kingdom.*

Introduction

Sirolimus is a powerful immunosuppressant widely used in renal transplantation to spare calcineurin inhibitor (CNI) therapy with the intention of improving renal function. We undertook a randomised controlled study of conversion of stable liver transplant recipients with impaired renal function.

Methods

Liver recipients at least 6 months post transplant with a glomerular filtration rate (GFR) less than 65mls/min were invited to participate. They were randomised to continue on CNI therapy or switch to sirolimus. Conversion involved abrupt cessation of CNI followed by commencement of 2mg/day sirolimus without an overlap period; target range was 5 to 15ng/ml. Concomitant immunosuppression and other medications were not altered. GFR was measured using radionuclide-labelled EDTA at baseline, 3 and 12 months after commencing the study.

Results

18 patients were randomised into the study. One declined further follow up when he was randomised into the CNI group. One who was converted to sirolimus returned to tacrolimus at 3 months because she felt tired since conversion, and another returned at 6 months due to feeling non-specifically unwell. One of the CNI group patients died at 13 months from complications of pulmonary hypertension.

GFR's at randomisation ranged from 15 to 63 mls/min. GFR increased significantly in the sirolimus treated patients by 3 months and was sustained to 12 months, see table.

	Change in mean GFR (mls/min)		
	SIROLIMUS	CNI	Significance
3 months	+ 10.7	+ 0.6	p = 0.004
12 months	+9.3	-5.7	p = 0.039

There was one rejection occurring 2 months following conversion, and associated with a sirolimus level of 3.8ng/ml which resolved with pulsed steroid therapy. The principle adverse events associated with conversion were rashes (4), mouth ulcers (4), herpes simplex stomatitis (2) and epistaxis (2). One patient in each group developed a de novo basal cell carcinoma during follow up. One patient each in the control group developed diabetes and gout.

Conclusion

Abrupt conversion to sirolimus appears safe and is associated with superior renal function. Side effects appear to be dose related and may be managed by dose reduction. These preliminary results suggest that sirolimus may have an important role in the maintenance phase after liver transplantation.

Abstract# 1597

STEROID-FREE REGIME VERSUS STANDARD TREATMENT IN LIVER TRANSPLANT RECIPIENTS. Alejandra Otero,[1] Evaristo Varo,[2] Jorge Ortiz de Urbina,[3] Rafael Martin-Vivaldi,[4] Valentin Cuervas-Mons,[5] Ignacio Gonzalez-Pinto,[6] Angel Bernardos,[7] Rosa Dominguez-Granados MD on Behalf of the Investigators of Study M67014.[8] *[1]H. Juan Canalejo, La Coruña, Spain; [2]H. Clinico Universitario de Santiago, Santiago de Compostela, La Coruña, Spain; [3]H de Cruces, Baracaldo, Vizcaya, Spain; [4]H. Virgen de las Nieves, Granada, Spain; [5]Clinica Puerta de Hierro, Madrid, Spain; [6]H. General de Asturias, Oviedo, Asturias, Spain; [7]H. Virgen del Rocio, Sevilla, Spain; [8]Roche Farma, S.A., Madrid, Spain.*

Objective: To evaluate the efficacy (proportion of patients experiencing a biopsy proven rejection) of a steroid-free regime (Daclizumab in combination with Mycophenolate Mofetil and Tacrolimus) versus standard treatment (Tacrolimus in combination with steroids) for the prevention of acute rejection episodes in recipients of primary hepatic allografts

Methods: First liver allograft recipients are enrolled in a Phase III, comparative, open-label study. Group A (n= 69) received: Daclizumab at a dose of 2 mg/kg 6 hours after surgery and then 1 mg/kg on the 7th day post-transplantation, MMF at 2 g/day. The aimed trough level of FK was 5 to 15 ng/ml during the first month after transplantation and 4 to 8 ng/ml from the first to the sixth month. Group B (n= 61) received: Steroids at 15-20 mg/day during the first week and decrease to withdrawal between Weeks 12 and 24. The aimed trough level of FK was 5 to 15 ng/ml. Treatment of rejection consisted of up to 3 full courses of high dose steroids (defined as up to 1g/day given over 3 days). Primary end point was acute rejection at 6 months.

Results: A group of 154 patients has been included in the study. The six month analysis has been completed in 130 patients. Patients were mainly male and the most frequent primary indication for transplantation was alcoholic cirrhosis followed by Hepatitis C. Seven patients (10.1%) from group A and 14 (22.9%) from group B suffered from acute rejection (p= 0.047). There were no cases of severe acute rejection. Most of them were treated by steroids pulses with good response. No patient required anti-lymphocite treatment. There were no differences between groups regarding patient and graft survival and opportunistic infections. Both treatments were well tolerated.

Conclusions: The steroid-free regime with Daclizumab, Mycophenolate Mofetil and Tacrolimus is more effective than the standard treatment (Tacrolimus and steroids). The combination of Daclizumab, Mycophenolate Mofetil and Tacrolimus was well tolerated without an increase neither in infection rate nor in adverse events.

Abstract# 1598

PERSPECTIVES IN CARDIOVASCULAR DISEASE AFTER LIVER TRANSPLANTATION. Olaf Guckelberger,[1] Alice Byram,[1] Jochen Klupp,[1] Ulf P. Neumann,[1] Matthias Glanemann,[1] Ruth Neuhaus,[1] Peter Neuhaus.[1] *[1]Dept. of Surgery, Charité Campus Virchow-Klinikum, Berlin, Germany.*

Increased prevalence of cardiosvascular risk-factors attributed to side-effects of immunosuppressive agents has been described in liver transplant populations. Long-term effects are controversial and the actual risk for cardiovascular events (CVE) in liver transplant recipients has not yet been established. Here we evaluate the development of cardiovascular risk-factors during a longitudinal follow-up of 10 years, report cardiovascular events during this period and compare numbers of coronary heart disease (CHD) events with numbers predicted from the PROCAM score (standard population).

Methods: 302 consecutive adult patients, surviving more than 6 months after liver transplantation (LT), have been evaluated for their cardiovascular risk profile in 1993 (T1). In 2003 (T2), patients have been re-evaluated and cardiovascular events have been recorded.

Results: 199 patients were eligible for re-evaluation at T2. During follow-up, 54 patients died (4 due to CVE), 13 underwent re-LT, 8 were "lost in follow-up", and 28 presented with incomplete sets of data. 20 non-fatal CVE occurred in 17 patients. Within 10 years, rate of steroid-free patients increased from 22% to 90%. Prevalence of overweight (17% vs 27%, p<0.001), hypertension (46% vs 63%, p<0.001), and diabetes (21% vs 25%, p<0.001) increased over time, while increased values of cholesterol (64% vs 37%, p<0.001) and triglycerids (40% vs 21%, p<0.001) were detected less frequent at T2. Gender did not affect those developments. In 2003, no significant differences were detected comparing patients under CsA or Tac treatment. In 75 middle-aged men, eligible for PROCAM score calculation at T1, the predicted risk of CHD events within 10 years was 7.4%. During follow-up, in 9 of those patients (12%) CHD events were observed.

Discussion: Although more than 10 years post LT, patients presented with elevated cardiovascular risk profiles. In parallel with steroid withdrawal, prevalence of impaired lipid metabolism decreased, while figures of overweight, hypertension and diabetes increased over time. The choice of calcineurin-inhibitor did not influence the cardiovascular risk profile long-term. Although liver transplant recipients represent a well selected population, cardiovascular morbidity matches numbers predicted from findings in risk-adjusted standard populations at 10 years post LT. Longer follow-up may even exceed those numbers.

Abstract# 1599

PRIMARY MANAGEMENT OF POST-TRANSPLANT DIABETES WITH ROSIGLITIZONE. Griselda Villanueva,[1] David Baldwin, Jr..[1] *[1]Section of Endocrinology, Dept. of Medicine, Rush University Medical Center, Chicago, IL.*

New onset diabetes mellitus is a common problem after solid organ transplantation. Post-transplant diabetes mellitus (PTDM) has been associated with an increased risk of infections, adverse cardiovascular outcomes, mortality and decreased graft survival. Our experience and that of other centers has been that the vast majority of patients with PTDM will require chronic insulin therapy. Since increased insulin resistance is believed to play a significant role in the development of PTDM, we have treated these patients with the insulin sensitizer rosiglitazone (ROSI) after liver and kidney transplantation.

METHODS: All patients with PTDM were initially stabilized with twice daily NPH/REG insulin. As long as heart failure was not present, ROSI 4 mg po per day was added. Patients were followed for a mean of 26 weeks and insulin was adjusted using home blood glucose data.

