British Transplantation Society

7th Annual Congress

The ICC, Birmingham
28-30 April 2004

Plenary Session II

Medawar Medal

Wednesday 28 April

11:30 – 13:30

O1

The Biphasic Haemodynamic Response To Brain Death Is Responsible For Acute Lung Injury (ALI) And Systemic Inflammatory Response Syndrome (SIRS) In The Transplant Donor

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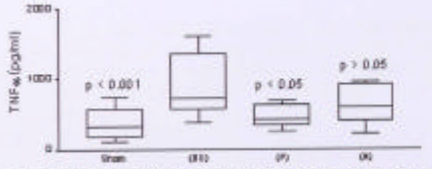
Objective: Brain death is accompanied by a sympathetic storm and hypertensive crisis, followed by neurogenic hypotension. Brain death is also followed by upregulation of proinflammatory mediators in all organs, and inflammatory ALI, which can adversely affect graft function post-transplantation. Until now, the mechanisms of the SIRS and ALI were unknown. We hypothesised that the haemodynamic changes associated with brain death are responsible for ALI and SIRS.

Methods: Brain death was induced by intracranial balloon inflation in 37 rats: in 13 (*BD*) an extreme hypertensive crisis and subsequent hypotension were observed; in 12 (*P*) the hypertensive response was prevented by pretreatment with phentolamine (α-blocker); in 12 (*N*) the hypotensive phase was corrected with noradrenaline. In 12 rats (*Sham*), a balloon was inserted intracranially, but not inflated. Arterial blood gases and neutrophil CD11b/CD18 expression were measured for 4 h. The lungs were excised, and bronchoalveolar lavage (BAL) of one lung was performed. The other lung was examined with transmission electron microscopy (TEM).

Results: The *BD* group had higher neutrophil CD11b/CD18 expression and BAL cytokine levels (TNF-α, CINC-1), and worse metabolic acidocis (MA) and oxygenation compared to *Sham*. Group *P* had lower BAL cytokine levels, lower CD18, and improved oxygenation compared to BD, but similar CD11b and MA. In group *N*, CD11b/CD18 expression was lower and MA and oxygenation improved compared to BD, but BAL cytokine levels were similar. TEM revealed areas of capillary-alveolar membrane rupture in *BD* and *N*, but not in *Sham* and *P*.

Conclusions: The hypertensive response to brain death causes haemodynamic capillary-alveolar membrane injury, with cytokine production, ALI and systemic neutrophil activation. Subsequent hypotension causes tissue ischaemia, MA and systemic neutrophil activation, further contributing to ALI.



TNF-α in bronchoalveolar lavage at 4 h (groups compared with BD).

O2

Tolerance To Composite Tissue Allografts In Miniature Swine Across A MHC Barrier

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Background: Composite tissue allotransplantation (CTA), such as hand or face transplantation, holds great potential for reconstructive surgery. However, the subject is highly controversial. Current hand transplants require life-long immunosuppression which places the recipients at risk of serious complications. In addition, chronic rejection is likely to negate any early favourable results. Tolerance, a situation where a recipient does not mount an immune response to donor tissue but remains responsive to all other stimuli, would obviate the need for long-term immunosuppression and prevent the occurrence of chronic rejection. Tolerance may be fundamental to the wide-spread clinical use of reconstructive allotransplantation. We investigated whether tolerance could be generated in swine to composite tissue allografts across a major histocompatibility (MHC) barrier. A clinically relevant tolerance protocol involving hematopoietic cell transplantation without the need for irradiation or myelosuppressive drugs was tested.

Methods: Seven recipient animals were transiently T cell depleted and a short course of cyclosporine was initiated. 24 hours later, a donor hematopoietic cell transplant consisting of either cytokine-mobilized peripheral blood mononuclear cells (C-MPBMC) or bone marrow cells (BMC) and a heterotopic limb transplant were performed. Anti-donor responsiveness was assessed by in-vitro assays. Acceptance of the limb allografts was determined by gross and histological appearance. Chimerism, the presence of donor cells in the recipient, was assessed by flow cytometry.in the peripheral blood and lymphohematopoietic organs.

Results: All experimental animals accepted the musculoskeletal elements of the allografts. All but one of the animals displayed donor-specific unresponsiveness in-vitro. The animals that received C-MPBMCs showed chimerism but had clinical evidence of graft-versus-host disease (GvHD). None of the animals that received BMCs showed stable chimerism or developed GvHD.

Conclusion: This protocol can achieve tolerance to the musculoskeletal elements of composite tissue allografts across an MHC barrier in miniature swine. Stable chimerism does not appear to be necessary for tolerance and may not be desirable due to the risk of GvHD. These studies could form the basis for the first clinical tolerance protocol for reconstructive transplantation.

O3

Evaluation Of Daclizumab To Reduce Delayed Graft Function In Non-Heartbeating Renal Transplantation: A Prospective Randomised Trial

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Introduction

Renal transplantation from NHB donors is one way of combating the global organ shortage. Unfortunately the large warm ischaemic insult sustained by the kidney leads to a high rate of delayed graft function (DGF), approximately 95% compared with 45% in heart-beating donors. In an attempt to minimise this period we have investigated the use of a delayed Tacrolimus based immunosupressive regime, covering the initial post-transplant period with an Il-2r monoclonal antibody.

Study Design and Objectives

Two centres in the United Kingdom with experience of NHBD transplantation (Newcastle and Leicester) enrolled 51 patients over two years. Recipients of NHB grafts were randomised into two treatment groups:

1. Daclizumab and daily MMF/Prednisolone. (26)
2. Tacrolimus, MMF and Prednisolone daily. (25)

Patients in the Daclizumab arm were converted to the control arm either when their serum creatinine went below 350 µmol/l or they had biopsy proven evidence of acute rejection. The patients had detailed follow-up for a period of three months post transplantation. The primary end point was the incidence and duration of DGF.

Results

There was one patient death in the daclizumab arm, during the study period, after a non-functioning graft was removed. Two (8%) and three (11.5%) of grafts failed to function in groups 1 and 2 respectively. The incidence of primary function was higher than expected overall (28%), therfore the proportion of recipients with immediate function was not significantly different between the groups (35% for group 1 and 22% for group 2). Sub-group analysis has shown a significant advantage for the delayed introduction of Tacrolimus for machine-perfused grafts ($p=0.015$ χ^2) (Table 1). There was no significant difference in the incidence of acute rejection or graft function (GFR) at three months.

Conclusion

The delayed introduction of Tacrolimus in conjunction with Il-2r blockade offers a safe alternative to traditional immunosupressive strategies and reduces the incidence of delayed graft function in machine perfused non-heartbeating kidney transplantation.

Machine perfused grafts subgroup	Group 1 (Daclizumab) n=17	Group 2 (Tacrolimus) n=19
Patient Survival	94% (1/17)	100%
Primary Non-Function	12% (2/17)	16% (3/19)
Immediate Function	**53%** (8/15)	**13%** (2/16) **p=0.015**(χ^2)

O4

Pre-Transplant Meld Score And Post Liver Transplantation Survival In The UK & Ireland

On behalf of the UK & Ireland Liver Transplant Audit

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Background: The Model for End-stage Liver Disease score (based on serum bilirubin, creatinine and INR) is a highly accurate predictor of survival for patients with liver disease without transplantation. A number of recent studies carried out in the United States have demonstrated that the MELD score is also associated with post-transplant survival. We evaluated how accurately the MELD score predicts post-transplant survival in adult patients with chronic liver disease in the UK and Ireland. We also explored whether the accuracy of the prediction of post-transplant survival could be improved by re-estimating the coefficients of the regression model from which the MELD score is derived.

Methods: Of the 3528 adult patients who received a first elective liver transplant between March 1994 and September 2002 in the UK and Ireland, 3210 (91%) had complete data for the variables in the MELD score. Patient survival was estimated using Kaplan-Meier methods according to the UNOS categories of the MELD score (≤10, 11-18, 19-24, 25-35 and 36). The area under the receiver operating characteristic curve (c-statistic) was used to express the ability of the MELD score to discriminate between patients who did and did not survive at least 90 days after transplantation.

Results: The overall patient survival at 90-days was 90.3% (95% confidence interval 89.3% to 91.2%). The 90-day survival varied according to the UNOS MELD categories (93.3%, 91.9%, 89.9%, 89.9% and 70.5%, respectively; $p<0.01$). This indicates that only those patients with a MELD score of 36 or higher (3% of the patients) had a survival that was markedly lower than the rest. Re-estimating the coefficients in the MELD regression model did not improve the discriminatory ability (c-statistic always <0.60).

Conclusion: The ability of the MELD score to predict post-transplant survival is poor. This result is in agreement with studies carried out in the United States. This suggests that the most appropriate way to select patients for transplantation may be to combine an appropriate pre-transplant survival model (MELD score) with a properly developed post-transplant survival model.

O5

The Beneficial Effect Of Remote Ischemic Pre-Conditioning Of The Hind Limb On Ischemia Reperfusion Injury Of The Liver Is Mediated By Nitric Oxide

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Background: Direct ischemic pre-conditioning (IPC) of the liver reduces ischemia reperfusion injury (IRI). Remote ischemic pre-conditioning (RIPC) of a distant organ may have a similar effect. The aim of this study was to determine the mechanism of RIPC in reducing liver IRI. Methods: 28 male New Zealand rabbits (3.2 kg ± 0.3 kg) were allocated into four groups; sham, IRI, RIPC + IRI, and RIPC. RIPC was induced in the right hind limb by three alternate cycles of 10 min ischemia followed by 10 min reperfusion. Liver IRI was produced by total inflow occlusion for 25 minutes. Markers of liver injury, systemic and hepatic haemodyanmics were measured up to 2 hours after reperfusion. Results: IRI after 2 hr of reperfusion was associated with increase in serum ALT and LDH levels and reduced PVF. RIPC before IRI reduced IR induced increase in serum ALT (27 ± 6 U/L vs. 43 ± 11 U/L for IRI; $p<0.05$) and levels and increased PVF ($p=0.001$) vs. IRI. At 2 hr of reperfusion hepatic venous nitrate and nitrite levels were greater ($p<0.05$) in the RIPC+IRI (22.0 ± 2.2 µmol/L) vs. IRI (28.3 ± 2.0 µmol/L). Conclusion: The beneficial effect of RIPC on liver IRI is mediated through an increase in hepatic availability of Nitric Oxide.

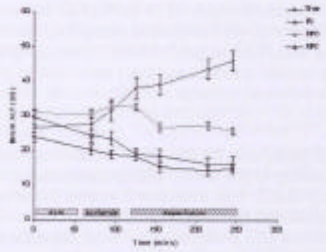

Figure 1. Serum ALT

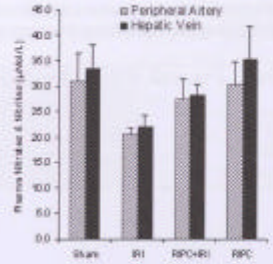

Figure 2. Plasma Nitrates and Nitrites (NO_x) after 2 hr of reperfusion.

O6

Transplantation Of Metanephroi To The Abdominal Cavity Of Adult Rats - A Potential New Form Of Transplantation For The Treatment Of End-Stage Renal Failure

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End-stage renal failure is a growing, worldwide problem. Current forms of renal replacement therapy (RRT), dialysis and whole organ transplantation, each have well known drawbacks.

Alternative forms of RRT are thus badly needed. The discovery that the foetal kidney rudiment, the metanephros, grows and develops if transplanted, arose from a series of studies on embryonic kidney development. This opens-up a further source of organs for transplantation.

We have transplanted 251 metanephroi, from embryos of gestational age between E14 and E15.5, to the abdominal cavity of 81 inbred (Lewis) rats. On re-exploration of transplanted animals after 17-21 days, we observed a mean successful growth rate of 68.9% . Transplants grew to a mean length and weight of 5.76mm (+/- 1.7mm) and 46.2mg (+/-31mg) respectively, a volume increase of > 1000 fold.

In this model, we demonstrate an essentially normal pattern of internal vascularisation and histological structure. After transplantation, 71.1% of transplants which grew were associated with visible dilated ureters (cysts) consistent with the production of urine. If not removed or connected to the urinary tract, transplanted metanephroi show progressive macroscopic and histological features of hydronephrosis consistent with accumulation of urine within an occluded ureter.

In selected animals, connection of the metanephric ureter to the host ureter by microsurgical, end-to-end anastamosis, was possible. These animals underwent a third, terminal clearance procedure at which urine of metanephric origin was produced and GFR estimated by inulin clearance. Values up to 62.6 (+/- 6.8) µls/min/100g b.w. were achieved. Tubular concentration of fluorescently-labelled inulin is further demonstrated.

This technology represents a potential alternative form of renal replacement therapy since even a modest gain of renal function may have therapeutic significance if multiple structures can be transplanted or the achieved GFR boosted. There may also be advantages to this approach in terms of the severity of the surgery, as well as that resulting from a vascular endothelium of at least partial recipient origin.

The combination of tissue engineering, transplantation and microsurgical connection, offers a variety of potential further approaches to addressing this expanding area of clinical need.

O7

Characterisation Of Human Hepatic Stem Cells Isolated From Foetal Liver By Fluorescence Activated Cell Sorting

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BACKGROUND

In humans, the identity of progenitor cells responsible for hepatic regeneration in response to injury remains to be elucidated. In the rat,oval cells have been demonstrated to be 'bi-potential' progenitor cells capable of developing into either hepatocytes or biliary epithelium. These oval cells express the stem cell marker CD90 (Thy-1). The liver is the principal organ of haematopoiesis in the developing human foetus. CD34 is a well-known marker of haematopoietic stem cells (HSCs). Recent evidence implies a role for HSCs from bone marrow in the regeneration of diseased adult liver. We therefore investigated $CD90^+$ and $CD34^+$ cells in human foetal liver in an attempt to characterise hepatic progenitor cells.

METHODS

Mid-trimester human foetal liver was subject to collagenase digestion and the resultant cells maintained in culture. Cells were sorted into $CD90^+$ and $CD90^+CD34^+$ populations using fluorescence activated cell sorting (FACS). Immunohistochemistry was performed using monoclonal antibodies against CD90, CD34, cytokeratin 18 (CK18, hepatocyte marker), cytokeratin 19 (CK19, biliary cell marker) and proliferating cell nuclear antigen (PCNA). mRNA expression was determined using reverse transcriptase polymerase chain reaction (PCR).

RESULTS

In the freshly isolated foetal liver, 0.54% of the cells were $CD90^+$, and 2.54% were $CD90^+CD34^+$. After culture with stem cell factor, Flt3-ligand and thrombopoietin, the sorted population of $CD90^+$ cells expanded 10-fold and immunohistochemistry showed co-expression of CD90 with CK18 and CK19. PCR confirmed mRNA expression for CD90, CK18 and CK19. Cell division in culture was verified by positive staining for PCNA. mRNA was extracted from the $CD90^+CD34^+$ population of cells one day after sorting and showed expression for the hepatocyte markers CK18, alpha-fetoprotein, transferrin and hepatocyte nuclear factor.