RESULTS: In 12 months time 30/107 liver (27%) and 8/205 kidney (4%) patients developed PTDM. In our center ~ 2/3 of renal transplant patients are managed without chronic glucocorticoid Rx, and ~1/3 without calcineurin inhibitors. Most liver transplant patients receive tacrolimus and prednisone. After a mean of 10 weeks of insulin and ROSI therapy, insulin was able to be discontinued in 32/38 (84%) patients with PTDM. 11/32 (34%) patients maintained normoglycemia (80-140 mg./dl.) with ROSI monotherapy; 21/32 (66%) required the addition of a sulfonylurea to meet this goal. 25/32 (78%) continued on 4 mg/day of ROSI ; 7/32 (22%) required an increase to 8 mg/day. There was no significant change in serum AST (43 U/L pre /48 U/L post) and ALT (79 U/L pre /51 U/L post) during the post transplant follow-up period. Mean serum creatinine was unchanged at 1.3 mg/dl after a mean of 26 weeks. 31/38 (81%) patients received tacrolimus and 7/38 (19%) received cyclosporine. As expected, there was a gradual decline in tacrolimus and cyclosporine doses over 26 weeks after transplant. Prednisone was able to be discontinued in 10/38 (26%) patients after 26 weeks.

CONCLUSIONS: Rosiglitazone is the first highly effective oral agent for PTDM. The majority of patients with PTDM may be safely treated with ROSI +/- a sulfonylurea, whereas our past experience indicates that sulfonylurea monotherapy is usually ineffective. After the expected 3-6 week delay in the onset of ROSI action, most patients with PTDM will no longer require long term insulin therapy.

Abstract# 1600

REDUCED RISK OF LIVER TRANSPLANT REJECTION IN THE ELDERLY. Meelie A. DebRoy,[1] Tempie E. Hulbert-Shearon,[1,2] Dawn M. Dykstra,[2] Robert A. Wolfe,[1,2] Friedrich K. Port,[2] Robert M. Merion.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]SRTR/URREA, Ann Arbor, MI.*

Liver transplantation is an accepted therapy in older patients. Older and younger recipients are usually given a similar intensity of immunosuppression. We propose that older patients may be relatively over-immunosuppressed and experience associated excess mortality.

SRTR data for 26,946 adult liver transplant (txp) recipients during 1993-2001 were used to determine post-txp survival and the incidence of rejection in the first year post-txp by age group. Patients missing immunosuppression therapy data or who died within 30 days post-txp were excluded. Logistic regression models were fit to determine the odds of treated rejection, adjusted for recipient race, ethnicity, sex, diagnosis, living or deceased donor, induction antibody treatment, and year of txp. Cox regression models from day 30 until 1 and 5 years post-txp were fit to examine all cause mortality risk by age group and that due specifically to infection and/or malignancy (Inf-Mal), adjusted for the factors above as well as recipient creatinine, life support, status 1, cold ischemia time, and ABO compatibility.

The incidence of treatment for rejection in the first year ranged from 44% for age 18-39 to 32% for age 60+. The adjusted odds of rejection in the first year were significantly lower for older than younger patients (see Table). Both overall and Inf-Mal specific 1-year adjusted mortality risks were significantly higher for older than younger patients (see Table). Mortality risk for the oldest patients was more than twice that for the youngest (overall mortality HR=2.28, p<0.0001; Inf-Mal-specific HR=2.07, p<0.0001). Results at 5 years showed 76% higher overall mortality risk and 62% higher Inf-Mal-specific risk for 60+ than 18-39 year olds (p<0.0001 for both).

Treated Rejection and One-year Mortality Risk After Liver Transplant by Age Group

	Treated Rejection	Relative Mortality Risk**	
Age group	Adjusted Odds Ratio*	All Cause	Infection and/or Malignancy
18-39 (ref.)	1.00	1.00	1.00
40-49	0.82	1.21	1.14
50-59	0.75	1.53	1.46
60+	0.68	2.28	2.07

* All pairwise comparisons significantly different at p<0.02. ** p<0.05 for 18-39 vs 40-49. All other pairwise comparisons significantly different at p<0.001.

Current immunosuppression regimens may expose older patients to excess mortality risk due to infection and malignancy, as a consequence of relative over-immunosuppression. We hypothesize that survival of older liver transplant recipients might be improved by reducing the intensity of immunosuppression.

Abstract# 1601

MYCOPHENOLATE MOFETIL IN LIVER TRANSPLANTATION FOR HEPATITIS C: EFFECT ON RECURRENCE AND PHARMACOKINETICS. Rafik M. Ghobrial,[1] Curtis D. Holt,[1] Theodore M. Sievers,[1] Sammy Saab,[2] Lucy Artinian,[1] Suzanne V. McDiarmid,[3] Ronald W. Busuttil.[1] *[1]Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Effects of Mycophenolate Mofetil (MMF) on hepatitis C virus (HCV) recurrence following orthotopic liver transplantation (OLT) remain controversial. Further, MMF pharmocpkinetics after OLT are yet to be investigated.

Methods: MMF effects on HCV recurrence following OLT for HCV was evaluated in a prospective randomized open-label study in OLT recipients. Protocol biopsies were performed at 3, 6, 12 and 24 months. At days 7 and 90 post-OLT, mycophenolic acid (MPA) concentrations were measured by a validated HPLC method with ultraviolet detection from serial blood draws over a 12 hr period following MMF dosing. Dose normalized area under the curve (AUC) were calculated by noncompartmental methods.

Results: Twenty patients were enrolled in each arm. In the first, recipients received Tacrolimus (Tac) and Prednisone that was tapered over a 3 months period, In the second arm patients received Tac, and Prednisone that was tapered over one month, and MMF that was orally given as 500 or 1000 mg (creatinine >2 or <2 mg/dl) twice daily for one year. Average age was 53±9.2 yrs and average follow-up was 185±195 days. Model for End Stage Liver Disease (MELD) scores, were 24.9 ± 6 and 23.9 ± 7.5, in each arm, respectively (*P* NS). Three deaths occurred in each arm that were caused by sepsis and multiorgan failure in 5 patients and severe pulmonary hypertension in one. One patient in each arm underwent re-OLT for recurrent disease. No significant difference was observed in HCV RNA, ALT, AST levels measured at 1, 3, 6 and 12 months post-OLT. However, evidence of histological HCV recurrence was detected at 3 months in both groups. Hepatitis activity index (HAI) for the two arms was 2.8±0.5 vs 3.1±0.4 (*P* NS) at 3 months, and 6.8±1.5 vs 7.5±1.4 at 6 months, respectively. Similarly, there was no difference in fibrosis scores 0.2±0.5 vs 0.15±0.5 (*P* NS) at 3 months, and 0.6±0.3.5 vs 1.5±1.4 at 6 months, respectively. Day 7 and day 90 AUC were 20.42±9.96 and 21.16±8.14 mg h/L. One patient with low AUC (8.8 mg h/L) experienced a rejection episode at day 40. AUC did not appear to significantly differ among patients based on level and timing of recurrence and did not correlate with trough drug levels.

Conculsions: MMF utilization does not appear to affect the rate of recurrence of HCV following OLT in the short-term. PK data demonstrate intra- and inter-individual variability post-OLT and does not correlate with trough levels.

Abstract# 1602

CORRELATION OF CELL-MEDIATED IMMUNE RESPONSE WITH LIVER ALLOGRAFT BIOPSY IN LIVER TRANSPLANT RECIPIENTS WITH HEPATITIS C. 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: Post-liver transplant immunosuppression management is difficult in recipients with hepatitis C (HCV). Over-aggressive immunosuppression may lead to severe HCV recurrence and sub-optimal immunosuppression may lead to graft rejection. Biopsies to assess HCV recurrence versus rejection are often difficult to interpret due to similar histopathologic processes. 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. Using this assay, the cell-mediated immune response in HCV post-liver transplant recipients was correlated with biopsy findings. We hypothesized that this assay would differentiate HCV recurrence compared to acute cellular rejection in HCV liver transplant recipients.

Methods: Blood from 24 liver transplant recipients with HCV undergoing protocol or diagnostic liver biopsy were obtained and assayed for cell-mediated immune response using the ImmuKnow™ assay. ATP release was classified as: low (<225ng/ml); moderate (226-524ng/ml); and strong (>525ng/ml). Biopsies were reviewed by pathologists blinded to assay results.

Results: In recipients with evidence of rejection on biopsy (n=6), 3/6 (50%) had a strong ATP release, 2/6 (33.3%) had a moderate release, and 1/6 (16.7%) had a low release. In recipients with recurrent HCV on biopsy (n=16), 0/16 had a strong ATP release, 4/16 (25%) had a moderate release, and 12/16 (75%) had a low release. Two recipients with HCV with evidence of neither rejection nor HCV recurrence on biopsy had low and moderate releases, respectively.

Conclusions: This initial evaluation suggests that rejection is predominately evident in HCV liver transplant recipients with strong/ moderate ATP release. This finding implies sub-optimal immunosuppression and relatively intact host immunity. HCV recurrence is predominately observed in recipients with low/moderate ATP release suggesting adequate and/or possible over-immunosuppression. In this latter group of recipients, reduction of immunosuppression may be necessary, in addition to, HCV anti-viral therapy. This assay when combined with histopathological findings may be helpful in post-transplant monitoring of immunosuppression in HCV liver transplant recipients.

Abstract# 1603

DRUG RESISTANCE MUTATION IN HUMAN CYTOMEGALOVIRUS ISOLATED FROM PATIENTS AFTER LIVER TRANSPLANTATION. Gregory A. Smallwood,[1] Ling Lu,[2] Todd Pillen,[2] Curt Hagedorn,[4] Thomas G. Heffron.[5] *[1]Department of Pharmacy, Emory University Hospital, Atlanta, GA; [2]Liver Transplantation, Children's Healthcare of Atlanta, Atlanta, GA; [3]Department of Medicine, Kansas University School of Medicine, Kansas City; [4]Department of Surgery, Emory University School of Medicine, Atlanta, GA.*

Background: In the viral DNA polymerase gene of human cytomegalovirus (CMV), mutations on 24 codons were experimentally proven to confer drug resistance to ganciclovir, cidofovir, and foscarnet (Chou et al., 1993, JID 2003:188).

Purpose: To evaluate the virus infecting liver transplant recipients that were slow to respond to antiviral therapy.

Methods and Materials: High risk liver transplant recipients were routinely followed (weekly to biweekly) by polymerase chain reaction (PCR) for the human CMV virus and at time of seroconversion, preemptive therapy is begun with ganciclovir 5mg/Kg Q12H. Patients were classified as responders, slow responders and clinically resistant patients. Serum samples of all patients (n = 12) converting between January and Nov. 2003 (n = 24) were collected and stored at -80° c. Long PCR was used to amplify CMV DNA of 2647 bp corresponding to nucleotides 897-2934 in the DNA polymerase gene of the serum samples collected. Amplicons were sequenced in ABI 3100 genetic analyzer. The sequence information was analyzed with DNASTAR software, i.e. in multiple sequence alignments; nucleotide positions against these mutations on the 24 codons were scrutinized to determine drug resistant CMV. Correlation with this lab results is then compared to clinical findings.

Results: Of the 12 seroconverting, 4 were pediatric (mean age: 7.1 years) patients with one seroconverting to Epstein Barr (EBV). Mean time to CMV conversion was 48.2 ± 6.5 days. Of the 12 patients, 6 responded within one week to ganciclovir with a drop in CMV by PCR levels. Of the remaining 6 patients, 3 patients were slow to respond to ganciclovir taking 10.6 ± 4.2 days to respond to ganciclovir and the remaining 3 patients were switched to foscarnet. Of the ones that were clinically switched to foscarnet only one mutation A692S was discovered in two consecutive samples from one child

patient. Based on the A692S mutation, the patient should have been resistant to foscarnet and in fact responded to foscarnet by sequential dropping of viral loads from <50,000 to 33,900 to < 200 copies/ml. Of the remaining samples, no further mutations were noted for nucleotides 897-2934 in the DNA polymerase gene.

Conclusion: Although previous transfer studies had proven that the A692S mutation conferred resistance to foscarnet, our results suggest that serum samples demonstrating this mutation may in fact respond to foscarnet. Clinical response should be primary determinate for resistant CMV.

LYMPHOCYTE ACTIVATION: EXPERIMENTAL THERAPEUTICS

Abstract# 1604

CD95/CD95L ENGAGEMENT IS A REQUIREMENT FOR STABLE EMBRYONIC STEM CELL ENGRAFTMENT IN ALLOGENEIC RECIPIENTS. Dorit Fabricius, Sabrina Bonde, Nicholas Zavazava. *Internal Medicine, University of Iowa & VA Medical Center, Iowa City, IA.*

Embryonic stem cells (ESC) have been recently shown to offer a possible solution for low risk induction of hematopoietic multilineage chimerism. This may ultimately lead to tolerance induction after transplantation without or with minimal host pre-conditioning. Here, we examined whether the constitutively expressed FasL (CD95L) in ESC is a requirement for the induction of stable ESC engraftment using the Fas-mutant MRL *lpr/lpr* mouse, which does not express functional Fas (CD95). Three mouse derived ESC lines, C57BL/6, 129SvJ R1 and the 129SvJ RW-4, were characterized and used for these studies. Characteristically, all cell lines expressed very low levels of MHC class I antigens, no class II antigens, high levels of stage-specific embryonic antigen-1 (SSEA-1) and FasL. One of the 3 cell lines, 129SvJ RW4 ESC (H-2K^b) showed high proliferation and virtually no class I expression. This cell line was further used for the induction of mixed chimerism. ESC were infused into the supra-orbital vein of allogeneic MRL (H-2K^k) *lpr/lpr* mice (n=7). The MRL wild type mice were used as controls (n=7). Chimerism was studied by flow cytometry of whole blood leukocytes on days 7, 14 and 28 post ESC infusion and defined as co-expression of H-2^b and any of the CD markers CD3, CD19, CD14, CD16&32 or NK1.1. At day 14, over 85 % of wild type mice showed mixed chimerism compared to less than 30 % in *lpr/lpr* mice. By day 28, still more than 70 % of wild type mice maintained chimerism, versus none in *lpr/lpr* mice. Mixed chimerism was generally between 5-10 % in peripheral blood. These results suggest that Fas-FasL interaction is a requirement for the induction of stable mixed chimerism in mice, presumably due to the induction of apoptosis to alloreactive T cells by ESC. Indeed, immunohistochemical staining of chimeric wild type mice showed massive induction of apoptosis in the thymus, spleen and liver, indicating the role of apoptosis in ESC engraftment. Thus, our studies provide a rationale for studying ESC as an alternative to bone marrow cells for the induction of mixed chimerism with minimal host pre-conditioning and underscore the role of Fas/FasL engagement in ESC engraftment.

Abstract# 1605

PIGS LACKING THE α-1,3GALACTOSYLTRANSFERASE GENE DEVELOP ANTI-GAL ANTIBODIES THAT ARE CYTOTOXIC TO WILD-TYPE (GAL$^{+/+}$) MHC-MATCHED PIG CELLS: A POTENTIAL MODEL TO STUDY ACCOMMODATION. Frank J. M. F. Dor,[1] Yau-Lin Tseng,[1] Jane Cheng,[2] Kathleen Moran,[2] Todd M. Sanderson,[2] Courtney J. Lancos,[1] Akira Shimizu,[2] Michel Awwad,[2] David H. Sachs,[1] Henk-Jan Schuurman,[2] Robert Hawley,[2] David K. C. Cooper.[1] *[1]Transplantation Biology Research Center, MGH/Harvard Medical School, Boston, MA; [2]Immerge Biotherapeuthics, Cambridge, MA.*

Background: Recently, pigs that lack the gene for α-1,3galactosyltransferase (GalT-KO pigs) have been bred using nuclear transfer technology on a MGH miniature swine background (SLA^{dd}). We studied whether GalT-KO pigs develop anti-Gal antibodies (Abs) (as do GalT-KO mice) and also their cellular response to wild-type (Gal$^{+/+}$) MHC-matched (SLA^{dd}) pig cells.

Methods: Tissues from GalT-KO pigs (n=5) were examined for their expression of Gal by the immunoperoxidase technique using the biotinylated lectin, *Griffonia simplicifolia* (IB4). The presence of anti-Gal Abs in the sera was determined by ELISA. Abs to MHC-matched (SLA^{dd}) Gal$^{+/+}$ miniature swine cells were determined by flow cytometry (FACS). Cytotoxicity assays were performed to assess whether the Abs, if present, were cytotoxic to Gal$^{+/+}$ SLA^{dd} peripheral blood mononuclear cells (PBMC). Cellular responses of GalT-KO miniature swine to Gal$^{+/+}$ SLA^{dd} miniature swine were examined by mixed lymphocyte reactivity (MLR) and cell-mediated lympholysis (CML) assays.