CONCLUSIONS

We have selected for two populations of cells expressing stem cell markers from human foetal liver and demonstrated the co-expression of liver-specific markers. In addition, the $CD90^+$ cells proliferated in culture with cytokines known to stimulate stem cells. Therefore, cells expressing the surface antigens CD34 and CD90 may be capable of regenerating diseased liver. Sources such as adult bone marrow may provide a potential therapeutic supply of these hepatic progenitor cells.

O8

Neo-Genesis Of Islet Cells From Adult Rat Stem Cells

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Aim

To isolate and characterise adult stem cells derived from pancreas with a view to producing de novo islets.

Introduction

World wide, 150 million people suffer from diabetes. Despite insulin therapy late complications such as retinopathy, nephropathy, neuropathy etc are not uncommon. This is due to lack of pulsatile insulin secretion from the pancreas, which offers minute to minute control of hyperglycaemia. We have sought to mitigate this condition through the isolation of adult stem cells and the de novo production of islets from such cells, using the rat as a model system.

Methods:

Adult rat pancreatic ducts were dissected, minced and cultured in CMRL medium for six weeks to generate a cell monolayer. Cells derived from this initial monolayer were then further differentiated through growth in DMEM medium and in matrigel, prior to analysis.

Results

We successfully derived a population of adult rat islet progenitor cells and have characterised these using a series of molecular and immuno-cytochemical markers. The progenitor cells are positive for the expression of stem cell markers Nestin and Oct-4.and negative for PDX-1 as determined by RT-PCR. Immunocytochemistry has also confirmed they are positive for the expression of nestin and NCAM. These cells, in keeping with a stem cell status, have been serially passaged in culture for 10 months without senescing. In accordance with previous published reports the cells are TH positive.

Significantly, two distinct populations of cells can be derived from this precursor population: islet like cell clusters and neurones. The islet like cell clusters appears to be positive for insulin and glucagon transcription, as judged by RT-PCR. They are also positive for insulin production on the basis of DTZ staining. Transmission electron microscopy has revealed the presence of dense granules, in keeping with an islet like status. The neuronal cells derived from the same precursor population are positive for neuronal markers nestin, cyc3 and NCAM. They are negative or PDX-1,MBOP, GFAP, AA3.

Conclusion:

We propose that derivation of islet cells from adult stem cells may be a viable alternative in this era where donor organs are in short supply. Transplantation studies have now commenced with these cells.

Parallel Session III(a)

Basic/Cell Science

Wednesday 28 April

15:30 – 16:30

O9

Identification Of Proteins Associated With Freedom From Cardiac Allograft Vasculopathy

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Cardiac allograft vasculopathy (CAV) is a major complication following cardiac transplantation. The aim of this study was to compare proteins from biopsies taken within the first two weeks post transplant and those taken nine years later or more from the same patient, and thus identify proteins that may act as markers of protection/promotion of CAV. Proteomics was used, allowing global analysis of alterations in protein expression. Six patients with CAV and 6 without CAV were investigated. Protein was extracted from each biopsy (n = 24) and 100 µg separated by two-dimensional gel electrophoresis. Protein spots were detected by silver staining and analyzed using Progenesis. Two sets of analyses were carried out: 1) Early vs. late biopsies in patients without CAV (comparison 1) and with CAV (comparison 2), using a paired Students t-test, and 2) early vs. early (comparison 3) and late vs. late biopsies (comparison 4), using an unpaired Students t-test. Here we report upon proteins that changed by 2-fold or more, $p < 0.05$. We found 69 proteins showing time dependent changes in comparison 1, and 38 changes in biopsies from comparison 2. Twenty-three proteins changed in comparison 3, and 21 in comparison 4. Aortic smooth muscle α-actin (SMαA), Troponin T, electron transfer flavoprotein (α-subunit, ETF), transferrin and phosphatidylethanolamine-binding protein (PEBP) were identified by MALDI-TOF. SMαA increased by 2.8-fold in late biopsies with disease compared with early biopsies from the same patient ($p < 0.01$). Troponin T significantly increased in late compared with early biopsies regardless of CAV status. ETF and PEBP were increased in late compared with early biopsies from CAV free patients. Transferrin was decreased in late compared with early biopsies from patients that developed CAV. A unique protein of molecular weight and pI of 21.4 kDa/6.48 was detected in all early biopsies, but was absent from late biopsies. More proteins are currently being identified. In conclusion, certain proteins, such as ETF, PEPB and transferrin are associated with survival whereas other such as SMαA (presumably reflecting thickening of blood vessels) are associated with CAV. The results will be validated by immunocytochemistry. This is a novel approach to chronic rejection and it promises novel therapeutic interventions.

O10

Expression of Genes Involved in Cell Interactions During Human Corneal Graft Rejection

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Purpose: The aim of this study was to identify genes that are differentially expressed in cell interactions in peripheral blood during corneal graft rejection .
Methods: cDNA array technology (Atlas Human Cell Interaction Array, BD Biosciences) was used to screen the gene expression changes in blood from corneal graft patients undergoing graft rejection compared to patients whose grafts were not rejected. Semi-quantitative RT-PCR was used to confirm differential expression of selected genes identified by the array results.
Results: Among 265 genes on the array, several showed a 2 fold or greater difference in expression. The most apparent change in the rejecting group was the up-regulation of the Rac2 gene, coding for a small guanosine triphosphatase which plays a role in T cell differentiation. There was also down regulation of the following genes; 1. LFA-1, a member of the integrin family, which binds ICAM-1 and is known to be involved in corneal graft rejection. 2. Paxillin, a focal adhesion protein that recruits adhesion and growth factor mediated signals from the extracellular matrix. 3. Rho A, a GTPase protein whose functions include roles in immune regulation, apoptosis, gene expression and cytoskeletal organisation.
RT-PCR further confirmed that there were significant differences between rejecting and non-rejecting groups, for all four genes. Serial samples were available for several patients who suffered an episode of rejection. The levels of Rac2 and LFA-1 mRNA were shown to return to normal after treatment and resolution of the rejection
Conclusions: Our data demonstrates that cDNA array technology can identify genes that may participate in the pathogenesis of corneal graft rejection. These genes may be useful prognostic markers of rejection or targets of new more specific treatments.

O11

Rapamycin Affects Dendritic Cells Function By Amplifying IL-12 Production

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Rapamycin (sirolimus) is a macrolide antibiotic, which potently inhibits T cell proliferation by inducing cell-cycle arrest in the mid-to-late G1 phase. It acts by inhibiting mTOR (mammalian target of rapamycin), a key kinase involved in regulating cell growth and differentiation. Although its effects on T cells have been well characterised, little is known about its effects on other cells involved in the initiation of anti-graft immune responses.
In this study we have focused on the effect of rapamycin on the maturation and function of antigen-presenting cells, specifically human monocyte-derived dendritic cells (DCs).
DCs were generated by the adherence method or by positive selection using anti-CD14 beads, then cultured in RPMI/10% FCS in the presence of IL-4 and GM-CSF. Rapamycin at a concentration of 10ng/ml was added at day 0 and replenished every two days. On day 5, DCs were rendered mature using lipopolysaccharide (LPS) 50ng/ml. The cells were washed to remove residual rapamycin prior to use in a mixed leucocyte reaction (MLR).
Somewhat counter-intuitively, rapamycin-treated DCs induced significantly greater T cell proliferation in an MLR when compared to untreated DCs. Furthermore, DCs treated with rapamycin exhibited diminished IL-10 production in response to stimulation by LPS or CD40 ligand. By contrast, production of IL-12 was significantly increased. This alteration in IL-12/IL-10 equilibrium appears to result in increased interferon-γ production and decreased IL-5 production by allogeneic naïve T cells. These data suggest that DCs in the presence of rapamycin can amplify the Th1 type of immune response. It is interesting that the net effect of rapamycin treatment is immunosuppressant despite these potentially deleterious effects on the afferent limb of the immune system which would tend to promote anti-graft immune responses.

O12

Costimulation On Murine Corneal Endothelium; An Implication For Corneal Allograft Rejection

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Objectives: To characterise the expression of costimulatory molecules on murine corneal endothelium and its response to pro-inflammatory cytokines as an in vitro model of allograft rejection.

Background: T lymphocytes play a central role in allograft rejection, including corneal transplants. On engagement of the T cell receptor by antigenic peptide - MHC complex, a second 'costimulatory' signal is critical to determine the fate of the T cell. This signal can be in the form of activation (positive signals; CD28-CD80/CD86, CD154-CD40 and ICOS-ICOSL) or inhibition (negative signals; CTLA4-CD80/CD86, PD-1-PD-L1/PD-L2) of T cell function. Antigen presenting cells including endothelium and in particular vascular, can provide this costimulation. This is not characterised on corneal endothelium and may be important, as it is a critical target in corneal allograft rejection.

Methods: Initial characterisation was carried out on two murine endothelial cell lines, cornea (MCEC) and vascular (sEnd.1) followed by primary corneal endothelium cultured in vitro. Expression on cytokine stimulated (TNFα, IL-1α, IFNγ) and unstimulated cells were determined by flow cytometry.

Results: As cornea is an immune privileged tissue we first studied the presence of ligands that act through negative costimulation. Neither PD-L1 nor PD-L2 was constitutively expressed on MCEC or primary corneal cells but PD-L1 was upregulated following cytokine stimulation. Similar expression was found on the sEnd.1. Of the positive costimulation ligands, CD40 expression was present on MCEC but not on primary corneal cells or sEnd.1 whereas ICOSL was absent on all cell types. CD80 and CD86 ligands can act as either positive or negative costimulation through CD28/CTLA4 respectively. There was low constitutive expression of CD80 on primary corneal cells that increased following cytokine stimulation, whereas CD86 was undetectable on any cell types.

Conclusions: Differential expression of these ligands on corneal endothelium may underlie some of the reasons why corneal grafts still undergo rejection. The presence of PD-L1 and CD80 may play a role in prolonging corneal allograft survival due to T cell inhibition. However the positive signals through CD80 (primary cells) and CD40 (MCEC) may trigger a rejection episode. These represent potential targets for immune modulation to prolong corneal graft survival.

O13

Cyclosporin Protects Human Hepatocytes By Inducing Heme Oxygenase 1

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BACKGROUND
Preconditioning of an organ to protect from injury is a developing concept which has the potential to improve outcomes in solid organ transplantation. Cyclosporin has been shown to be protective in animal models of ischaemia-reperfusion, but the mechanism is unclear. Preliminary work demonstrated that cyclosporin preferentially induced the cytoprotective enzyme Heme Oxygenase 1 (HO-1). We hypothesised that cyclosporin could protect isolated human hepatocytes via an HO-1 dependent mechanism.

METHODS
Isolated human hepatocytes obtained from patients undergoing liver resection and were cultured in standard conditions prior to treatment with a range of doses of cyclosporin or matched vehicle controls. RNA was extracted from treated cells for quantitative analysis by Real Time PCR. Protein was prepared from whole cell extracts for Western blotting. A model of oxidative injury using glucose oxidase to generate H_2O_2 was used to assess protection against oxidative injury. HO-1 dependence was confirmed using specific inhibitors.

RESULTS
Heme Oxygenase mRNA levels were significantly elevated following cyclosporin treatment ($p<0.05$). HO-1 protein levels were also raised. Cyclosporin pretreatment prior to oxidative stress resulted in significantly improved survival compared with either vehicle or no treatment ($p<0.05$). This protection was additive to that seen with heat stress. Addition of an HO-1 inhibitor (ZnPPIX) removed the protective effect of cyclosporin treatment. HO-1 activity generates carbon monoxide, which functions as an intracellular signalling messenger. In cells where HO-1 was inhibited, addition of dichloromethane to generate carbon monoxide restored the protective effect of cyclosporin treatment.

CONCLUSIONS
Cyclosporin administration induces heme oxygenase 1 in human hepatocytes and protects them from oxidative injury. This preconditioning effect is mediated by carbon monoxide, a product of Heme Oxygenase 1 activity. Cyclosporin has clinical potential as a preconditioning agent.

O14

Factors Affecting Rejection Of Second Corneal Transplants In Rats

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Background. Second and subsequent corneal transplants in the same eye are more prone to rejection reactions and failure than first grafts. This may be due to local ocular changes associated with transplantation that prejudice survival of a later graft or to systemic sensitisation to antigen shared by first and subsequent donors. Because HLA typing is not routine in corneal transplantation a clear correlation between accelerated rejection and specific sensitisation has not been established.

Methods. LEW (RT1l), AO (RT1u) or PVG (RT1c) strain corneas were transplanted to PVG strain rats, followed by a LEW strain cornea in the ipsilateral or contralateral eye six weeks later. Graft survival was evaluated by slit lamp biomicroscopy. Proliferation of lymph node cells of recipients was tested against LEW, AO and PVG stimulator cells 14-16 days after second transplantation.

Results. The second allograft, whether in the ipsilateral or contralateral eye, was rejected in an accelerated fashion (median of 8.5 days compared with 14 days for first grafts; $p<0.001$) that did not depend on MHC compatibility between first and second grafts. Lymphocyte proliferation to third party (AO strain) cells showed secondary kinetics in animals previously exposed only to LEW strain antigens. A second allograft was rejected more rapidly if performed in the ipsilateral eye compared to the contralateral eye, but this difference was not significant ($p=0.16$).

Conclusions. Systemic sensitisation to donor antigens, rather than local changes induced by first transplantation, contributed to accelerated rejection of a second graft. The lack of HLA specificity of sensitisation could be due to indirect presentation of "public" MHC determinants shared by the nominally mismatched donors or of minor antigens shared by first and second donors, or to cross reactivity of T cells to epitopes on AO and LEW grafts. HLA mismatching of first and subsequent donors may not prolong corneal graft survival.

Parallel Session III(c)

Live Donation

Wednesday 28 April

16:00 – 16:30

O15

Analysis Of Potential Living Kidney Donors Who Do Not Subsequently Donate

K Brown[1], G Campbell[1], J Traynor[1], I Galbraith[2], K Simpson[3], D Deardon[1] and C C Geddes[1]

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Improved access to kidney transplant may be achieved by increasing resources to facilitate living kidney donation. There is limited published information to quantify the investigation of potential living donors (PLD) and the reasons for PLD drop-out

The LDT process in our centres involves several steps before transplant: 1) inform PLD and screen for history of known contraindications; 2) establish blood group compatibility; 3) preliminary lymphocyte cross-match; 4) screening blood tests, electrocardiogram, chest radiograph, creatinine clearance, urine protein excretion, nephrologist review; 5) MRA renal arteries, glomerular filtration rate, review by independent physician; 6) transplant surgeon review and final lymphocyte cross match. The PLD proceeds to the next stage if the preceding stages are satisfactory.