Results: None of the GalT-KO pigs showed any Gal expression on histological staining of all major tissues. Their sera contained anti-Gal IgM Abs by ELISA. FACS demonstrated binding of GalT-KO sera to Gal$^{+/+}$ SLA^{dd} PBMC. IgM Abs were present in the sera of 2 pigs and both IgM and IgG Abs in 3 pigs. Sera with IgM Abs alone were weakly cytotoxic, while those with IgM+IgG were strongly cytotoxic to Gal$^{+/+}$ PBMC, but treatment with dithiothreitol (DTT) rendered the sera non-cytotoxic, revealing that most of the cytotoxicity was due to IgM Abs. MLR and CML assays revealed no T cell response by GalT-KO pigs to their Gal$^{+/+}$ MHC-matched counterparts. There was no correlation between the age of the pig and the Ab status.

Conclusions: GalT-KO pigs develop cytotoxic anti-Gal Abs, primarily IgM, but have no increased T cell reactivity to MHC-matched Gal$^{+/+}$ pigs. These data suggest that the transplantation of organs from Gal$^{+/+}$ pigs into MHC-matched GalT-KO pigs may be a clinically-relevant large animal model in which to study mechanisms of accommodation or B cell tolerance.

Abstract# 1606

INABILITY TO CLEAR POLYOMA VIRUS IN MHC DISPARATE MURINE KIDNEY TRANSPLANT MODEL RESULTS IN ACCELERATED GRAFT REJECTION. Eun D. Han Lee,[1] Jun Wang,[1] Ying Dong,[1] Aron E. Lukacher,[1] Kenneth A. Newell,[1] Tom C. Pearson,[1] Christian P. Larsen.[1] *[1]Surgery, Emory University, Atlanta, GA.*

BK virus (BKV) is a significant factor in the failure of renal transplants. Polyomavirus establishes a persistent silent infection in most immunocompetent individuals. However, in the setting of immunosuppression, viral replication and pathology may ensue. To date no animal model has been described to investigate the pathogenesis of BKV associated nephropathy.

Methods: Transplantation of either B6 or C3H kidneys into B6 recipients with or without native nephrectomy was performed with or without polyoma infection. Viral titers and virulence were measured by real time PCR and plaque assay. Renal allograft was assessed histologically and functionally (serum creatinine). Cytotoxic T lymphocyte (CTL) assay and flow cytometry was used to quantitate antiviral and anti-donor responses.

Results: Mice with renal allografts develop chronic rejection but survive >60 days without immunosuppression. Polyoma virus precipitates accelerated destruction of allografts (MST 11±2) but not isografts (MST>60). Furthermore, we found >1000-fold higher levels of virus in allogeneic as compared to syngeneic kidney transplants. Tissue damage was markedly greater and creatinine significantly higher in polyoma infected renal allografts compared to uninfected allografts or infected isografts (n=5, 1.7 vs. 0.3, 0.5 mg/dL, P<0.0007, 0.0019). To show recipient restricted viral clearance, mice were acutely infected after orthotopic allogeneic kindey trasplantation. Allograft kidneys had markedly elevated levels of virus compared to the native kidney ($1x10^8$ vs. $1x10^4$ copies/mg, P<0.02). Lastly, we observed that the anti-donor CD8+T cell response was greater in renal allograft recipients with infection compared to uninfected recipients. However, ICCS and CTL assay with donor specific stimulation showed that infection alone does not induce cross-reactivity.

Conclusion: These data indicate that polyoma virus promotes rapid destruction of renal allografts. Nephropathy is associated with an inability to clear virus from an MHC disparate organ, but not a syngeneic transplant or native kidney. Polyoma infection significantly augments the anti-donor T cell responsive to a kidney allograft. However, infection without an allogeneic kidney transplant fails to induce virus-specific T cells that are cross-reactive against the donor. Using this novel model of polyoma associated nephropathy, we hope to discover insights into the pathogenesis and develop new therapies for this major clinical problem.

Abstract# 1607

BLOCKADE OF CO-STIMULATORY MOLECULES USING INTRACELLULAR FUSION PROTEINS. Andrew J. T. George,[1] Peng H. Tan,[1] John Yates,[1] Robert I. Lechler,[1] Giovanna Lombardi.[1] *[1]Department of Immunology, Imperial College London, London, W12 0NN, United Kingdom.*

We have developed a novel strategy to inhibit expression of B7 molecules (CD80/86) by transfecting with a gene construct encoding a modified CTLA4 molecule (CTLA4-KDEL). The KDEL sequence serves to retain this molecule in the endoplasmic reticulum (ER), where it binds to CD80/86 molecules, preventing them from moving out of the ER, through the Golgi and onto the cell surface. Human APCs, including B cells and dendritic cells, expressing this construct did not express CD80/86 on their surface. They showed normal expression of other cell surface molecules. They were unable to stimulate allogeneic and peptide-specific T cell responses and induced antigen specific anergy of the responding T cells. Cells expressing CTLA4-KDEL did not upregulate the indoleamine 2, 3-dioxygenase (IDO) enzyme, unlike the cells treated with soluble CTLA4-Ig. This gene-based strategy to inhibit expression of key surface receptors is an attractive alternative to the use of immature DCs (iDCs) for prevention of transplant rejection.

Abstract# 1608

ROLE OF NFAT TRANSCRIPTION FACTORS IN SKIN AND VASCULARIZED CARDIAC ALLOGRAFT REJECTION. Takuya Ueno,[1] Nader Najafian,[2] Akira Yamada,[1] Laurie H. Glimcher,[2] Mohamed H. Sayegh,[2] Hugh Auchincloss, Jr..[1] *[1]Transplantation Unit, Surgery, Massachusetts General Hospital, Boston, MA; [2]Transplantation Research Center, Brigham and Women's Hospital & Children's Hospital, Boston, MA.*

The nuclear factor of activated T cells (NFAT) proteins are a family of transcription factors whose activation is controlled by calcineurin, a calcium-dependent phosphatase. Both cyclosporine and tacrolimus, commonly used in clinical transplantation, are known to inhibit the activation of NFAT family of transcription factors. Nevertheless, the role of individual NFAT family members has not been explored in transplantation. In a previous study, our group demonstrated that naive T precursor cells that are doubly deficient in NFATc2 and NFATc3 intrinsically differentiate into Th2-secreting cells. In this study we sought to explore the role of these molecules in both skin and cardiac transplantation models in vivo. To this end, NFATc2/c3 double-deficient BALB/c mice (DKO) received full thickness trunk skin or vascularized cardiac allografts from fully MHC-mismatched BALB.B donors. The allograft survival was significantly prolonged in DKO recipients of skin grafts (18.9±2.9, n=8 versus 12.1±1, n=9 in controls p<0.0001) and somewhat less in recipients of heart allografts (15.4±0.6, n=6 versus 8±0.3, n=5, p=0.0002). The delay in allograft rejection was not due to inability of DKO T cells to respond to alloantigens, as mixed lymphocyte reaction of purified DKO CD4+ T cells to irradiated BALB.B splenocytes was comparable to that of wild-type control CD4+ T cells. To elaborate the role of IL-4 in prolonging allograft survival, DKO mice were next crossed with BALB/c IL-4 deficient mice to generate mice triply-deficient for NFATc2, NFATc3 and IL-4. TKO mice rejected both skin (13.5±0, n=4, p=NS) and cardiac allografts (7.5±0.4, n=8, p=NS) as promptly as wild-type BALB/c controls. This is the first report demonstrating that NFATc2 and NFATc3 play an important role in transplant rejection, at least in part through their ability to control IL-4 production. Better understanding of the contribution of NFAT molecules to the regulation of the immune response will help develop better strategies for tolerance induction in future.

Abstract# 1609

THROMBOPHILIA ASSOCIATED WITH ANTI-CD154 MONOCLONAL ANTIBODY TREATMENT AND ITS PROPHYLAXIS IN NONHUMAN PRIMATES. Svetlan Boskovic,[1] Tatsuo Kawai,[1] David Andrews,[2] Ichiro Koyama,[1] Ognjenka Nadazdin,[1] Robert B. Colvin,[3] David H. Sachs,[4] A. Benedict Cosimi.[1] *[1]Transplant Unit, Massachusetts General Hospital, Boston, MA; [2]Department of Blood Transfusion, University of Miami, Miami, FL; [3]Pathology, Massachusetts General Hospital, Boston, MA; [4]Transplant Biology Reseach Center, Massachusetts General Hospital, Charlestown, MA.*

Background: Blockade of the CD40/CD154 pathway has provided one of the most robust strategies to regulate auto or alloimmune responses. The successful clinical application of this strategy for organ transplantation or autoimmune disease therapy, however, was delayed when initial clinical trials suggested an unusually high incidence of thromboembolic complications in patients receiving anti-CD154 monoclonal antibody (mAb)treatment. Thrombophilia was also associated with anti-CD154 mAb treatment in nonhuman primate renal allograft recipients.These studies were undertaken to clarify the mechanisms involved and to define an effective prophylactic regimen for this complication.**Methods**: All monkeys were treated with a nonmyeloablative conditioning regimen which consists of low dose TBI (1.5 Gy x2), thymic irradiation(7 Gy), ATG, combined donor bone marrow and kidney transplants with or without splenectomy (day 0), followed by a one-month course of cyclosporine. A short course (20mg/kg/day x 2 or 6) of anti-CD154 mAB (5c8) was added in eighty recipients.**Result:** Without 5c8, no recipients developed thromboembolic complications (0/67). Among 11 recipients who received 5c8 without anticoagulation, 2/6 without splenectomy and 3/5 with splenectomy developed thromboembolism. Initial in vitro studies suggested that CD40 expressed on platelets may interact with the mAb possibly leading to a lowered threshhold of activation by platelet agonists such as epinephrine.With addition of perioperative heparin, the thromboembolic incidence was decreased to 2/10. No further improvement was observed by adding intraoperative prostaglandin E1 (PGE1). However, addition of Ketorolac, a non-steroidal anti-inflammatory drug, to PGE1 and heparin decreased the incidence of thrombosis (1/8). Most recently, we have found that Ketorolac administration alone resulted in no thrombosis in 25 consecutive recipients. **Conclusion**: Ketorolac is remarkably effective in preventing thromboembolism associated with anti-CD154 mAb treatment, further suggesting the mechanism underlying this complication is related to platelet activation leading to enhanced aggregation.

The incidence of thromboembolism

		No Splenectomy		With Splenectomy	
		# Tx	# thrombosis	# Tx	# thrombosis
no 5c8		3	0	64	0
+ 5c8	No anticoagulation	6	2	5	3
+5c8	Heparin	10	2	-	-
+5c8	Heparin+PGE1	24	6	2	2
+5c8	Heparin+PGE1+Ketorolac	4	0	4	1
+5c8	Ketorolac	25	0	-	-

Abstract# 1610

IN VIVO IMMUNE MODULATION BY IN VITRO-GENERATED TOLEROGENIC DENDRITIC CELLS IN MURINE HEART TRANSPLANTATION. Mu Li,[1] Dameng Lian,[1] Thomas E. Ichim,[1] 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]Department of Surgery, Microbiology and Immunology, and Pathology, University of Western Ontario, London, ON, Canada.*

BACKGROUND: Tolerogenic dendritic cells (Tol-DC) play an important role in transplant tolerance through a self-maintaining regulatory feedback loop between Tol-DC and T regulatory cells (Treg) (*J Immunol 2003, 170:1304*) based on experiments in vitro. This study was designed to evaluate the in vivo immunomodulatory ability of Tol-DC using a murine allogeneic heart transplant model. **METHODS:** Dendritic cells were isolated from the donor strain (C57/BL6, H-2^b) bone marrow progenitors. Tol-DC were generated by culture in the medium conditioned with LF15-0195, a tolerance-inducing immune suppressant. Tol-DC were intravenously administrated to recipients (Balb/c, H-2^d) 7 days prior to MHC-fully mismatched heterotopic heart transplantation. The abilities of Tol-DC to induce immune modulation were assessed in vitro and in vivo. **RESULTS:** In vitro-generated Tol-DC possessed tolerogenic properties as witnessed by decreased expression of MHC II and co-stimulatory molecules, as well as, impaired allo-stimulatory capacities. Administration of in vitro-generated Tol-DC ($5x10^6$ cells) prior to transplantation resulted in significant prolongation of allograft survival (MST 48 ± 14 days), as compared to the survival of recipients treated with the same number of control DC (MST 13 ± 1 day). In addition, $CD4^+CD25^+$ T cells were significantly increased in the Tol-DC treated recipients but not in control DC treated recipients. $CD4^+CD25^+$ T cells isolated from Tol-DC treated recipients had regulatory ability in an in vitro inhibitory MLR assay. Furthermore, the cytokine profile of T cells in the Tol-DC treated recipients revealed a Th2 differentiation. **CONCLUSIONS:** In vitro-generated DC initiate a regulatory feedback loop in vivo through promotion of Treg generation and Th2 differentiation, which subsequently prevented allograft rejection.

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Abstract# 1611

SINGLE INFUSION OF PHARMACOLOGICALLY-MODIFIED, ALLOANTIGEN PULSED RECIPIENT DC PROLONGS TRANSPLANT SURVIVAL BY INDUCING AG-SPECIFIC T CELL HYPORESPONSIVENESS. T. Taner, H. Hackstein, Z. Wang, A. W. Thomson. *T E Starzl Transplantation Institute, U of Pittsburgh, Pittsburgh, PA.*

Dendritic cells (DC) are professional antigen-presenting cells with the unique ability to initiate and regulate immune responses. In the transplantation setting, both donor-derived and recipient DC contribute to allorejection by initiating T cell activation and proliferation. DC, particularly in an immature state, are also inherently tolerogenic and can prolong allograft survival. Sustained immaturity in vivo, however, remains to be achieved. Among many approaches to generation of stably immature DC, pharmacologic modification stands out as a promising and clinically applicable option. Rapamycin (Rapa) is a potent immunosuppressant with well-recognized T cell inhibitory effects. We have recently shown that Rapa can also impair DC maturation and function. Here, we examined the impact of in vitro-generated, Rapa-treated, alloAg-pulsed recipient myeloid DC (Rapa-DC) on murine vascularized heart graft survival and the underlying immunological mechanisms. DC were generated from C3H/HeJ (H-$2K^k$) mouse bone marrow in the presence of clinically relevant concentration of Rapa (10 ng/ml). Following pulsing with alloAg [freeze-thawed donor (C57BL/10; H-$2K^b$) splenocytes], the DC were infused i.v. ($1.5x10^6$ cells) to recipient C3H mice. Rapa-DC were able to localize to T cell areas of the spleen, similar to control DC. Unlike the latter, however, they prolonged graft survival significantly when infused 7 days prior to transplantation (MST 23.9 vs. 10.4 days, p<0.005). This effect was Ag-specific (MST 12 days for Rapa-treated, 3rd party Ag-pulsed DC) and could be enhanced by administration of minimally-effective dose (1 mg/kg/day, i.m.; 10 days) of FK-506 (MST 47.8 days). Studies to elucidate the underlying mechanisms showed that Rapa significantly inhibited spontaneous and LPS-induced maturation of DC, while it did not block alloAg uptake. Rapa-DC were poor stimulators of syngeneic T cells, presumably due to their impaired ability to present alloAg via the indirect pathway. T cells primed by Rapa-DC showed decreased capacity to produce IL-2 and IFN-γ, and were hyporesponsive to rechallenge in an Ag-specific manner. This hyporesponsiveness was reversible by exogenous IL-2. Together, these results show that a single infusion of pharmacologically-modified, alloAg-pulsed DC can induce Ag-specific T cell hyporesponsiveness via the indirect pathway and prolong graft survival significantly. This strategy constitutes a novel approach to cell-based therapy of graft rejection applicable in the clinic.

Abstract# 1612

THE ROLE OF CD30 IN ALLOIMMUNE RESPONSES. Jay Campisi,[1,2] Monika Zwierzchoniewska,[2,3] Eugenia V. Fedoseyeva,[2,3] Sheri M. Krams,[1,2] Robert C. Robbins,[3] Carlos O. Esquivel,[2] Olivia M. Martinez.[1,2] *[1]Department of Surgery; [2]Program in Immunology; [3]Department of Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, CA.*

It is now well established that the co-stimulatory molecules CD28 and CD40L are necessary for complete T cell activation and play a crucial role in mediating alloimmune responses. However, the role of other co-stimulatory molecules in these responses has yet to be fully elucidated. The TNFR member CD30 is a co-stimulatory molecule that is induced on alloactivated T cells, marks the major proliferating T cell population in response to allogeneic APC and CD30+ T cells are the primary source of IFN-γ and IL-5. Although this evidence suggests that CD30 co-stimulation might modulate alloimmune responses, the function of the CD30 molecule on alloactivated T cells remains unclear. Therefore, the current study was designed to test the hypothesis that CD30 acts as a co-stimulatory molecule in allogeneic responses and directly modulates T cell proliferation and cytokine production. Negatively selected T cells from C57Bl/6 wild type (H-2^b) or CD30$^{-/-}$ mice (H-2^b) were co-cultured in vitro with mitomycin C treated Balb/C (H-2^d) splenocytes as allogeneic stimulators. Cells were harvested after 1, 2, 3, 4, and 5 days of culture and the extent of proliferation and cytokine production was assessed via [^{3}H]thymidine incorporation and ELISA, respectively. In parallel, the frequency of cytokine producing T cells was assessed by ELISPOT. Separate groups of animals were used to examine the frequency of cytokine producing T cells in response to allostimulation in vivo. Cell proliferation was reduced by 51% in CD30$^{-/-}$ compared with wild type T cells at the peak of proliferation (day 3) following allogeneic stimulation. IFN-γ production was abrogated in CD30$^{-/-}$ T cells compared with wild type T cells on day 5 (399 pg/ml vs. 1583 pg/ml; 74%) following in vitro stimulation, while no differences were observed in IL-2 production. Moreover, CD30$^{-/-}$ T cells displayed a lower frequency of IFN-γ production (406 spots/106 cells vs. 764 spots/106 cells; 46%) then wild type controls. A similar trend of reduced frequency of IFN-γ producing cells in CD30$^{-/-}$ mice was observed after in vivo allostimulation. No differences were observed in the frequency of cytokine produced per T cell, for IL-2, IL-4, or IL-5 following in vitro or in vivo allostimulation. Taken together these results indicate that CD30 contributes to alloimmune responses by modulating T cell activation and IFN-γ responses to alloantigen.