It is difficult to quantify how many PLD may have dropped out in the first 2 stages as this may occur in a variety of settings. The aim of the present study was to examine blood group compatible potential LDT who had a preliminary lymphocyte crossmatch (stage 3) in the 2 years between April 2001 and March 2003 to determine the number of PLD reaching each stage, the number of actual transplants achieved and identify the reasons for PLD drop-out.

110 PLD to 80 potential recipients (PR) reached the stage of having a preliminary lymphocyte cross match (1-3 PLD per PR). 74 PLD reached stage 4; 49 reached stage 5; 32 reached stage 6; 23 PLD in this cohort have donated a kidney, 3 have firm arrangements for LDT in the near future and 4 will probably donate at a suitable time in the future.

The commonest reasons for PLD drop-out were: donor medical issues (n=25; 22.7%), donor or PR withdrew voluntarily (n=15; 13.6%), positive lymphocyte crossmatch (n=14; 12.7%) and other suitable PLD (n=13; 11.8%). 3 PR received a cadaveric transplant and 2 PR died during the PLD investigation.

49 (44.5%) of the PLD were male and 11 (42.3%) of the actual kidney donors were male. A greater proportion of male PLD dropped out because of voluntary donor or recipient withdrawal from the process (24.4% male v 4.9% female; p=0.003 chi square test).

These data show that when allocating resources to increase the number of LDT it must be acknowledged that only 27.3% of PLD that are investigated will subsequently donate. The reasons for PLD drop-out deserve further study.

O16

Perspectives, Problems and Pitfalls: Eight Years Experience of Reimbursement Costs Related to Living Kidney Donors

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The Transplant Framework focuses on increasing living donor transplantation as part of a strategy to boost renal transplantation in the UK. One area of contention has been reimbursement of donors for their loss of earnings (LOE)and other directly related expenses incurred, since these costs may be a disincentive to donation. The DoH guidelines encourage donor reimbursement and we relate our highly successful experience in dealing with the Health Authorities(HA) and Primary Care Trusts (PCT) of live donor recipients in gaining reimbursement over the past eight years.

From 1995 the Transplant Unit has been proactive in approaching HA/PCTs for reimbursement through the efforts of our dedicated transplant social worker. Of 113 donors, only 29 (25.6%) needed recompense for loss of earnings (19) and travel and/or accommodation costs (10). Eleven were female donors and 18 were male. Three donors were living in the USA. Loss of earnings ranged from £225 up to £6648 (average £1866) whilst travel/accommodation costs ranged from £42 to £6934 (average £948). Of those who had LOE repaid, 8 were self-employed and others were generally low paid. Time off work ranged from 5.5 weeks to 31 weeks (average 12 weeks). One patient had 31 weeks off work due to the need for repair of an incisional hernia and another 24 weeks following a pulmonary embolus.

Problems faced were the retrospective nature of reimbursement, leading to financial hardship since many were low paid; current lack of co-ordinated policy amongst PCTs; validation of LOE for some some self-employed , especially as earnings vary from year to year.

NICE has estimated that the costs of dialysis is £22000 per annum so the actual costs of reimbursement are small in comparison, leading to considerable cost saving alone, and notwithstanding the better outcome for transplant recipients. Ninety-six live donor transplants are still functioning and, therefore, off dialysis.

In conclusion, live donation and live donor nephrectomy is a stressful and painful process with significant risks for the donor. Financial support for donors must not be an obstacle if the government's targets and the patients' needs are to be met. Society as a whole gains from these acts of altruism. Happily,no HA or PCT in the area covered by our transplant unit has refused reimbursement thus far.

O17

Living Kidney Donors – The UK Register

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Background

In 2000 it was agreed that a national register be set up to record donation and follow-up data for living kidney donors. This pooling and sharing of information would help highlight any problems that may need to be addressed and aid those involved in the donation process.

Method and data

Data collection forms were designed and agreed and issued for all living kidney donors in the UK since November 2000. These forms gather details about the donor at the time of donation and assessment data each year thereafter. The information is collected as part of the National Transplant Database.

Three years into the project over 750 one-year follow-up forms have been issued but only about two thirds have been returned so far. Approximately half as many two-year follow-up forms have been issued.

Results

The age of donors ranges from 19 to 73 years, the most common decade being the forties. In 40% of cases the relationship of donor to the recipient is that of parent and the donor was genetically unrelated in 24%. Although approximately twice as many recipients are male as female, the gender of the donor is more evenly distributed with no evidence of sex-matching. Nearly 15% of living kidney donors are from ethnic minority groups whereas they constitute less than 3% of cadaveric donors. Peri- and post-operative complications were rare, wound infection being the most common in 3% of donors. Nearly all (95%) of donors were discharged from hospital within 9 days and 70% had returned to normal general activity within three months. Serum creatinine levels at one year post-donation (mean 113 µmol/l) were slightly but significantly higher than at the time of donation (mean 86 µmol/l) and still remained significantly raised at two years (mean 109 µmol/l). Haemoglobin was unchanged at one year (mean 13.6 g/dl) compared to pre-operatively (mean 13.9 g/dl). Approximately 10% of donors had elevated blood pressure one year post-donation. Urine dipstick testing showed 6% of donors positive for blood and 14% positive for protein at one year. Very few related medical conditions were reported one year post-donation, complications with the wound being the most common (6%).

Conclusions

The outcome for living kidney donors is generally satisfactory but there is a small risk of morbidity. The data in the registry will become even more valuable as more follow-up information is collected.

Parallel Session IV(a)

Immunogenetics

Wednesday 28 April

18:00 – 18:30

O18

Serial Analysis of HLA Specific Antibodies in Renal Allograft Recipients - Their Natural History

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INTRODUCTION: In renal transplantation, donor specific HLA antibody is associated with graft rejection. Whether they are cause or consequence of the rejection process is uncertain. Their presence can cause hyperacute rejection and although in some patients antibody production ceases following removal of the immune stimulus, in others it persists. Thus the majority of donors are unsuitable because of positive crossmatch. Excluding restimulation via transfusion, why persistence occurs remains speculative. Such patients are often highly sensitised and to manage them effectively a full understanding of the principles underlying persistence is essential. In an attempt to address this, we determined both IgG/IgM PRA levels and determined antibody specificity serially over an extended period.

METHODS: IgG/IgM PRA was determined against 2 pools of LCL's using a dual stain flow cytometry technique. Antibody specificity for HLA class I and class II was determined using ELISA. Serial analysis for class I specific HLA antibody was performed using single antigen flowbeads.

RESULTS: HLA IgG antibody is persistent in some patients for more than 10 years. Persistence is often maintained by transfusion. In some patients however, IgG persistence is maintained without transfusion. Like IgG antibody, IgM antibody can also be transient and in some cases IgM antibody persists. Serial analysis of HLA IgG antibody specificity shows that changes in PRA can be the result of changes in antibody specificity. However, changes in PRA do not necessarily equate to changes in HLA antibody specificity.

SUMMARY: HLA IgG antibodies can be transient in some patients and persistent in others. IgM antibodies are never observed persistently in the absence of IgG antibodies. IgG HLA specific antibodies are maintained long term due to pregnancy and transplantation. The typical response following graft failure is characterised by a spike of IgM antibody production and persistent IgG. A typical response to transfusion is usually a spike of IgM antibody and/or IgG which rapidly disappears. Changes in antibody specificity are not always concurrent with changes in PRA. Conversley, changes in PRA do not necessarily indicate new emerging antibody specificities.

O19

Sensitised Patients With Anti-HLA Class I Antibodies Of IgG Isotype Skewed Solely Towards The IgG_1 Subclass Have Poorer Graft Survival Following Renal Transplantation

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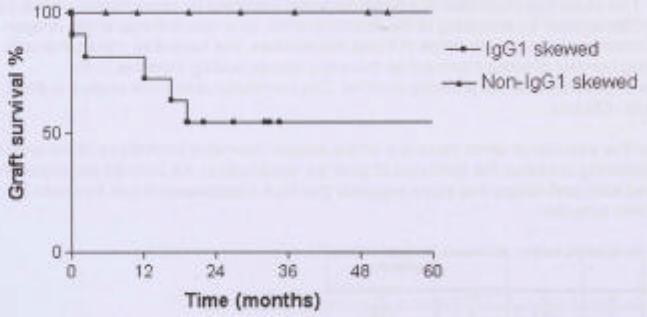
Sensitised patients with lymphocytotoxic IgG anti-HLA antibodies have an increased risk of rejection and poorer graft survival following renal transplantation. Little is known, however, about the correlation between IgG antibody subclass and clinical outcomes.

We identified 20 sensitised renal transplant recipients (PRA >15%) all of whom had anti-HLA class I antibodies of an IgG isotype with known specificity prior to transplantation but who had received a crossmatch negative graft. We analysed the degree of skewing either solely towards IgG_1 (N=11) or to other IgG subclasses with or without IgG_1 (N=9) and correlated these findings with graft survival (see figure).

At last follow-up (median follow-up 28 months), 6/11 patients (55%) with anti-HLA antibodies skewed towards IgG_1 had lost their graft compared with 0/9 (0%) in non-IgG_1 skewed patients ($p=0.01$ Log Rank test).

Anti-HLA antibodies of an IgG_1 subclass may be a novel marker predicting poor graft outcome. Such patients appear to be at increased immunological risk and may warrant a more aggressive immunosuppressive protocol following transplantation.



O20

HLA Matchmaker As A Predictor Of Post Renal Transplantation Sensitisation

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Introduction: Recently published data have demonstrated a significant association between the production of donor HLA specific antibodies and subsequent graft failure. However not all patients whose grafts failed produced antibodies and those who did produce antibodies did not produce them to all transplant (tpx) mismatches. HLA Matchmaker is a computer algorithm where donor-recipient HLA compatibility is assessed at the structural level. Intralocus and interlocus comparisons are made of polymorphic amino acid triplet sequences, the software then determines which triplets on mismatched HLA molecules are different or shared between donor and recipient. This study aimed to use the HLA Matchmaker algorithm to see if it was predictive of post-tpx sensitisation.

Materials and Methods: The study group comprised 35 adult recipients of primary renal tpx (transplanted between 1981 and 1998) whose grafts had failed. Sera taken pre-transplant, 1,3,6 months post-transplant and annually thereafter until graft failure were tested by enzyme-linked immunoabsorbent assay (ELISA) for the presence of HLA class I and class II specific antibodies. Antibody specificity was defined by a combination of cytotoxicity, ELISA and flow cytometry techniques. Antigen mismatches were analysed for each locus individually for immunogenic triplets according to the HLA Matchmaker software.

Results: All recipients were negative for donor specific antibodies pre-tpx; post-tpx 20 were positive and 15 were negative. The table below summarizes the correlation between triplet mismatches and antibody production.[table nn here]

Summary: This study has illustrated that donor-recipient matching by conventional criteria can be further differentiated by analyzing at the structural level, as a conventional single antigen mismatch corresponds to a wide range of triplet mismatches. We have also shown that with an increasing number of triplet mismatches there is a corresponding increase in the percentage of patients who are antibody positive. This correlation was most evident in the HLA-DR and –DQ loci.

Conclusion: For each locus when there is a single antigen mismatch knowledge of the level of triplet mismatching indicates the likelihood of post-tpx sensitisation. As post-tpx sensitisation is associated with graft failure this study suggests that HLA Matchmaker might therefore be used for donor selection.

HLA Locus	No. Ag MM	No. Trp MM	No. Patients	Recipients Antibody Positive (%)
HLA-A	0	0	12	0
	1	2 to 4	8	12.5
	1	5 to 12	14	35
	2	17	1	100
HLA B	0	0	8	0
	1	2 to 4	8	11
	1	5 to 12	14	35
	2	10 to 15	5	60
HLA-DR	0	0	18	0
	1	2 to 4	6	33
	1	5 to 9	10	60
	2	12 to 15	2	100
HLA-DQ	0	0	24	8
	1	3 to 19	10	60
	2	20	1	100

Parallel Session IV(b)

Paediatric/Adult Interface

Wednesday 28 April

17:40 – 18:20

O21

Hepatic Artery Thrombosis in 500 Paediatric Liver Transplant Recipients – the Role of Protocol Ultrasound Scans and Heparin/Aspirin Prophylaxis

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Hepatic artery thrombosis (HAT) has been a particular problem in paediatric liver transplantation with an overall incidence of 7.8% in the 500 children transplanted on our unit since 1983. HAT during the first week after transplantation is usually the result of technical problems and may be corrected by urgent intervention provided that it is recognised promptly. In 1998 we modified our protocol to include routine Doppler ultrasound scans immediately after abdominal closure and on a daily basis for the first five days post-transplant. Additional scans were performed if clinically indicated. Patients with high risk of HAT (long arterial conduit, small recipient artery) were given prophylactic low-dose heparin for seven days post transplant. All children with platelets count more than 75000/mm3 were given on low-dose aspirin. Two children had a false positive scan.Hepatic artery thrombosis has occurred in nine of 195 children (4.5%) transplanted since 1998. Three children developed late HAT and were retransplanted. The other six cases (occurring on post-operative days 1,2,2,3,3 &,5) were all detected on ultrasound scan during the first five days post-transplant. One child did not have exploration but later required biliary reconstruction for biliary stricture. The other five were re-explored and four grafts were salvaged. Revascularisation failed in one child who ultimately survived after emergency retransplantation. . We conclude that heparin and aspirin prophylaxis in paediatric liver recipients (and, perhaps, adults with risk factors for HAT) may reduce the incidence of HAT, and that doppler ultrasound surveillance during the first five days post-transplant may allow prompt intervention and graft salvage.