Predictors of Kidney Graft Function and Survival

Abstract# 1613

IMPACT ON CLINICAL STUDIES OF THE CHOICE OF EQUATIONS TO ESTIMATE RENAL FUNCTION. Kevin C. Mange,[1,2] Alden Doyle,[1] Simin Goral,[1] Marty Sellers,[3] Roy D. Bloom.[1] *[1]Medicine, University of Pennsylvania Medical Center, Philadelphia, PA; [2]Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; [3]Surgery, University of Pennsylvania Medical Center, Philadelphia, PA.*

Background: Various equations have been utilized to estimate renal function (fcn) among renal allograft recipients. Implications of equation choice to clinical studies are unknown. Three equations (MDRD(4-variable), Cockgroft-Gault (CG) and the Nankivell A (NankA) formulae are frequently used, and were compared in this study.

Methods: From 1990-2000, there were 387 renal allograft recipients from a living donor transplanted at UPMC. Among these persons, 287 had data that allowed for the calculation of the 3 formulae understudy. Cross-sectional comparisons were made at 3 months and longitudinal changes were made over the first year. Potential relevance of the variances between equations was explored by using: 1) threshold of 70 ml/min for study entry; 2) proportion of subjects in strata (>90, <=90& >70, <=70 & >50, <=50 & >=30, <30 ml/min) that allow for stratified randomization, or for sampling strategies; 3) change of renal function as an outcome.

Results: See Table. Means of the renal fcn at 3 months are different (P<0.0001, F test) with higher estimates by MDRD and CG compared to Nankivell (P<0.001, P<0.001), and by MDRD compared to CG (P<0.0001); proportions of subjects having >=70ml/min are different (P<0.001, chi square); proportions of subjects within strata are different with Kappa statistics indicating only fair agreement across formulae considering chance; and change of renal fcn over one year is significantly different (P=0.04, F test).

Conclusions: The choice of equation and the clinically relevant differences between them can have a significant impact in clinical trials or observational studies on: 1) eligibility rates; 2) efficiency of sampling or randomization strategies; 3) power; and 4) comparability of results between studies. 5) A measure(s) of cross-sectional and longitudinal renal fcn, across the distribution of GFR, which is confirmed to be a valid and reliable standard among recipients of renal allografts should be developed.

	Comparison of Formulae			
	NankA-MDRD	**NankA-CG**	**MDRD-CG**	
Mean Difference (95%CI)	8.95	4.7	4.3	P<0.001 (all comparsions)
	(-7.3-25.2)	(-17.5-26.9)	(-21.6-30.1)	
rho value (correlation)	0.772	0.771	0.721	P<0.001 (all comparisons)
Strata Agreement				
Agreement by Chance	32.4%	32.0%	31.6%	
Overall Agreement	51.9%	57.1%	51.3%	
Kappa Statistic	0.2883	0.37	0.2887	
	NankA	**MDRD**	**CG**	
% >70 mL/min	30.3%	16.4%	25.4%	P <0.001
Mean(SD)Change Renal Function	-0.68 (11.4)	0.34 (11.7)	-3.1 (12.7)	P =0.04

Abstract# 1614

FAVORABLE OUTCOME IN KIDNEY TRANSPLANT RECIPIENTS ENROLLED IN CLINICAL TRIALS. Sali Aswad,[1] Ellen Liao,[1] Fred Aswad,[1] Shirley Mirador,[1] Rafael G. Mendez,[1] Hamid Shidban,[1] Mazen Sabawi,[1] Robert Mendez.[1] *[1]Transplantation, National Institute of Transplantation, Los Angeles, CA.*

Background:

Outcome following renal transplantation is influenced by several interdependent factors. This study was designed to test the hypothesis that graft outcome can be enhanced by close follow-up thus increased compliance of patients.

Aim: To assess graft survival of patients enrolled in post-transplant studies (high compliance group) versus control group not enrolled in any post-transplant studies (low compliance group). Patients enrolled in studies had more aggressive follow-up then non-study patients.

Patients and Methods:

From Jan 1996 to Dec. 2002, we performed 1718 renal transplants. Patients were enrolled in 20+ post-transplant Phase II and III clinical trial studies (high-compliance group) at 23% (n = 389) of our transplanted population and were compared to control group of 77% (n=1328) patients not enrolled in any post-transplant studies. Anova, T-Test and Kaplan Meier were used for analyses.

	High Compliance Group	Control Group	P
N	389(23%)	1328(77%)	
Mean Recipient Age@TX (yrs)	48.8±13.3	47.7±12.4	0.16
Donor Type(Living/Cadaver)	280(72%)/109(28%)	910(69%)/418(31%)	0.19
Mean CIT (hrs)	26.3±10.5	24.7±10.6	0.03
Total Mismatch	3.4±1.6	3.2±1.7	0.34
T-Warm PRA(%)	3.4±15.8	4.4±17.0	0.41
Mean Follow-Up Time (mos)	34.1±22.7	33.6±23.0	0.69

Results:

Demographic profile of high compliance group not significantly differerent from control group in all categories except mean CIT. In contrast, graft survival of the high compliance group (89% /1 yr, 73% /5 yrs) was significantly better than control group (88% /1 yr, 63% /5 yrs) (p=0.04). One and 5 yrs. patient survival in study group was 90%, 80% respectively vs. 95%, 78% in control (p=0.68).

Conclusion:

Since donor and recipient profile of each group was similar, difference in graft outcome can be attributed to a higher level of compliance by patients enrolled in post-transplant studies versus those that were not. As patient follow-up includes many factors in addition to immunosuppressive therapy, results suggest improved graft outcome may not be based exclusively on immunosuppressive therapy. Further studies should be performed to determine what aspects of compliance have a direct effect on graft survival. Furthermore, our study shows that close follow-up of patients following transplantation is a determining factor that can help prolong the survival of renal grafts by up to 10%.

Abstract# 1615

NON-LINEAR ASSOCIATION BETWEEN ESRD DURATION AND GRAFT AND RECIPIENT SURVIVAL. Donald Morris,[1] Alfred K. Cheung,[1,2] Lev Barenbaum,[3] John Hurdle,[2,4] Bradley Baird,[1] Alex S. Goldfarb-Rumyantzev.[1] *[1]Medicine/Nephrology, University of Utah, Salt Lake City, UT; [2]VA Salt Lake City Health Care System, Salt Lake City, UT; [3]RenalService.com, Inc., Salt Lake City, UT; [4]Medical Informatics, University of Utah, Salt Lake City, UT.*

The effects of preemptive transplant (Tx) and the duration of end-stage renal disease (ESRD) on the allograft outcome have been described before. However, the role of relatively short duration of pre-Tx dialysis (3 months or less) as compared to preemptive Tx is unclear; it is also unclear, if the effect of ESRD duration on the outcome is linear. The goal of the present project was to perform a retrospective study of US Renal Data System (USRDS) data to re-evaluate the role of pre-Tx ESRD course duration and in particular interrogate the relationship between the duration of ESRD and the outcome of the Tx. Using Cox regression, we studied USRDS records from patients receiving kidney or kidney-pancreas Tx between 1/1/1990 through 12/31/1999, with the follow-up period through 12/31/2000. Patients with prior history of kidney Tx were excluded, 81,130 records were used for analysis (age 44.1±14.3, pre-transplant ESRD duration 27.1±26.4 months, 29% diabetic, 24% black, 60.5% males, 25.7% living donors). Outcome variables were the allograft survival (time between the transplant and graft failure) and recipient survival (modeled separately as time between the transplant and recipient death and as time between the ESRD onset and recipient death). Duration of ESRD as a continuous variable as well as divided into categories (≤14 days, 15-60 days, 61-180 days, 181-365 days, 1-2 years, 2-3 years, 3-5 years, >5 years) was the primary risk factor of interest. Cox model was adjusted for donor and recipient characteristics (demographic data, comorbidities) and Tx procedure characteristics. When ESRD duration was analyzed as a continuous variable in relation to allograft outcome, the hazard ratio (HR) for graft failure was 1.02 per 1 year increase in ESRD duration ($p<0.001$). When it was studied as a categorical variable with 14 or less days on dialysis as a reference, the risk of allograft failure reached statistical significance only when the time on dialysis was 181 days or greater. When recipient survival was analyzed, the duration of ESRD was a significant risk for patient death (HR 1.04 $p<0.001$), however, the risk reached statistical significance only when patient was on dialysis for >1 year. While preemptive Tx has been shown to have superior outcome, the results of this analysis suggest that short (3 months or less) dialysis course may have no detrimental effect on the graft and patient survival.