O22

Mycophenolate Mofetil (MMF) For Renal Dysfunction Following Paediatric Liver Transplantation

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Background: The calcineurin inhibitors cyclosporin (CyA) & tacrolimus (Tac) provide effective immunosuppression following liver transplantation (OLT) but may cause renal dysfunction. MMF does not inhibit calcineurin and does not affect renal function but its long term use has not yet been fully evaluated in children

Aim: To evaluate the safety and efficacy of MMF in children with renal dysfunction following OLT

Method: A retrospective review was performed of all children who commenced MMF between January 1997 and December 2001 for renal dysfunction. Glomerular filtration rate (cGFR) was estimated using the Schwartz formula (height (cm) x 40 / creatinine (µmol/l)). Renal dysfunction was defined as cGFR ≤ 65ml/min/1.73m^2. cGFR and liver function were measured at baseline, 1, 2, 3, 6 and then 6 monthly following MMF. Results were analysed by the Wilcoxon Signed Rank test and general linear model analysis of variance

Results: 48 children received MMF for renal dysfunction (23M; 25F). Median age at starting MMF was 11.2 yrs (0.9-18.1 yrs) and median time from OLT to starting MMF was 4.0 yrs (0.3–12.4 yrs). Median follow-up was 3.0 yrs (1.5–6.7 yrs). Immunosuppression following MMF was MMF montherapy (40), MMF + Tac (6), MMF + CyA (1) and MMF + sirolimus (1). cGFR increased significantly by 1 month of MMF therapy (median increase 11.5%; $p<0.001$) and again between 1 and 2 months (median increase 4.6%; $p<0.01$). The most significant changes occurred in younger children ($p=0.022$) and those closer to the time of OLT when transferred to MMF ($p=0.047$). Four patients did not respond (median baseline cGFR 37.1, range 31.2-48.7) and required renal transplantation. Abnormalities in liver function occurred in 5 children who received MMF monotherapy - transiently raised ALT (2), acute rejection (1), chronic rejection (2 - of whom 1 required retransplantation). Side effects included bone marrow suppression (3), headache (1) and nausea (1). All responded to decreasing MMF dose. Gastrointestinal bleeding requiring discontinuation of MMF occurred in 1

Conclusions: MMF significantly improved renal function if started early after transplant and before irreversible renal dysfunction. In order to prevent allograft rejection, steroid therapy during transfer from calcineurin inhibitors to MMF is recommended

O23

Chronic Allograft Nephropathy (CAN) And MMF Introduction In Paediatric Renal Transplant Patients

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Background: In the first decade after renal transplantation CAN is the most common cause of graft loss. Introduction of mycopenolate mofetil, MMF, along with reduction in calcineurin inhibitors has previously been shown to improve renal function in adults with CAN.

Aims: To examine the effect of MMF introduction in paediatric renal transplant recipients with evidence of CAN.

Methods: A retrospective analysis of paediatric transplant recipients with CAN who commenced MMF at a single centre. Changes in calculated GFR were plotted for each patient before and after MMF introduction. Cyclosporin & MMF doses, rejection episode rate, blood pressure and side effects were recorded.

Results: 19 children (M:F=11:8) satisfied the inclusion criteria. The mean age at transplant was 10.0 ± 5.1 (range 1.5 - 18) years. The ratio of cadaveric:live donors was 9:10. MMF was introduced 25 ± 23.3 (range=2.6 - 81.7) months after transplantation and patients followed up for a mean of 13.2 ± 12.3 (range 1.2 - 51) months. All patients underwent renal biopsy to confirm CAN prior to MMF introduction. The mean intial MMF dose was 660 ± 238 mg/m^2/d. This increased to 1042 ± 206 mg/m^2/d one year later. Cyclosporin doses were gradually reduced from 110 ± 65 mg/m^2/d at MMF introduction to 76 ± 61 mg/m^2/d six months later. This further decreased to 49 ± 32 mg/m^2/d one year later. Over a six month period prior to MMF introduction there was a deterioration in GFR of -0.09 ± 0.07 ml/min/1.73m^2/d. After MMF introduction a significant improvement in GFR of +0.02 ± 0.02 ml/min/1.73m^2/day over the ensuing six-month period occurred ($p<0.001$). This significant improvement in GFR continued at +0.06±0.13 ml/min/1.73m^2/day for the six months after MMF introduction to the last follow up ($p=0.001$). MMF introduction did not significantly alter the rejection rate ($p=0.11$). Systolic blood pressure was significantly lower one year after MMF introduction than before MMF was introduced ($p=0.01$). Heamatological parameters did not significantly differ six months before and six months after MMF introduction. During the study period MMF administration was halted in one patient due to gastrointestinal disturbance.

Conclusion: MMF introduction in paediatric renal transplant recipients with CAN causes a significant improvement in GFR in both the long and short term, as well as having a beneficial effect on blood pressure.

O24

Small Bowel Transplant (SBTx) In Children: A Decade Experience From A Single Centre

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Introduction: SBTx is a life saving option for children with short bowel syndrome and PN complications. Surgical techniques and immunosuppressive regimens have developed over the past decade but experienced centres still report 50% mortality at three years post-transplant.

Aim: To analyse the long-term outcome in children after SBTx.

Subjects & Methods: Retrospective review of all children who underwent SBTx at a single centre from 1993 to 2003. Patient demographics, indications for transplant, morbidity, mortality and long-term survival were analysed according to the type of graft.[table nn here]

Results: 23 children underwent SBTx. with short bowel syndrome (n=12), intestinal pseudo-obstruction (n=7) and mucosal disorder (n=4). 14 (60%) children died at a median of 8 months (range 7 days to 6.5 years) post-transplant as a result of VRE sepsis (n=3), chest infection (n=3) PTLD (n=3) chronic rejection (n=2), gut perforation (n=2) and sub-acute bacterial endocarditis (n=1). 9 children are alive at a median of 4 years (range 3 months to 5.6 years) post transplant. In this group there was high incidence of acute rejection (n=17), infections (CMV n=4, EBV n=8, VRE n=3, Cryptosporidia n=2) and PTLD (n=6). **Conclusion:** SBTx is the only therapeutic option for a small number of children with irreversible intestinal failure and complication of PN. The majority survive the first year post-transplant but long-term survival is still compromised by rejection, infection and PTLD.

		Isolated bowel	Liver &Bowel, n=17	Liver &Bowel, n=17	Liver &Bowel, n=17
			Classical	En-Block, n=15	En-Block, n=15
		n=6	n=2	Full n=3	Reduced n=12
Graft surviving >3 months		5	1	3	10
Recipient	Age median & (range) year	3.5 (0.8-4.6)	2.5,3.8	1.9(1.1-7.1)	0.9(0.8-11)
Recipient	Weight median (range) kg	11.3(9.2-19.8)	14,13	9.6(8.8-23)	7.8(7-34)
Indication	Lack of CV access	4	1	-	1
Indication	Recurrent repsis	1	-	-	-
Indication	TPN liver disease	1	1	3	11
Actuarial survival	1,3,5 years	42%,33%,33%	70%,40%,40%		

Parallel Session IV(c)

Clinical Audit Session

Wednesday 28 April

18:00 – 18:30

O25

Improving The Educational Needs Of Patients Who Develop Diabetes Post Liver Transplantation Through Audit

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Introduction
It is recognised that tacrolimus in combination with azathioprine and prednisolone is diabetogenic. Post transplant diabetes mellitus (PTDM) is another complication for patients to cope with while recovering from major surgery. In our centre the median post transplant hospital stay is 12 days, however this is often delayed due to patients developing PTDM. Access to diabetic services is constrained by excessive demand from the whole hospital. The training provided is based at another hospital. The patients are usually discharged home on the same day and will have had minimal experience in monitoring their diabetes and injecting their insulin. The patients are given time to digest and assimilate the information about their liver transplant however this is not achieved with their newly diagnosed diabetes.

Aims
1. Evaluate the incidence of PTDM since changing from Neoral to tacrolimus.
2. Identify factors that may precipitate patients becoming diabetic post liver transplantation.
3. Quantify delays in discharge and identify ways to improve the educational needs of patients with post transplant diabetes.

Methodology
A retrospective notes review of 171 grafts was performed, over two twelve month periods. During the first period Neoral was our first line calcineurin inhibitor and during the second period this had been replaced by tacrolimus.

Results
15.5% of patients on tacrolimus became diabetic post liver transplant compared to 9.6% on Neoral (p=n.s). Predisposing factors were identified as patients aged >50yrs ($p<0.08$) and 40% ($p<0.005$) of those who required sliding scale insulin became diabetic. Delays in discharge averaged 4 days, while patients waited to undertake diabetic education. This resulted in an increased financial cost to the liver unit of £11,800 over the study period. However this excludes all hidden costs (e.g. transporting patients between hospitals, readmissions due to uncontrollable blood sugars, etc).

Conclusion:
There is a recognised association between tacrolimus and PTDM. Patients over 50 and those requiring sliding scale insulin regimes are at greater risk. We have identified a need to improve the quality and timing of education delivered by the ward nurses and have identified the need for a diabetic specialist nurse to support this group of patients. A business plan has been submitted.

O26

Eastern Co-Operative Oncology Group (Ecog) Performance Status Is Associated With Post Liver Transplantation Mortality

on behalf of the UK & Ireland Liver Transplant Audit
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Background: The Eastern Co operative Oncology Group (ECOG) Performance status is a simple tool to assess a patient's disease progression, assess the impact of disease on the daily living abilities of the patient, and to determine appropriate treatment and prognosis for cancer patients. It is measured on a scale from 0 to 4 with 0 being patients who are fully active and able to carry on normal activity without restriction and 4 for completely disabled, unable to carry on any selfcare, totally confined to bed or chair. It is evident that the ECOG score reflects the physiological status of a patient. The objective of this study is to investigate the possible association between ECOG performance status and post liver transplantation mortality in patients receiving a first liver transplantation in the UK Ireland.

Methods: We included all 4112 adult patients who underwent a non-urgent first liver transplantation between 1 March 1994 and 31 March 2003. As a first step the impact of ECOG performance score on 90-day patient mortality was assessed by univariate analysis. Multivariate logistic regression modelling was then employed to adjust the ECOG score for other risk factors using bootstrap sampling techniques.

Results: The overall 90-day patient mortality was 9.7% (95% confidence interval 8.8% to 10.6%). When compared with patients with a score of 0 the unadjusted odds ratios for 90-day mortality for scores of 1, 2, 3 and 4 were 1.2, 1.8, 2.3 and 6.8, respectively ($p<0.001$). After adjustment for other risk factors the ECOG score remained a significant predictor of 90-day mortality ($p<0.001$). Recipient female sex, serum creatinine, cold ischaemia time, abnormal donor organ appearance and use of partial organs were also significantly associated with mortality ($p<0.05$).

Conclusions: The ECOG performance status indicator is a simple score, which can be easily measured. Patients with higher ECOG scores have a worse outcome after liver transplantation compared with those with lower scores. The results of this study suggest that performance status of the patient should be taken into consideration when selecting patients for liver transplantation.

O27

The Influence Of Cold Ischaemia Time On Kidney Transplant Survival

J Connolly, RJ Johnson, P Dyer, SV Fuggle, L Marson, R Thuraisingham and JLR Forsythe

On behalf of the UK Transplant Kidney and Pancreas Advisory Group, UK Transplant, Bristol, BS34 8RR, United Kingdom

Background: National analyses of the influence of cold ischaemia time (CIT) on kidney transplant outcome have not been possible until now due to incomplete reporting of CIT data. From 2000, CIT has been well reported and validated and this study investigates the effect of CIT on post-transplant survival for recent transplants, adjusting for other known risk factors.

Methods: Data were obtained for 2348 first cadaveric heartbeating donor kidney only transplants in adults in the UK, January 2000 - June 2002. A valid CIT was reported for 2282 (97%). Median CITs for locally retained and exchanged kidneys were compared and the influence of CIT on transplant survival time was investigated. Transplant survival was defined as the time from transplant to transplant failure, this being the earlier of a return to regular dialysis or patient death. Unadjusted survival rates were obtained from Kaplan-Meier estimates and Cox regression models were fitted. The one-year follow-up rate was 96%.

Results: Overall the median CIT was 19 hours (inter-quartile (IQ) range 16-23 hours). Median CIT did not vary significantly between kidneys retained at the retrieval centre (18 hours, IQ range 15-22) and those exchanged with other centres (19 hours, IQ range 16-24 hours). Univariate analysis showed significant differences in one-year transplant survival according to five CIT groups ranging from 90% (95% CI 88-91%) for transplants with CIT<20 hours (n=1273) to 82% (95% CI 75-89%) for transplants with CIT>34 hours (n=115), (p=0.04). Cox regression modelling found a highly statistically significant effect of CIT on outcome having adjusted for donor and recipient age, donor-recipient gender match and HLA match (p<0.0001). Compared with transplants with CIT<=27 hours, the relative risk of transplant failure in the first post-operative year for transplants with CIT over 27 hours was 1.9 (95% CI 1.4-2.5). Analysis of post-transplant epochs showed that the effect influenced transplant survival in the first 3 months, after which time the survival differences remained unchanged.

Conclusion: Complete and accurate CIT data on a national basis confirm that there is a highly statistically significant detrimental effect of long CIT on one-year transplant survival. This is an important finding as CIT is potentially a controllable risk factor: measures should be taken to minimise CIT and improve post-transplant outcome.

Parallel Session I(a)

Best Abstracts

Thursday 29 April

09:00 – 10:00

O28

Complete Inhibition Of Acute Humoral Xenograft Rejection By Regulated Endothelial Cell Expression Of Novel Anticoagulant Fusion Proteins

D Chen[1], J McVey[2], G Kemball-Cook[2], E Tuddenham[2], R Lechler[1] and A Dorling[1]

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Acute humoral xenograft rejection (AHXR) remains a significant immunological problem after xenotransplantation. Histologically, organs rejected by AHXR show prominent widespread microvascular thrombosis, often accompanied by systemic coagulation disturbances in the recipient. This has led to the hypothesis that abnormal activation of clotting might be of primary importance to the pathophysiology of AHXR. We have tested the effect of inhibiting coagulation in a mouse heart-to-rat model of AHXR. Control C57BL/6 hearts, transplanted heterotopically without immunosuppression were rejected after a mean of 2.8 (+/-0.4) days. Histology showed widespread intravascular fibrin deposition and features typical of AHXR. Hearts were harvested from one of two novel strains of transgenic mice expressing membrane-tethered human tissue factor pathway inhibitor or hirudin fusion proteins under the control of a modified CD31 promoter for expression on activated endothelial cells (EC). These hearts survived for a mean of 6.6 (+/-0.49) days and 6.4 (+/-1.02) days respectively. Histology on the day of rejection showed no evidence of thrombosis but the grafts were infiltrated with CD3+ cells. Experiments were repeated under cover of daily cyclosporin. Control hearts were still rejected at 2-3 days by AHXR. In contrast, hearts from both transgenic donors are still beating more than 28 days post-transplantation. These experiments are ongoing. These data show that efficient inhibition of intravascular coagulation by expression of anticoagulants on EC completely inhibits AHXR in this small animal model, implying that activation of coagulation factors is an important element in the pathophysiology of humoral rejection.