Abstract# 1616

SURROGATE MEASURES OF LONGTERM KIDNEY ALLOGRAFT SURVIVAL. Marcello Tonelli,[3] Brian G. Pereira,[2] John S. Gill.[1] *[1]Division of Nephrology, University of British Columbia, Vancouver, BC, Canada; [2]Division of Nephrology, Tufts University, Boston, MA; [3]Division of Nephrology, University of Alberta, Edmonton, AB, Canada.*

Background: Surrogates of long-term allograft survival are needed to assess the efficacy of new therapeutic strategies in kidney transplant recipients. The purpose of this study was to evaluate the performance of indices of graft function (serum Cr (SCr) and MDRD estimated GFR at 6,12,24 months after transplant and the annualized change in GFR (GFR slope, in ml/min/1.73m²/yr) over the first 2 post transplant years) to predict graft failure.

Methods: We studied all adult (18-70 year old) first, kidney only transplant recipients between 1987-1996 with at least two years of allograft survival in the United States Renal Data System (USRDS). The primary outcome measures were graft loss from all causes (GL) and death censored graft loss (DCGL) within 7 years of transplantation. Receiver operating characteristic (ROC) curves were constructed to demonstrate the relation between various levels of renal function and outcomes. Individual ROC curves were plotted for each index of renal function, using diagnostic thresholds that were selected *a priori*. For each threshold, sensitivity, specificity, positive predictive value and negative predictive value were calculated and used to construct an ROC curve for each index of renal function. To evaluate the relative predictive power of each index of renal function for graft outcomes, areas under the ROC curve (AUC) were compared.

Results: AUC for ROC describing GL for SCr and GFR at 6,12,24 months for GL were 0.57,0.60,0.64 and 0.59,0.60,0.65 respectively. AUC for GFR slope was 0.59. AUC for all indices were higher for DCGL (data not shown). The table shows the sensitivity (SN) and specificity (SP) of various thresholds for GL.

Conclusions: Indices of graft function predict DCGL better than GL. No single index of graft function is both sensitive and specific for graft failure. The commonly reported cutoff of SCr ≥1.5 mg/dl is not predictive of allograft failure. Cutoffs for SCr of ≥1.0 and ≥3.0 are highly sensitive and specific, respectively, for allograft failure within 7 years of transplantation.

	6 mo (SN)	6 mo (SP)	24 mo (SN)	24 mo (SP)
SCr ≥ 1.0	93.4%	8.4%	95.2%	8.0%
SCr ≥ 1.5	59.3%	51.7%	65.2%	54.0%
SCr ≥ 3.0	5.2%	98.1%	11.3%	98.7%
GFR ≤ 75	94.7%	6.0%	95.9%	5.6%
GFR ≤ 30	14.8%	93.4%	27.7%	93.6%
GFR slope < 0	(any GFR loss)	71.3%	39.7%	
GFR slope ≤ -4	(GFR loss > 4 ml/min/yr)	51.6%	62.4%	

Abstract# 1617

DONOR AGE AND IMPROVEMENT IN GRAFT SURVIVAL OVER TIME. John S. Gill,[1,2] Nathan Johnson,[1] Brian J. G. Pereira,[2] Sundaran Hariharan.[3] *[1]Nephrology, University of British Columbia, Vancouver, BC, Canada; [2]Nephrology, Tufts-New England Medical Center, Boston, MA; [3]Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Continued improvement in graft survival may be limited by the increased use of older donors in recent years. To determine the impact of older donors on the improvement in graft survival over time, we evaluated all adult cadaveric kidney recipients in the United States Renal Data System from 1996-2000 with information regarding donor age. **Methods:** Among the 36,417 recipients studied separate multivariate regression analyses were performed for recipients from donors < 55 and ≥ 55 years. Variables included donor: race and gender; recipient: age, gender, race, transplant number, cause of ESRD, HLA match, PRA, CIT, DGF, use of induction immunosuppression and type of calcineurin inhibitor (tacrolimus or cyclosporine). Adjusted survival estimates and projected graft half lives for each transplant year were derived from these multivariate analyses. **Results:** The table shows that 15-16% of donors in each year were ≥ 55 years. From 1996 –2000, the 1-year kidney graft survival remained steady at 91- 92% for donors < 55 yrs and improved from 83%-85% for donors ≥ 55 yrs. There was a significant improvement in graft half-life for donors <55 yrs from 13.3 to 18.4 years, a change of 38%, $p<0.0001$. For donors ≥ 55, half-life improved from 8.9 to 10.7 years, a non significant change of 21% $p = 0.28$. **Conclusion:** The overall 1-year and long-term graft survival rates were lower with the use of donors ≥ 55 yrs. long-term survival improvements were greater for donors < 55 years. Given the organ shortage and the need to consider the expanded use of older donor organs, future research should focus on modifying the course of renal disease in transplant recipients of older donor organs.

	1996	1997	1998	1999	2000
Donor <55 yrs	N = 5914	N = 5963	N = 6265	N = 6340	N = 6272
1 yr survival %	91	92	92	92	92
Half-life (95% CI)	13.3 (11.9-14.6)	14.8 (13.5-16.1)	16.5 (14.9-18.1)	18.4 (16.0-20.8)	n/a
Donor ≥ 55 yrs	N = 1066 (15%)	N=1127 (16%)	N=1209 (16%)	N=1175 (16%)	N=1127 (15%)
1 yr survival %	83	90	86	86	85
Half life (95% CI)	8.9 (7.5-10.2)	9.4 (8.3-10.6)	10.0 (8.7-11.5)	10.7 (8.6-12.8)	n/a

Half life estimated from multivariate model

Abstract# 1618

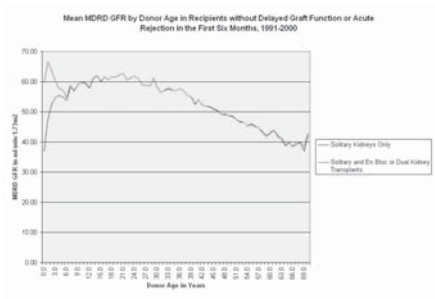
THE IMPACT OF DONOR AGE ON GFR AT SIX MONTH IN CADAVERIC KIDNEY RECIPIENTS. Douglas S. Keith,[1] Angelo deMattos,[1] Muralikrishna Golconda,[1] Jonathan Prather,[1] Douglas Norman.[1] *[1]Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, OR.*

Donor age and six month graft function are important factors in long-term cadaveric renal graft and patient survival. To determine the independent effect of donor age on post-transplant renal function at six months, patients with a history of delay graft function, acute rejection in the first six months post-transplant or graft survival less than six months were excluded. Using OPTN data as of 8-27-03, six-month cadaveric renal allograft function was determine in 20,637 adult cadaveric kidney transplants between 1991-2000 using the abbreviated MDRD formula. Among patients who received solitary kidneys, the peak GFR was 62.8 ml/min/1.73m² at a donor age of 21 years old (figure 1). After the age of 21 there was a highly linear decline in GFR with a slope of -0.512 ml/min/1.73m²/year in donor age and $R^2=0.9735$. When both solitary and en bloc or dual kidney transplants are included in the analysis the mean GFR by donor age showed a bimodal curve with a peak GFR of 66.5 ml/min/1.73m² at donor age of 1 and a second peak at donor age of 21 years of 62.8 ml/min/1.73m². For all pediatric donor ages in which more than 15 en bloc transplants were performed, the mean GFRs were substantially higher than solitary transplants, particularly donors under the age of 4 (table 1). In conclusion, donor age is an important factor in determining post-transplant graft function. En bloc kidney transplant of young pediatric donors substantially improves renal function when compared to solitary recipients. After donor age of 21 years six month renal allograft function declines by 0.51 ml/min/1.73m² per year.