O29

Basiliximab (Simulect) With Ciclosporin(Neoral) As A Strategy For Steroid Avoidance In Renal Transplantation

NR Parrott[1], AQ Hammad[2], CJE Watson[3], PJA Lodge[4] and C Andrews[5]

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Historically, ciclosporin monotherapy has produced excellent long-term graft and patient survival rates when used as either initial or maintenance (>1 yr) immunosuppression. Conversely, addition of steroids results in a dose-related reduction in survival figures. However, amongst patients started on Neoral alone, rejection rates are relatively high and fewer than 50% remain steroid-free in the long-term. In order to investigate the utility of CD25 antibody (anti-IL-2R) as a strategy for avoidance of steroids or other additional immunosuppression, we conducted a prospective, multicentre, randomised, double-blind, placebo controlled, 12 month study of basiliximab induction on 108 kidney transplants receiving ciclosporin for microemulsion (Neoral) monotherapy. Patients were randomised pretransplant to receive a two dose course of basiliximab (n=52) or placebo (n=56).

Requirement for oral steroids at any time in the study was lower in the basiliximab group (33% vs 61%; P=0.004). Maintenance steroid use was lower with basiliximab than placebo at 6 months (26% vs 60%; P<0.001) and at end of study (25% vs 61%; P<0.001). More basiliximab patients than placebo patients were still maintained on Neoral monotherapy at 6 months (52% vs 29%; P=0.018) and at the end of study (46% vs 27%; P=0.046). 73% of the basiliximab group and 61% of the placebo group continued with Neoral as sole agent or with adjuncts until the end of study. The main reasons for changing from Neoral monotherapy were acute rejection and delayed graft function. Rejection occurred in 29% basiliximab patients and 43% placebo patients (P=0.17). One year graft and patient survival were 88% and 98% for basiliximab and 88% and 96% for placebo. The mean and median values for serum creatinine were consistently lower in the basiliximab group at every timepoint in the study: at 12 mo! nths median creatinines were 141 vs 164 mol/l for the basiliximab and placebo groups respectively (P=0.55)

Conclusion. This is the first reported study of basiliximab induction with Neoral monotherapy immunosuppression. This strategy of Simulect induction significantly reduced the need for added maintenance immunosuppression allowing approximately 50% of the patients to be maintained on Neoral monotherapy, and 75% to be maintained on steroid-free, tailored immunosuppression at 1 year post transplant.

O30

National Potential Donor Audit

KM Barber, JC Hussey, D Collett, CJ Rudge and SJ Falvey

UK Transplant, Bristol, BS34 8RR, United Kingdom

Background: UK Transplant's (UKT) national potential donor audit (PDA) began in January 2003, as part of a series of measures to improve organ donation.

The PDA aims to provide an up-to-date assessment of the potential for solid organ donation from Intensive Care Units (ICUs) throughout the UK. The audit will identify the number of patients who could be heartbeating or non-heartbeating solid organ donors and will establish the obstacles to donation.

Methods: Since January 2003, data have been collected by donor liaison nurses in the ICUs where they are in post (34) and by donor transplant co-ordinators, and/or link nurses in all other units. An audit form developed in collaboration with other appropriate personnel is being used. One form is completed for each patient death in an ICU. All completed forms are returned to UKT for data input, validation and analysis.

Results: An evaluation of data from April-June 2003 showed that 256 hospitals (309 ICUs) had reported at least one patient death. Of the 5,096 audited deaths, death was confirmed by brain stem tests in 278 (5%). In 34 (12%) of 278 families there was no record of any discussion of donation with relatives. Of the 244 patients for whom the possibility of organ donation was suggested to relatives, consent was given for 126 (52%) and not given for 118 (48%) patients. Of the 126 patients for whom consent was given, 114 (90%) became cadaveric heartbeating donors. A further nine non-heartbeating donors were identified during this period.

From this preliminary analysis, an overall relative refusal rate of 48% (95% confidence interval 42-54%) was observed. Relative refusal rates by former NHS region ranged from 37-63%. However, due to the small number of patients included in the analysis across regions, these results must be viewed with caution.

Conclusions: It is of concern that one of the main findings was a high relative refusal rate. We are looking at ways to address this and are currently analysing the data in more detail. However, once more validated data are available for analysis a truer picture should emerge.

UKT hopes that the PDA will continue to raise the profile of organ donation and heighten awareness of donation issues amongst critical care staff. It will also provide a realistic estimate of the true potential for organ donation and will allow both local and national obstacles to realising the potential to be identified.

O31

Adoptive Cell Therapy With Cultured, Donor-Specific Regulatory T Cells To Promote Transplantation Tolerance

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The key goal in clinical transplantation is the induction of donor-specific tolerance to minimise the morbidity and mortality associated with long-term immunosuppression. A consistent feature of transplantation tolerance in experimental models is the presence of donor-specific regulatory T cells that can transfer the tolerant state to naive animals. Furthermore, these regulatory cells appear to have indirect allospecificity for donor antigens (i.e. they recognise allogeneic major histocompatibility complex (MHC) molecules as peptides presented by recipient MHC class II molecules). A population of dedicated, naturally occurring regulatory T cells has recently been defined in mice and humans that co-express CD4 and CD25. These CD4+CD25+ T cells play a crucial role in the maintenance of peripheral tolerance. The question that we addressed in this study is whether CD4+CD25+ T cells can be subverted to limit alloresponses. Using autologous CBA/Ca ($I\text{-}A^k$) dendritic cells pulsed with a peptide of $H\text{-}2K^c$, we generated mouse cell lines from purified CD4+CD25+ T cells. The cell lines could be expanded to large numbers in the presence of IL-2 and maintained the phenotype and potent suppressive capacities characterising freshly isolated CD4+CD25+ T cells. When these expanded regulatory T cells were co-cultured with CD4+CD25- T cells, 90% inhibition of proliferation and cytokine production was observed at a 1:8 ratio; this suppression was greater than that obtained with fresh CD4+CD25+ T cells. Furthermore, the inhibition was not cytokine mediated but dependant on cell contact between the regulatory cells and the responders as suggested by transwell and blocking experiments. Following intravenous injection, the regulatory cell lines expanded, homed to lymphoid tissues, and accumulated in the lymph nodes draining the site of injection of dendritic cells pulsed with the cognate alloantigen. In early transplant experiments the *in vitro* expanded CD4+CD25+ T cells prolonged skin allograft survival in the absence of any other immunosuppression. Taken together, these data suggest that the selection and expansion of dedicated regulatory T cells is a clinically applicable strategy for the induction of transplantation tolerance.

Parallel Session I(b)

Transplant Coordinators Session

Thursday 29 April

09:30 – 10:00

O32

Does Donor Cause Of Death Affect Survival In Heart Transplantation?

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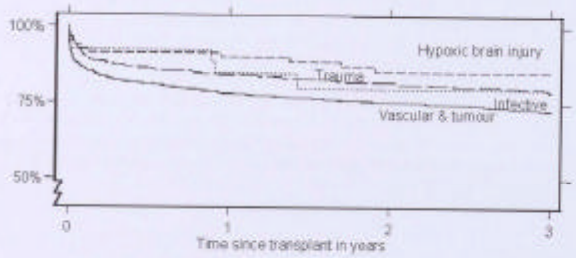
Objectives: Donor cause of death (DCD) may be a risk factor for early mortality following heart transplantation (HTx), but its effect on longer-term survival is uncertain.

Methods: We investigated the influence of DCD on survival to 3 years, using a national prospective database in 1233 adult recipients of cadaveric HTx between July 1995 and June 2002. DCD was categorised a priori to: vascular and tumour (V, n=725), trauma (T, n=402), hypoxic (H, n=80) and infective causes (I, n=26). Risk factors for early (30-day), late (30-days to 3-year) and overall mortality were identified using Cox regression.

Results: V donors were older (median 40 years vs. <26 years); V and H groups had proportionally fewer males (p<0.001); and V recipients had a higher incidence of previous heart surgery (p=0.02). There were 286 total deaths. Unadjusted 3-year survival varied significantly with DCD (p=0.01). Cox analysis identified donor age, organ ischaemia time, recipient creatinine clearance, recipient diagnosis, peripheral vascular disease, ventilation, diabetes, and donor-recipient size mismatch as risk factors for early, late and/or overall mortality (p<0.10). After adjusting for these factors, DCD was no longer a significant predictor of recipient death (early death, p=0.5, late death, p=0.7, overall mortality, p=0.14).

Conclusions: We have confirmed the previous observation of an apparent association between cause of donor death and post transplant survival but this was not maintained after adjustment for confounding variables. DCD is not an independent risk factor for mortality up to 3 years after HTx.



O33

An Efficiency-Equality Model Of Equity, Applicable To Cadaveric Transplant Allocation

RM Higgins[1], RJ Johnson[2], MNA Jones[2] and CJ Rudge[2]

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Equity is achieved when each patient is treated fairly relative to others. It is proposed that equity in cadaveric organ allocation is a trade-off, or compromise, between equality and efficiency. This is in contrast to some previous views, which suppose an inherent conflict between efficiency and equity.

The 'efficiency-equality model' quantifies, using value judgements, the best trade-off between efficiency and equality for each of several factors impacting on transplantation. It can also be used to test an allocation algorithm.

Previously published views of public, patients and professionals appear to regard both efficiency and equality as important principles in fair transplant allocation. Desire for efficient transplant allocation was particularly strong amongst potential transplant recipients and the general public.

The kidney transplant allocation algorithm currently used in the UK was tested in an efficiency-equity model. In a modelling exercise of 2000 past UK donors and a dynamic waiting list of 5000 potential recipients, 4000 transplants were allocated according either to the current United Kingdom national matching (NAT) system; and by an equal allocation (EQ) model (a lottery); and by an efficiency (EF) model. The latter used a risk score constructed from recent data on the effects of recipient age and diabetes and donor-recipient HLA matching on graft survival. Diabetic recipients made up 7.4% of transplants in the NAT scheme, 8.6% in EQ model, and 0% in EF model, and paediatric recipients made up 6.8 % of NAT scheme, 0.6% in EQ model, and 0.7% in EF model. For HLA matching, there were 77.9% favourable or 000 matches under the NAT scheme, compared to 3.0 in EQ model and 53.1% in EF model. The predicted survival of each transplanted cohort was estimated using a 'prognostic risk score' which showed significantly better outcomes in the EF model (p<0.0001) compared with the NAT, while the EQ cohort had outcomes inferior to those of the NAT model cohort (p=0.05).

The NAT allocation system favours paediatric recipients and does not deny diabetic recipients the chance of a transplant, which would broadly be in line with public and professional opinions voiced in previous research. The NAT scheme achieves better HLA matching than the EF model, and this suggests that the rationale for allocation based primarily on HLA matching could be re-examined.

O34

An Education Programme Objectively Improves Understanding Of Issues Related To Organ Donation Among Schoolchildren

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The number of transplants is limited by the availability of donor organs. Public education, to promote awareness and reduce misconceptions about organ donation, is an important responsibility of the transplant co-ordinator. School education programmes have been in existence for a number of years but their effectiveness has never been objectively assessed. This study assessed the effectiveness of a school education programme given by transplant co-ordinators in Scotland.

Methods

The study was conducted in 23 schools among 1185 pupils (aged 15-17 years). The pupils completed questionnaires before and 1 week after a 45-minute educational presentation given by a transplant co-ordinator. The questionnaire was previously developed and validated in a separate cohort and was designed to test understanding and common misconceptions of organ donation and changes in these indices following education.

Results

Both questionnaires were completed by 697 students (59% completion rate). Before the talk 69% had seen an organ donor card while after the presentation, 85.5% recognised the card ($p<0.03$). Half of subjects either did not know or believed that they were too young to be an organ donor, this fell to 24% following the presentation ($p<0.02$). Before the presentation 16% of students believed that they were more likely to require a transplant than to become an organ donor, this changed to 39% after the talk ($p<0.01$). While 80% of students believed that organ donation was a good thing, only 5% carried a donor card, however, one week after the talk, 25% carried organ donor cards. Questionnaire 2 revealed that in the week following the education session 54% of pupils had discussed the topic of organ donation with family and/or friends.

Conclusions

1. This tool can be used as an objective measure of understanding in schoolchildren regarding issues in transplantation.
2. Education of schoolchildren by transplant co-ordinators provides objective improvement in understanding of transplant and donor issues and results in increased uptake of donor cards and discussion of the issues of organ donation within families.

Parallel Session II(b)

Immunosuppression

Thursday 29 April

11:00 – 12:00

O35

Campath 1h (Alemtuzumab) In Renal Transplantation: Comparative Follow Up At 5 Years

CJE Watson[1], J Firth[2], J Bradley[2], KGC Smith[2], NV Jamieson[1], PJ Friend[3], S Thiru[1], C Taylor[1], G Hale[4], H Waldmann[5] and JA Bradley[1]

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Alemtuzumab is a powerful lymphocyte depleting monoclonal antibody that is licensed for the treatment of lymphoreticular malignancy and is under evaluation in transplantation. This paper describes the 5-year follow up of a study which comprised alemtuzumab followed by low dose ciclosporin monotherapy. These patients have been compared to contemporary controls. The only selection criteria for alemtuzumab was the giving of informed consent.

Method Cadaveric renal transplant recipients (n = 33) were given two doses of 20mg alemtuzumab followed 48 hours later by low dose ciclosporin monotherapy. A cohort of patients (n = 66) transplanted in the same time period in the same centre and receiving conventional ciclosporin-based triple therapy (n = 61) or sirolimus, ciclosporin and prednisolone (n = 5) were selected for comparison. Patients receiving living donor or multi-organ transplants were excluded.

Results Follow up was complete in the alemtuzumab group and while 2 of the control group were lost to follow up at varying intervals post transplant; both had normal graft function at the time. Table 1 illustrates the outcomes.
One patient in each group died from PTLD. The other deaths were due to ischaemic heart disease (n = 2) and calciphylaxis (n = 1) in the alemtuzumab group, or to ischaemic heart disease (n = 6), cerebrovascular disease (n=1), sepsis (n = 2), cerebral tumour (n = 1) and unknown (n=1) in the control patients. Notable events in alemtuzumab treated patients include one de novo autoimmune haemolytic anaemia and one ileocaecal TB infection.
In spite of profound initial lymphocyte depletion in the alemtuzumab group, the total lymphocyte counts in both groups were similar by 6 months. Renal function remained similar in each group throughout. The incidence of acute rejection was also similar in both groups, but time of the first acute rejection episode was much later in alemtuzumab treated patients (medians 170 days vs 16 days).

Conclusion Alemtuzumab combined with low dose ciclosporin is a safe and effective regimen that is well tolerated and avoids steroids. Patients need to be followed up closely for late acute rejection.

Table 1. Patient and Graft survival.

		Alemtuzumab	Controls	
Graft survival	1 year	94%	83%	
(Censored for death)	5 years	79%	75%	P = 0.57
Patient survival	1 year	97%	88%	P = 0.13
	5 years	88%	83%	P = 0.28

O36

Liquid Chromatography with Tandem Mass Spectrometry: A Precise and Specific Method for Routine Therapeutic Monitoring of Tacrolimus

GD Chusney[1], RJ Borrows[2], TDH Cairns[2], M Griffith[2], N Hakim[2], AWK James[1], J Lee[1], AG McLean[2], A Palmer[2], V Papalois[2], JL Stichbury[1] and D Taube[2]

[1]Brent Laboratory, 1st Floor Mint Wing, St.Mary's Hospital, London, W2 1NY, United Kingdom and [2]Renal and Transplant Unit, St.Mary's Hospital, London, W2 1NY, United Kingdom

Therapeutic drug monitoring of Tacrolimus (Tac) is routinely measured in whole blood by immunoassay. The technique of liquid chromatography-tandem mass spectrometry (LC-MS) is considered to be the 'gold standard', however recent developments in the design, simplification of operation and reduction in cost have made these instruments more suitable as a routine analytical tool. We have validated a LC-MS method for the measurement of whole blood Tac concentrations in renal graft recipients. This method was then compared to the current method, a microparticle enzyme immunoassay (MEIA, Abbott Diagnostics). The LC-MS system was a Micromass Quattro-Micro tandem mass-spectrometer coupled to a Waters AllianceHT 2795 separation module. Sample extraction required only 25µL of whole blood. LC-MS is highly selective for Tac which is achieved by monitoring the specific fragmentation of the ammoniated ions of both Tac and the internal standard, ascomycin. Throughput was 24 samples per hour. Assays were calibrated with the MEIA Tacro II calibrators and were used as supplied. The LC-MS assay was linear up to a concentration of 50µg/L using in-house calibrators. Intra-assay variability, determined by analysis of 3 patient samples (n=20), was 3.9% @ 5.0µg/L, 3.0% @ 9.5µg/L and 4.2% @ 15.3µg/L. The inter-assay variability, using MEIA Tacro II controls was (LC-MS vs MEIA) 8.0% vs 13.7%, 6.5% vs 8.3% and 5.5% vs 10.9% at target concentrations of 5.0µg/L, 11.0µg/L and 22.0µg/L respectively.
The accuracy of LC-MS was assessed by the recovery of Tac from spiked samples provided by the International Tacrolimus Proficiency Testing scheme (Analytical Unit, St.George's Hospital Medical School, UK) mean recovery (LC-MS vs MEIA) was 100.0% (n=20, range 88.1 – 114.2%) vs 116.0% (n=20, range 92.8 – 143.3%).
Tac concentrations were measured by LC-MS and MEIA in 950 samples, from 232 renal transplant patients, and compared using Passing-Bablok regression and Bland-Altman difference plots. Regression analysis gave an intercept (mean (±95% CI)) of –0.105 (-0.333 – 0.155) and slope of 0.864 (0.839 – 0.889) and indicated proportional bias. The MEIA assay overestimated Tac by 16.2% compared to LC-MS using the Bland-Altman plot.
In conclusion LC-MS is both a more specific and precise method for Tac analysis and is suitable for routine therapeutic monitoring.

O37

Randomised Study Comparing Cyclosporin With Azathioprine One Year After Renal Transplantation - 15 Year Outcome

N Joss

On behalf of the Glasgow Transplant Group, Renal Unit, Western Infirmary, Dumbarton Road, Glasgow, G11 6NT, United Kingdom

The introduction of cyclosporin (CsA) has improved the 1-year graft survival and reduced the incidence of acute rejection episodes after renal transplantation. CsA is associated with many side effects including hypertension and nephrotoxicity. Reducing the exposure of this drug after the first year may be beneficial on patient and graft survival. 216 patients were enrolled in a single centre study. After 1 year, if serum creatinine was less than 300µmol/l and there were no acute rejection episodes in the previous 6 months, the patients were randomised to continue cyclosporin (114) or to be converted to azathioprine (102). Analyses were performed on an intention to treat basis and we present follow up data at 15 years post transplant.

The patients were well matched at baseline There was no difference in patient survival at 15 years, 64.3% in the CsA group and 64.4% in the Aza group. Fifteen-year transplant survival (including death with a functioning graft) was 40% for the CsA group and 47.2% for the Aza group (p=0.7). Fifteen year graft survival censoring for death with a functioning graft was 57% in the CsA group and 72% in the Aza group (p=0.5). The graft survival for the patients who remained on their assigned treatment was higher in the Aza group (87%) compared to 65% in the CsA group, although this was not significant (p=0.1). The median (range) CsA dose was 3 (1.4-7.1) mg/kg at randomisation and 2.6 (1.8-3.9) mg/kg in the patients who remained on CsA at 15 years.

The estimated glomerular filtration rate (EGFR) at year 2, 5 and 10 was significantly lower in the CsA group; however, by 15 years this effect was lost. More patients in the CsA group were on antihypertensive agents. Cox regression analysis was performed to determine which factors predicted death and graft failure. EGFR at year 1 (p=0.003, RR 0.97) and age (p=0.003, RR 0.97) predicted graft survival (censoring for death). Age (p<0.001, RR 1.07) and SBP at year 1 (p=0.03 RR 1.01) predicted patient survival. Assigned drug had no effect on graft or patient survival.

In conclusion, conversion from CsA to Aza at 1 year after transplantation in patients with a serum creatinine less than 300 µmol/l was not associated with any adverse effects on patient or graft survival at 15 years. The most important factor predicting graft survival was renal function at time of randomisation.

O38

Factors Influencing 12 Hour Trough Mycophenolic Acid Levels In Renal Transplantation

R Borrows, G Chusney, J van Tromp, A James, J Stichbury, T Cairns, M Griffith, N Hakim, A McLean, A Palmer, V Papalois and D Taube

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The purpose of this study was to evaluate factors influencing mycophenolic acid [MPA] levels in renal transplantation. Despite recent observations that levels of MPA, the active metabolite of mycophenolate mofetil [MMF] may be predictive of rejection episodes and side effects in transplant patients, longitudinal study of MPA levels and their determinants are scant.
We have analysed 3700 serial 12-hour trough MPA levels by immunoassay in 140 renal transplant recipients, treated with tacrolimus, MMF [initial daily dose 1.5g, increasing to a maximum of 3g daily, and reducing in the event of side effects], and steroid avoidance [steroids for the first 7 days only].
The mean daily MMF dose rose from 1.5g during week 1 to 1.6g from week 4 until the third month. A subsequent reduction in daily MMF dose was seen between months 5 and 12 [1.4g], related to the development of MMF side effects. Beyond the first year post transplant the mean daily dose again rose to 1.6g.
Mean MPA levels progressively rose during the first 30 days post transplantation [1.56 mg/l in week 1 to 2.15 mg/l by week 4], and then stabilised at a mean level of 2.3 mg/l from the second month onwards. Thus the dose-corrected level rose from 1.02 mg/lg^{-1} in the first week to 1.4 mg/lg^{-1} between months 2 and 4, and then again to 1.68 mg/lg^{-1} beyond month 4. High variability [%CV=78] in the dose-corrected MPA level was seen at all times. The relationship between the MMF daily dose and MPA level was linear when overall mean values were considered, with a close correlation between the total daily dose and MPA level [r=0.94]. However, due to the high variability, a poorer correlation was seen between dose and level when individual patient samples were considered [r=0.44].
Age, gender and ethnicity did not influence dose-corrected MPA levels, and no difference was seen in the first 2 weeks between patients requiring post transplant dialysis and those with immediate graft function. Beyond the first month, the administration of oral antibiotics [augmentin, ciprofloxacin and metronidazole either alone or in combination] led to a reduction in mean dose-corrected MPA level from 1.62 mg/lg^{-1} to 0.91 mg/lg^{-1}. This may be due to interference of MPA enterohepatic recirculation.
This study shows that the relationship between MPA levels and MMF dose changes with time post transplantation and co-administration of other medications.

O39

Phase III Prospective, Randomised Study To Compare Conversion From Calcineurin Inhibitors To Rapamune® Versus Standard Therapy In Established Renal Allograft Recipients With Mild To Moderate Renal Insufficiency At 6 Months

K Baboolal

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Objective: To determine the efficacy and safety of conversion from calcineurin inhibitor (cyclosporin or tacrolimus) based therapy to rapamycin based therapy in patients with mild to moderate renal allograft insufficiency.
Methods: We present a six-month interim analysis of established renal allograft patients with mild to moderate renal insufficiency, recruited from centers in the UK and Ireland. Patients at 6 months to 10 years following renal transplantation, with a calculated creatinine clearance (Cockcroft and Gault) of ≥ 20 ml/min and ≤ 70 ml/min and treated with a calcineurin inhibitor (CNI) for a minimum of 3 months were eligible for the study. Patients were randomized (3:2) to either have their CNI-based therapy switched to Rapamycin (Group A) or to continue on CNI-based therapy (Group B). Patients randomized to Group A received a loading dose of 12 mg Rapamycin on Day 1 and then concentration-controlled Rapamycin to achieve trough levels of 8-16 ng/ml. Patients randomized to Group B continued on their CNI aiming for trough levels of 50-150 ng/ml or 3-8 ng/ml for cyclosporine of tacrolimus respectively.
Results: Fifty five patients were randomized to Group A (n=30) or Group B (n=25) of whom 48 completed 6 months of study therapy. There were no differences in demographic variables between Groups A and B. Baseline values for creatinine clearance were similar between Groups A and B (44 mL/min vs 44 mL/min respectively). At 6 months, the mean creatinine clearance was significantly higher in Group A vs. Group B (45 mL/min vs 38 mL/min respectively ps0.001). There were no significant differences in systolic or diastolic blood pressure between the groups. Serum triglycerides (2.83 mmol/L vs. 1.90 mmol/L) and cholesterol (5.9 mmol/L vs. 5.1 mmol/L) were more elevated at 6 months for patients in Group A than Group B respectively, but were not statistically significant. Four patients discontinued therapy in Group A and three patients in Group B. There were no episodes of acute rejection in either treatment group. Patient survival was 100% in both groups. Graft survival was 100% for Group A and 92% for Group B.
Conclusions: These data suggest that switching patients from CNI's to Rapamycin is safe and does not compromise immunosuppressive efficacy. Converting patients to Rapamycin is associated with significant improvements in renal function.

O40

Conversion To Sirolimus Following Renal Transplantation - Preliminary Results From A Randomised Trial

CJE Watson[1], J Firth[1], J Bradley[1], PF Williams[2], JC Smith[1], CR Palmer[3] and JA Bradley[1]

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Sirolimus is a powerful immunosuppressant that does not share the nephrotoxicity associated with calcineurin inhibitors (CNI). We undertook a randomised controlled study of conversion of renal transplant recipients with impaired graft function from CNI therapy to sirolimus-based therapy.

Methods Renal transplant recipients at least 6 months post transplant with a glomerular filtration rate (GFR) less than 65mls/min were randomised to continue on CNI therapy or switch to sirolimus. Conversion involved abrupt cessation of CNI followed by commencement of sirolimus without an overlap period; an initial dose of 8mg was followed by 4mg/day with subsequent adjustment to keep sirolimus concentrations within a range 5 to 15ng/ml. Concomitant immunosuppression and other medications were not altered. GFRs were measured using radionuclide-labelled EDTA at baseline, 3 and 12 months after commencing the study.

Results 37 patients have been randomised into the study of whom 31 have reached three-months, and 14 one-year. One patient refused to switch to sirolimus after randomisation, and remained on a CNI. Three others were intolerant of sirolimus and reverted to CNIs at 2 to 3 months. All patients are alive, one of the CNI patients returned to dialysis 13 months after randomisation.
Mean (sd) GFR at randomisation was 42 (12) mls/min. GFR increased significantly in the sirolimus treated patients by 3 months and was sustained to 12 months; GFR fell in the CNI group (intention to treat analysis) - see table.
In addition there was a significant fall in uric acid in the sirolimus group at 3 and 12 months, and a fall in leucocyte count significant only at 3 months. There were no rejection episodes in either group. The main adverse events associated with conversion were rashes, mouth ulcers, and herpes simplex stomatitis.

Conclusion Abrupt conversion to sirolimus appears to be safe, and is associated with superior renal function compared to continuation on CNI therapy. Side effects seem to be dose related and can sometimes be managed by dose reduction. These preliminary results suggest that sirolimus may have an important role in the maintenance phase after renal transplantation.

Table: Change in mean (sd) GFR from baseline at randomisation

	SIROLIMUS	CNI	Significance
3 months n = 16 SRL, 15 CNI	+ 7.1 (4.3)	– 2.2 (8.7)	p = 0.001
12 months n = 7 each	+ 7.7 (8.6)	- 11.0 (13.3)	p = 0.01

Parallel Session IV(a)

Renal Transplantation

Thursday 29 April

15:00 – 16:00

O41

Serum Creatinine In The First Year Post-Transplant As A Predictor Of Long-Term Renal Transplant Outcome

RJ Johnson, CJ Rudge, D Collett and JLR Forsythe

On behalf of the UK Transplant Kidney and Pancreas Advisory Group, UK Transplant, Bristol, BS34 8RR, 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.

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. Analysis of transplant survival (death with function treated as a failure) considered 3 and 12-month serum creatinine, the change between 3 and 12-month serum creatinine (Δ creatinine) and a number of donor and recipient-related factors. Data on patient size, ethnicity, cold ischaemia time and sensitisation were not recorded. All other 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 an adverse association between outcome and creatinine deterioration, 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 ($p<0.1$). Significant effects of donor age and donor-recipient gender match 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.

O42

Renal Function In Kidneys From Controlled And Uncontrolled NHBD

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Introduction

NHBD are utilised to alleviate the critical donor shortage. The yield from NHBD is poor in uncontrolled donors than controlled donors. But there is a larger pool of uncontrolled donors. Kidneys from NHBD suffer from warm ischaemia at the cardiac arrest which is reflected as ATN of allograft resulting in a period of DGF.

The aim of the study was to determine the renal function of kidneys from the different Maastricht categories (II, III and IV).

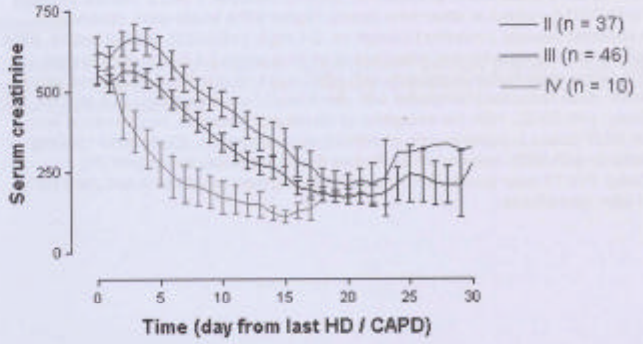
Materials and methods

Since 1998, 144 kidneys were procured from 72 NHBD resulting in 93 transplants. The renal function was determined (serum creatinine) and recorded retrospectively and results shown.

Results (see graph)

Conclusion

Kidneys from the different Maastricht categories recover at different rates though they are all similar at 3 months.