Mean GFR in Pediatric Donors, Solitary vs. En Bloc Transplants

Donor Age in Years	Mean GFR En Bloc Transplants	Number of En Bloc Tranplants	Mean GFR Solitary Transplants	Number of Solitary Transplants
0	61.2	57	36.9	4
1	71.8	104	47.7	29
2	72.0	84	52.5	64
3	65.0	67	54.7	65
4	62.0	32	55.5	68
5	72.0	19	54.7	114

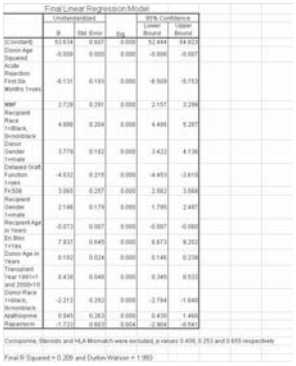
Mean MDRD GFR in ml/min/1.73m2



Abstract# 1619

IMPACT OF ACUTE REJECTION, DELAY GRAFT FUNCTION, MAINTENANCE IMMUNOSUPPRESSION, AND HLA MISMATCH ON SIX-MONTH RENAL FUNCTION IN CADAVERIC RENAL TRANSPLANTS, AN ANALYSIS OF OPTN DATA. Douglas S. Keith,[1] Angelo deMattos,[1] Muralikrishna Golconda,[1] Jonathan Prather,[1] Douglas Norman.[1] *[1]Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, OR.*

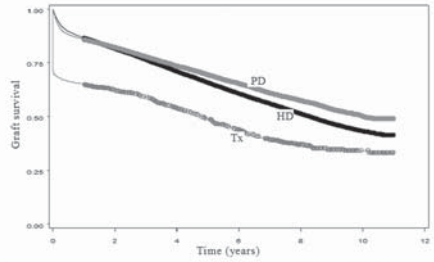
Graft function at six months post-transplant is a powerful predictor of long-term graft and patient survival. Using a multivariant linear regression modeling, the impact of acute rejection, delayed graft function, maintenance immunosuppression, en bloc vs. solitary transplantation and HLA mismatch on the abbreviated MDRD GFR was determined in 40,155 patients who underwent cadaveric renal transplant between 1991 and 2000. Only patients with graft survival at least six months, and who had complete data regarding donor and recipient age, gender and race, acute rejection in the first six months, delayed graft function, HLA mismatch, solitary vs en bloc transplant and creatinine at six months were included. Figure 1 shows the final model. Acute rejection in the first six months, delayed graft function and rapamycin treatment were with associated with a 6.13, 4.03 and 1.72 ml/min/1.73m^2 reduction in GFR, respectively. En bloc transplantation, treatment with FK506, MMF and azathioprine were associated with 7.94, 3.07, 2.73 and 0.95 ml/min/1.73m^2 improvement in GFR, respectively. Over the decade, 3.94 ml/min/1.73m^2 improvement in GFR was unaccounted for by factors in the model. Cyclosporine treatment, steroid treatment and HLA mismatch did not significantly effect GFR. In conclusion, acute rejection and delayed graft function are associated with significant reductions in GFR at six months. Even after adjusting for clinical acute rejection in the model, FK 506, MMF and azathioprine were associated with significant improvements in GFR suggesting that other immunologic and nonimmunologic factors like reduced subclinical rejection and nephrotoxicity may be important benefits of these medications. Further study is required to determine if this translates into better long-term graft and patient survival.



Abstract# 1620

THE ROLE OF PRE-TRANSPLANT RENAL REPLACEMENT THERAPY (RRT) MODALITY IN KIDNEY ALLOGRAFT AND RECIPIENT SURVIVAL. Alex S. Goldfarb-Rumyantzev,[1] John F. Hurdle,[2,3] Lev Barenbaum,[4] Zhi Wang,[1] Bradley Baird,[1] John D. Scandling,[4] Alfred K. Cheung.[1,2] *[1]Medicine/Nephrology, University of Utah, Salt Lake City, UT; [2]VA Salt Lake City Health Care System, Salt Lake City, UT; [3]Medical Informatics, University of Utah, Salt Lake City, UT; [4]RenalService.com, Inc., Salt Lake City, UT; [5]Nephrology, Kidney and Pancreas Transplant Program, Stanford University, Stanford, CA.*

The effect of pre-transplant renal replacement therapy (RRT) modality on allograft and recipient survival outcome is not clear. We studied allograft and recipient survival using USRDS records from patients receiving kidney or kidney-pancreas transplant (Tx) from 1/1/90 through 12/31/99, with the follow-up period through 12/31/00 (n=92,844, 60% males, 70% white, 23% black). Pretransplant and predominant RRT modality during end-stage renal disease period, as well as specific combinations of the RRT modalities were evaluated. Cox models were adjusted for donor and recipient characteristics (demographic data, comorbidities) and Tx procedure characteristics. For graft survival (reference modality - hemodialysis (HD)) the Cox model demonstrated the disadvantage of a Tx immediately prior to the Tx of interest (HR 1.65, p<0.001), while peritoneal dialysis (PD) as a modality immediately prior to Tx predicts better graft outcome (HR 0.97 p<0.05) (Figure). When the predominant modality was analyzed, both PD (HR 0.97 p<0.05) and Tx (HR 0.86 p<0.001) had a protective effect for the graft survival compared to HD. Any combination or any single modality (except for Tx only, PD+HD and None) had protective effects on graft survival compared to HD alone. PD+HD, and "None" were associated with non-significantly higher risk than HD. When recipient survival was evaluated, both prior Tx (0.798 p<0.005) and PD (0.94 p<0.005) had a protective effect compared to HD. Better recipient survival was also associated with both PD (HR 0.96 p<0.05) and Tx (HR 0.82 p<0.001) as predominant pre-Tx RRT modalities. Results suggest the beneficial role of PD for allograft and recipient survival.



	p	HR	95% CI	
RRT modality prior to transplant - PD	0.0402	0.971	0.944	0.999
RRT modality prior to transplant - TX	<.0001	1.651	1.514	1.799

Abstract# 1621

CYTOMEGALOVIRUS AND HLA-DR MISMATCHES IN US RENAL TRANSPLANTATION. Mark A. Schnitzler,[1] Karen L. Hardinger,[2] Daniel C. Brennan.[3] *[1]Health Administration, Washington University, St. Louis, MO; [2]St. Louis College of Pharmacy, St. Louis, MO.*

In our center we have observed a powerful combined impact on graft failure of CMV-disease and HLA-DR mismatching in renal transplant recipients who survived beyond the end of prophylaxis at six-months post-transplant. Five-year graft survival was 16% in patients with CMV-disease and 2 HLA-DR mismatches and 78% otherwise. No pattern of benefit from HLA-DR matching was observed unless a patient had CMV-disease. We sought to determine if this is occurring nationwide.

METHODS: Patient records for all adult renal transplant recipients surviving six-months post-transplant were drawn from the Scientific Renal Transplant Registry database between 1997 and 2002. CMV-disease was not recorded in the database so the association of graft survival with HLA-DR mismatches was compared in patients believed to be at high risk of CMV-disease (CMV D+/R-) with patients believed to be at low risk (D-/R-). Donor CMV-serology was not available for the large majority of living donors; thus recipients of living donor kidneys were excluded.

RESULTS: Outcomes of 8,157 D+/R- and 5,932 D-/R- cadaveric renal transplant recipients were examined. No association with death with a functioning graft was observed between the interaction of CMV-serology and HLA-DR mismatches. However, death censored, so called "immunologic", graft failure was significantly associated with the interaction of CMV and HLA-DR mismatches. The relative hazard of immunologic graft failure for D+/R- compared to D-/R- patients was increased by 55% (P = 0.007) with 2 HLA-DR mismatches, 30% (P = 0.038) with 1 HLA-DR mismatch, and 3% (P = 0.77) with 0 HLA-DR mismatches. Multivariate analysis confirmed the size and significance of this interaction. Assuming a 20% CMV-disease incidence in D+/R- and 5% in D-/R- patients, compard to patients without CMV-disease our observed results would be consistent with a 550% increased hazard of graft failure with CMV-disease and 2 HLA-DR mismatches and a 350% increased hazard of graft failure with CMV-disease and 1 HLA-DR mismatch.

CONCLUSION: National data is consistent with our center's observation of an interaction between CMV-disease and HLA-DR mismatches that is associated with graft failure. While the mechanisms of this interaction require further study, we find it of extreme importance that CMV-disease be avoided in patients with HLA-DR mismatches. Treatment regimens to extend graft survival are needed for the unfortunate HLA-DR mismatched patients experiencing CMV-disease.

Notes