O43

12 Hour Trough Mycophenolic Acid Levels Predict Rejection, Bone Marrow Suppression, Viral Infections And Diarrhoea In Renal Transplant Recipients Immunosuppressed With Mycophenolate Mofetil And Tacrolimus

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The purpose of this study was to correlate serum mycophenolic acid [MPA] levels with adverse events in renal transplant recipients.
We have analysed serum 12-hour trough MPA levels by immunoassay in 140 renal transplant recipients treated with tacrolimus and MMF as maintenance therapy in a steroid avoidance regime [steroids for the first 7 days only]. The initial dose of MMF was 1.5g daily, increasing to a maximum daily dose of 3g, depending on side effects. The transplant team was blinded to the results of the MPA levels, but were able to adjust MMF dosage on clinical grounds. MPA analysis was performed at least three times per week for the first 2 weeks post transplantation, twice weekly for the following month, weekly for the next month, and then at each visit. Median follow up for the group was 26 months [range 3-42 months]. A total of 3700 samples were analysed, with each patient having at least 15 samples [median 23; range 15-50].
12 episodes of biopsy proven acute rejection occurred, 7 within the first month. The median trough MPA level prior to the rejection episode in these 7 patients was lower than the median level in the first month for patients not experiencing rejection [0.76 mg/l vs. 1.8 mg/l; p=0.02]. Higher MPA levels were observed in leucopaenic patients between 1 and 6 months [3.5mg/l vs. 2.4 mg/l; p<0.0001;], but not at other time points. Higher MPA levels were observed in patients with anaemia beyond 3 months [3.6mg/l vs. 2.4 mg/l; p=0.0078], but not before. MPA levels were higher in patients with viral infections at all time points [4.0 mg/l vs. 2.5 mg/l; p=0.008]. MPA levels were higher in patients with MMF-induced diarrhoea [diarrhoea which settled with MMF dose reduction] compared with diarrhoea of other aetiology [3.4 mg/l vs. 1.7 mg/l respectively; p=0.0002]. With the exception of diarrhoeal episodes, no difference was seen between MMF doses in patients with or without adverse events. [Daily dose 1800mg vs. 1500mg in patients with MMF-induced and infective diarrhoea respectively; p=0.01].
This study shows that 12-hour trough MPA level monitoring may be of use in reducing early rejection and later side effects.

O44

Excellent Transplant Results From Controlled Non Heart-Beating Kidney Donors

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The shortage of cadaveric organs for transplantation demands that non heart-beating donor (NHBD) outcomes are carefully evaluated. With UKT support there has been a significant expansion in this area and many centres will be planning to start a programme in the near future.

Our previous experience (since 1988) has demonstrated that the best NHBD results are achieved with controlled donors (Maastricht Category 3 and 4) aged 15-50 with warm ischaemic time less than 30 minutes. We report the clinical outcomes from such donors in South Thames renal transplants between January 01 and June 03.

51 Transplants were performed in 2 centres from 26 donors during this period. Mortality in the first year was 6%, graft survival 92% and censored graft survival 98%. The incidence of delayed graft function was 78% and its average duration 12 days. The incidence of primary non function was 2% and cold storage time was significantly shorter than for our heart-beating donor kidneys (16 hours v 22 hours). Comparison of renal function with heart-beating donor transplants showed no significant difference at one year despite the routine use of calcineurin inhibitors.

Summary

Controlled NHBD kidneys produce excellent results if carefully selected and storage time is minimised. They should be part of the transplant programme of all units. There are, however, major logistical implications.

O45

BK Virus-Associated Nephropathy: The Oxford Experience

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BK virus-associated nephropathy (BKVAN) occurs in up to 5% of renal transplant recipients. However, a recent survey of the UK experience, including 80% of units but not Oxford, found a much lower incidence, identifying only 34 cases of BKVAN. In this study we review the cases of BKVAN diagnosed on our unit over 3-years (1/3/00-1/3/03).

During this period 248 patients were transplanted, of whom 12 (5%) were diagnosed with BKVAN on renal biopsy. The median time from transplant to diagnosis was 6.5 months (range 3-18 months). Full immunosuppression (IS) history and follow-up were available on 10 patients: in 9/10, BKVAN followed ATG or OKT3 therapy, for steroid-resistant rejection (6) or as part of a delayed function protocol (3), 6/10 received a tacrolimus-based regimen, 4/10 ciclosporin, and 9/10 received MMF. Following diagnosis, IS was reduced in all patients; no antiviral agents were used. All grafts are functioning at a mean of 15 months following diagnosis of BKVAN (mean sCr 274µmol/l), renal function improving or stabilising in 9/12 and deteriorating in 3/12.

All biopsies showed tubulointerstitial inflammation and active tubular injury, with viral cytopathic changes in 11/12. The diagnosis was confirmed by immunohistochemistry (IH) in all 12 cases. Following diagnosis, IH was performed on previous biopsies from these patients. The day 28 protocol biopsy was negative for viral protein in all patients, but in one patient, diagnosed with BKVAN at 6 months, a 4-month biopsy (originally diagnosed as Banff borderline changes) was positive for BKV.

Quantitative PCR for BKV was performed retrospectively on stored patient serum from the time of transplantation to after the biopsy diagnosis in 5 patients (median 8 measurements/patient). In all 5 patients, PCR was negative in the first month post-transplant, but became positive prior to biopsy diagnosis and viral load fell following reduction in IS.

The recorded incidence of BKVAN is higher in Oxford than elsewhere in the UK and anti-T cell antibody therapy appears to be the strongest risk factor for its development. Our experience indicates that IH for BKV should be performed on all biopsies performed after 2 months post-transplantation in which there is inflammation or tubular damage. Measurement of BKV viral load by PCR is useful for monitoring response to therapy. A good outcome is achievable in most patients without the use of anti-viral agents.

O46

HLA Matching And Outcome Of Living Donor Kidney Transplants In The UK

RJ Johnson, SV Fuggle, RA Hodge, CJ Rudge and JLR Forsythe

On behalf of the UK Transplant Kidney and Pancreas Advisory Group, UK Transplant, Bristol, BS34 8RR, 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 different donor types and the influence of HLA match and other factors on outcome of all living donor transplants in the UK.

Methods: HLA mismatches of 2075 living related and unrelated kidney donor transplants and 10786 cadaveric HBD transplants in 1995-2002 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 analysed.

Results: As expected, transplants from living unrelated donors were significantly less well matched. 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 two HLA-DR mismatched living unrelated donor transplants (90%, 95% CI 85-95%) and 000 mismatched cadaveric HBD transplants (89%, 95% CI 87-91%). Cox regression modelling showed older donors ($p<0.01$) and female recipients ($p<0.03$) to adversely affect 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. 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.

Parallel Session IV(b)

Liver Transplantation

Thursday 29 April

15:00 – 16:00

O47

Auditing The 50% Five Year Survival Criterion For Registering Elective Liver Patients In The UK

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Background: Since November 1999, the criteria for registering patients onto the national liver transplant list have included the requirement that patients should be offered a transplant only if the clinician feels that they have a greater than 50% probability of survival at five years post-transplant. A statistical model to audit registrations made onto the liver transplant list has been developed to assess adherence to this criterion.

Methods: Data were obtained from the National Transplant Database on 3221 adult Group 1 elective liver recipients transplanted in the UK, 1994-2002. First cadaveric heartbeating liver only transplants were considered. The data were divided into three datasets. A *modelling set* comprising 1289 recipients transplanted 1994-1997, *set 1* (pre-introduction of criterion) comprising 776 recipients transplanted 1998-1999 and *set 2* (post-introduction of criterion) comprising 1156 recipients transplanted 2000-2002. A multifactorial Cox model was developed using the *modelling* dataset to identify patient factors that significantly affect five-year liver transplant survival. The model was then fitted separately to *sets 1* and *2*, and patients were allocated into one of four pre-defined groups: 'met the criterion', 'borderline but met the criterion', 'borderline but did not meet the criterion' and 'did not meet the criterion'.

Results: The factors included in the final model were recipient primary liver disease, urea and albumin. For *set 1*, 674 (87%) of 776 individual patients were deemed to have met the criterion, 55 (7%) were borderline but met the criterion, 32 (4%) were borderline but did not meet the criterion and 15 (2%) did not meet the criterion. Of those 15 who did not meet the criterion, 9 (60%) have since experienced graft failure or died within two years post-transplant. An analysis of *set 2* showed similar results.

Conclusions: A model capable of auditing whether transplanted recipients met the 50% five year survival criterion has been developed. This model classifies patient types and individual patients into one of four levels of adherence to the 50% criterion. Only 2% and 1% of recipients transplanted in *sets 1* and *2*, respectively, were classified as not having met the 50% five year survival criterion. The listing of such patients could be questioned, but in general the results suggest that the 50% criterion is being adhered to.

O48

Superior Renal Function Following Conversion To Sirolimus After Liver Transplantation - Preliminary Results From A Randomised Trial

CJE Watson[1], GJ Alexander[1], MED Allison[1], P Gibbs[1], JC Smith[1], CR Palmer[2] and AE Gimson[1]

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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.
GFRs 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.

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 seems to be 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.

O49

Early Stress Protein Gene Expression In A Human Model Of Ischemic Pre-Conditioning Of The Liver

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[1]Tissue Injury and Repair Group, MRC Centre for Inflammation Research, University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG, United Kingdom and [2]Department of Surgery, University Hospital Maastricht, P Debyelaan 25, PO Box 5800, Maastricht, 6202 AZ, Netherlands

BACKGROUND: Intermittent vascular inflow occlusion has been reported to precondition organs resulting in improved outcome following transplantation. This study investigated the early expression of cytoprotective stress proteins during portal ischemia- reperfusion in a model of ischemic pre-conditioning of the human liver.

METHODS: Ethical permission was obtained to perform repeated liver sampling from 6 patients undergoing liver resections for secondary cancers in otherwise normal livers. Samples were taken before and after each event in a two cycle ischemia-reperfusion protocol using 15 minutes of portal clamping followed by 5 minutes of reperfusion. Liver tissue was analysed by fluorescence detection real time PCR for heme oxygenase-1 (HO-1) and heat shock protein 70 (HSP70) mRNA expression. Extracted protein was analyzed by western blot for HO-1, HSP70 and nuclear extracts were analyzed by DNA mobility shift assay for hypoxia inducible factor-1α (HIF-1α) and heat shock factor–1 (HSF-1).

RESULTS: Within minutes of portal clamping significant increases in HO-1 mRNA expression were detected and these increased though the protocol ($P<0.01$). Protein expression of HO-1 was similarly increased between the start and end of ischemia- reperfusion (40 minutes $P<0.03$). Immunohistochemistry demonstrated that early HO-1 expression was confined mainly to Kupffer cells. Binding of active HIF-1α to its consensus sequence was increased within 15 minutes of the start of the ischemia-reperfusion cycle. At the same time point transcriptionally active forms of HSF-1 were detectable, however, no changes were evident in HSP70 mRNA or protein expression in the time frame studied.

CONCLUSIONS: Expression of the cytoprotective protein HO-1 is significantly upregulated within minutes of vascular occlusion in the human liver. This process is transcriptionally regulated probably by HIF1α. Since HO-1 orchestrates antioxidant, cGMP and iNOS mediated effects, augmented expression may have an important role in protection of organs following transplantation. The rapidity of onset of HO-1 expression suggests that intraoperative preconditioning may be feasible.

O50

Long-Term Transplant Survival For Liver Recipients In The UK

KM Barber, CJ Hamilton, D Collett and AD Mayer

on behalf of UK Transplant Liver Advisory Group, UK Transplant, Bristol, BS34 8RR, United Kingdom

Background: The National Liver Transplantation Programme in the UK was established in 1983. The aim of this study was to investigate the long-term transplant survival of these liver transplants.

Methods: Data on elective liver transplants performed in the eight designated liver centres in the UK from 1 January 1985 to 31 December 2002 were obtained from the National Transplant Database (NTxD). Long-term transplant survival estimates were obtained using the Kaplan-Meier method. Transplant survival time was calculated as time from first graft to patient death or graft failure.

Results: Between 1985 and 2002, 6197 first cadaveric heartbeating liver only transplants were performed: 5369 (87%) in adult and 828 (13%) in paediatric patients. Transplant survival estimates and corresponding 95% confidence intervals (CI) were 63% (CI 62-65%) at 5 years, 52% (CI 51-54%) at 10 years, 42% (CI 38-46%) at 15 years and 38% (CI 33-44%) at 18 years post-transplant. The median transplant survival time was 11 years (95% CI 10.2-12.4 years). There was some evidence to suggest better long-term transplant survival for paediatric recipients compared with adult recipients: 56% and 35%, respectively, of transplants still functioning after 18 years, $p=0.06$.

Analysing these data by year of transplant (in three-year groups from 1985 to 2002) showed that transplant survival was significantly different between the groups, $p<0.0001$, with more recent transplants doing better. This was mainly due to a significant improvement in transplant survival in the first year post-transplant for the years analysed. One year transplant survival was 54% (95% CI 48-59%) for transplants in 1985 to 1987 compared with 85% (95% CI 83-87%) for transplants in 2000 to 2002, $p<0.0001$. Analysis of long-term transplant survival of those transplants functioning after one year showed no statistically significant effect of transplant year.

Conclusions: 50% of first cadaveric heartbeating liver only transplants recorded on the NTxD since 1985 were still functioning after 11 years. Long-term transplant survival has significantly improved over the last 18 years. This improvement has been principally due to fewer graft losses in the first post-transplant year. However, there has been no appreciable improvement in transplant survival for those transplants still functioning after one year.

O51

The Use Of Microdialysis For Monitoring The Metabolic Changes That Occur In The Liver At Transplantation

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Background: A microdialysis catheter was inserted into the graft immediately following reperfusion at orthotopic liver transplantation (OLT). Metabolites of the ischemia reperfusion injury and selected amino acids were studied.

Methods: The study included fifteen patients (8 males) undergoing elective OLT with a median age of 52 (38-62) years. The microdialysis catheter was perfused with an isotonic solution for 48hrs. The dialysate was collected at 1 hourly intervals and lactate, pyruvate, glycerol and glucose levels were measured. In addition, concentrations of amino acids alanine (ALA), arginine (ARG), citrulline (CIT), γ-amino-butyric acid (GABA), glutamate (GLU), glutamine (GLN), glycine (GLY) and taurine (TAU) were determined. Routine liver function tests and indocyanin green (ICG) clearance were also done.

Results: Median cold ischemia time (CIT) was 10 hrs 28 min, warm ischemia time (WIT) was 39 minutes. All grafts worked well. 24 and 48hr ICG clearance correlated significantly with post operative transaminase levels. High lactate, pyruvate and glycerol levels were observed in the immediate post operative period. These showed a significant decrease and stabilized to baseline levels within 3-4hrs. A rise in median glucose and pyruvate levels were noted after 40hrs. For ALA, CIT, GLN and GLY, no significant changes were observed. There was a significant decline in the levels to a baseline of TAU, GABA and GLU. In contrast, ARG levels were low immediately post reperfusion and progressively increased reaching significantly higher values from 24hrs onwards.

Conclusions: These data may represent 'normal' changes seen in the immediate post transplant period since all grafts functioned well. The rise in both glucose and pyruvate levels beyond 40 hrs may represent glycogen break down and increased rate of glycolysis. Two most important metabolic fates of ARG in the liver are in the detoxification of ammonia via the urea cycle, and in the synthesis of nitric oxide. Low extracellular ARG may reflect influx of the amino acid into hepatocytes resulting in either formation of supra physiological levels of NO in the presence of inducible NO synthase or conversion to ornithine, in the presence of arginase in the urea cycle. As the organ stabilises, restriction of ARG uptake may give rise to the observed rise in extracellular ARG.

O52

Magnesium Prevents Deterioration In Right Ventricular Diastolic Function During Liver Transplantation

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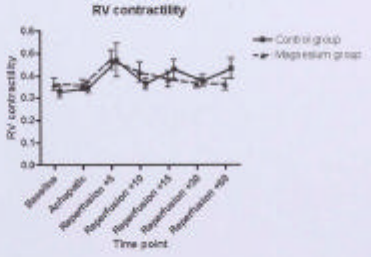
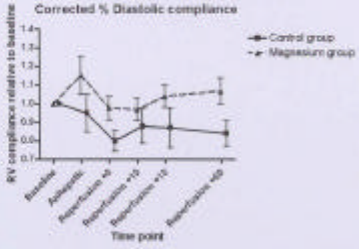
Magnesium administration is associated with preservation of ventricular diastolic function in myocardial ischaemia. Right ventricular dysfunction has been described as a limiting factor at graft reperfusion during orthotopic liver transplantation. Therefore, we have investigated the hypothesis that magnesium supplementation protects right ventricular function at liver graft reperfusion.

Patients and Methods
After ethical approval, 40 patients undergoing elective liver transplantation gave informed consent to a randomised double blind study. All patients received standardised anaesthetic (air-oxygen-isoflurane-alfentanil) and surgical (orthotopic graft with venovenous bypass) management. During the anhepatic phase, group M received 50 mm0l of magnesium sulphate 50% solution by infusion, and group C received saline placebo. Right ventricular function was assessed by right ventricular ejection fracyion catheter, allowing determination of RV volumes and pressures. Pressure volume histories were constructed for baseline, pre-reperfusion, and 5, 10, 15, 30 and 60 minutes post reperfusion, and compared between groups.

Results
There were no demographic differences between groups at baseline. Magnesium administration was associated with vasodilatation and a reduced mean arterial pressure. Group M had a higher RVEF than group C (P=0.02). M and C had identical RV contractility as measured by the ejection systolic point (figure 1). Group C showed a 20% deterioration in diastolic function at graft reperfusion, whereas this was maintained in group M (P=0.03). These changes were sustained with time (figure 2).

Conclusions
Magnesium supplementation maintains RVEF at graft reperfusion, apparently by attenuating the impairment in RV diastolic function. Ther is little effect on systolic function. This could be due to antagonism of calcium influx, altered cellular energetics, a lusitropic or a pseudo-lusitropic effect. Further work is required to establish the clinical significance of these observations.





Parallel Session IV(c)

Alloimmunity

Thursday 29 April

15:00 – 16:00

O53

Chemokine Presentation During Allograft Rejection: The Role Played By Cell-Surface Glycosaminoglycans

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Chemokines bind both to their specific receptors and to glycosaminoglycan (GAG) molecules. In this study we examined the significance of these interactions for the regulation of inflammation during renal allograft rejection.

The expression of CC chemokines was examined in transplant biopsy sections. The chemokines CCL2, 3, 4 and 5 were expressed predominantly within the tubular basement membrane. All four chemokines were elevated after transplantation but only CCL2 and 4 showed further upregulation during rejection. In the normal kidney it was found that heparan sulphate (HS) was largely restricted to the tubular basement membranes, whilst the chondroitin sulphate (CS) species C4S and C6S were expressed at lower levels within the interstitial tissues. The expression of all three GAGs was increased during acute rejection, but HS remained predominant. Co-localisation studies showed that CCL5 was associated with the HS-rich tubular basement membrane; however, the distribution of CCL5 was not uniform, with evidence of focal accumulation.

Similar focal accumulation of chemokines was observed on the apical surface of monolayer cultured endothelial cells. Treatment of endothelial cells (HMEC-1) with IFN-γ and TNF-α increased their capacity to bind exogenous chemokines. Analysis by real-time PCR showed that proinflammatory cytokines increased expression of N deacetylase/N-sulphotransferase-1, leading to an increase in the number of chemokine-binding, N-sulphated HS epitopes on the endothelial cell surface.

These data suggest that alteration of the expression and composition of HS provides a mechanism for increasing chemokine sequestration within allograft tissues leading to efficient presentation to leukocytes during rejection. Modulation of this process might provide a novel target for therapeutic intervention.

O54

Human T Cells Can Become Allogeneic Antigen Presenting Cells And Amplify The Direct Alloresponse

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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 B7 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 various immunosuppressive drugs 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.

O55

Treatment Of CD4+ T Cells With Anti-CD4 In Vitro Induces The Generation Of Cells Capable Of Suppressing Alloreactive Responders In Vitro And In Vivo

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Strategies for the generation of regulatory T cells in vitro have great potential in transplantation. It has been shown that in vivo administration of alloantigen under the cover of an anti-CD4 antibody results in the long-term acceptance of cardiac allografts in mice. This pretreatment induces the generation of a population of cells with suppressive capacity within the CD25+CD4+ population. In this study we have investigated if CD25+CD4+ regulatory T cells can be generated by exposing naïve T cells to donor alloantigen and anti-CD4 in vitro. CD4+ T cells from naïve CBA mice were cultured with irradiated antigen presenting cells from C57Bl/10 mice in the presence of 5µg/ml of 177 YTS anti-CD4 antibody. After 8 days in culture these cells were studied for their regulatory properties.
In vitro assays: Total CD4+ or CD62L+CD25+CD4+ CBA T cells treated with anti-CD4 were cultured with naïve CFSE stained CD4+ T cells from CBA mice at different ratios in the presence of allogeneic antigen presenting cells from C57Bl/10 or BALB/c mice. Suppression was assessed by analysing CFSE profiles, thymidine incorporation and cytokine expression.
In vivo assays: Total CD4+ or CD62L+CD25+CD4+ CBA T cells treated with anti-CD4 were co-injected with CD45RBhighCD4+ cells into CBA Rag-/- mice. One day after reconstitution these animals received an allogeneic skin transplant: C57Bl/10 or BALB/c.
Our results show that after treatment with anti-CD4, CD4+ cells do not proliferate in vitro and are able to block the proliferation of naïve responders. T cells treated with anti-CD4 express low levels of IL2 and when co-cultured with CD4+ naïve cells can block the production of IL2 by the responders. When administered in vivo, T cells treated with anti-CD4 are responsible for the development of tolerance to allogeneic skin transplants. Furthermore, higher suppressive activity was found within the subpopulation of CD62L+CD25+CD4+ cells following treatment with anti-CD4 in vitro.
These data show that in vitro treatment of CD4+ cells with anti-CD4 induces the generation of a population of cells with suppressive properties possibly by selecting high affinity T cells. This is the first study showing the generation of a population of regulatory cells capable of suppressing the proliferation of alloreactive responders in vitro and in vivo.

O56

The Specificities Of A Peptide Dependent Alloreactive CD8+ T Cell Line Can Be Identified Using HLA Class I Tetramers

A M E Whitelegg[1], L E M Oosten[2], S Jordan[1], J A Madrigal[1], E A J Goulmy[2] and L D Barber[1]

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Allogeneic transplants stimulate a vigorous T cell response to foreign HLA molecules. There are two models to account for the magnitude of the alloresponse. Peptide-independent alloresponses involve recognition of polymorphic residues on the surface of foreign HLA molecules and high antigen density accounts for the vigorous response. Alternatively, peptide-specific alloresponses involve recognition of numerous antigenic targets created by the diverse range of peptides presented by allogeneic HLA molecules. We have explored the molecular basis of CD8+ T cell allorecognition using recombinant HLA class I multimeric complexes. Peptide-independent allorecognition was investigated using artificial antigen presenting constructs (aAPC) consisting of latex beads coated with HLA-A*0201 complexed with minor histocompatibility antigen (mHag) derived peptide, and the costimulatory molecules CD54 and CD80. These aAPCs stimulated production of IFN γ by T cells specific for mHag derived peptide presented by A*0201. However, these aAPCs were not recognized by an anti A*0201 alloreactive CD8+ T cell line, whereas it did produce IFN γ after stimulation with cells naturally expressing A*0201. This suggests that a diverse range of A*0201-bound peptides was required. Peptide-specific allorecognition was examined using a panel of fluorochrome-labelled A*0201 tetramers representing self-peptides known to bind endogenously to A*0201. The anti A*0201 alloreactive CD8+ T cell line contained small subsets of T cells that bound each tetramer. A cocktail of the A*0201 tetramers bound anti A*0201 alloreactive CD8+ T cells in numbers that equalled the sum of the individual tetramer subsets suggesting that each tetramer binds a discrete population. The subsets were expanded by stimulating responder T cells with the antigen-processing deficient A*0201 positive cell line T2 loaded with synthetic versions of each self-peptide. Each T cell subset showed precise specificity for the self-peptide. Only the tetramer complexed to the relevant peptide bound each subset and IFN γwas only produced in response to the appropriate stimulating peptide.
Our results provide evidence to support the theory that the vigorous alloreactive T cell response is caused by the summation of numerous responses to each of the peptides bound by allogeneic HLA molecules.

O57

Fas-Ligand Alters The Susceptibility Of The Graft To Rejection

MD Dooldeniya, I Berton, F Morgan and AN Warrens

10s5 Commonwealth Building, Imperial College, Hammersmith, Du Cane Road, Acton, W12 0NN, United Kingdom

BACKGROUND: The Fas/FasL receptor/ligand combination contributes to immune privilege and acts as a homeostatic mechanism within the immune system. In the light of evidence of alteration of the immune response by the expression of FasL, we hypothesised that graft parenchyma uses FasL as a protective mechanism during rejection.

METHODS AND RESULTS: Male FasL deficient and wild type H-2b (C57BL/6) mice were transplanted on to female H-2b recipients. The survival time of the grafts was observed, as well as stained for FasL upregulation. Graft parenchymal expression of FasL was seen to be upregulated in this model of rejection. The upregulation of FasL on the grafts improved the survival time of the grafts (p=0.0003) (figure 1). We next wanted to see if graft FasL would affect the susceptibility of the graft to rejection. To determine this, the recipient mice received a second graft of either wild type or FasL deficient skin. The presence of FasL on the graft parenchyma led to an improvement in survival time (p= 0.0002). Recipient mice were then bled and analyzed for the HY restricted Db (MHC Class I)–specific CD8+ T cell expansion, to see if FasL altered CD8 cell numbers. We saw more T cell expansion in the female mice who had received the FasL-deficient grafts The grafts were also removed at different time points for immunohistochemical and TUNEL staining, again to see if FasL alters infiltrating cell numbers and if this was secondary to the induction of apoptosis. We saw decreased graft infiltrating cells within the wild type compared to the FasL deficient skin grafts.

CONCLUSIONS: Graft expressed FasL is upregulated during the immune response and acts to induce apoptosis in graft infiltrating cells and so protect the graft from rejection.

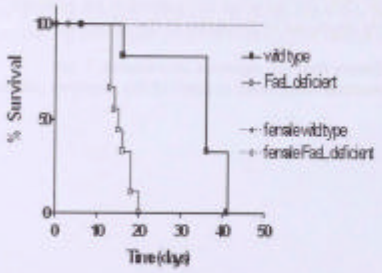

FIGURE 1: Improved graft survial with male wild type grafts during the anti HY response compared with the FasL-deficient grafts.

O58

Effect Of Cognate Interactions Between Human CD4+ T Cells And Endothelial Cells On Chemokine Production

S.S. Tay, A. McCormack and M.L. Rose

Transplant Immunology, Imperial College, Heart Science Centre, Harefield Hospital, Harefield,Middlesex, UB9 6JH, United Kingdom

Interactions between recipient leukocytes and donor endothelium are important in allograft rejection. Cognate recognition of EC by CD4+ T cells leads to T cell proliferation, cytokine secretion and retards transmigration, with less known about chemokine induction. HLA-DR restricted alloreactive CD4+ T cells were cocultured with MHC class II-expressing allogeneic Eahy.926, aortic or heart microvascular EC. Chemokine mRNA was detected by RTPCR. mRNA for CCL2, CCL3, CCL4, CCL5, CXCL8, CXCL10, CXCL11 and XCL1 were detected in noncognate T:EC cocultures. Cognate interaction induced additional expression of CCL7, CCL8 and CXCL9 mRNA, and higher levels of CCL3, CXCL8 and CXCL10 mRNA. In contrast, CCL11, CCL13, CCL19, CCL21, CXCL5 and CXCL12 were not detected. ELISA revealed that up to 10ng/ml of CCL3, CCL8 and CXCL10 were secreted into supernatants as a result of cognate interactions. The supernatants were chemotactic for T cells and monocytes. By titrating T or EC numbers, it was found that CCL8 and CXCL10 were EC-derived whilst CCL3 was T cell-derived. Blocking antibodies to HLA-DR and LFA-3 abrogated production of all three chemokines. Antibodies to ICAM-1, IFNgamma and TNFalpha could inhibit EC-derived CCL8 and CXCL10 production, with less effect on CCL3 production by T cells. Hence, cognate interaction between alloreactive CD4+ T cells and MHC class II-expressing endothelium results in a specific pattern of chemokine production. These interactions are likely to be responsible for directing recruitment of leukocytes into the allograft.

Parallel Session V(c)

Heart/Lung Transplantation

Thursday 29 April

16:50 – 17:20

O59

Development And Validation Of A Statistical Model To Predict 30-Day Mortality Following Heart Transplantation In The UK

JS Ganesh, CA Rogers, NR Banner and RS Bonser

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Objective: Prognostic models are of importance to clinicians, patients, and purchasers of heart transplantation (HTx).

Methods: We used data from a national prospective cohort study of 1173 first-time adult HTx between Jul 95 and Mar 01 to identify pre-operative and operative risk factors associated with death within 30-days of transplantation. The model was validated using a further 168 transplants between Apr 01 and Sept 02. 18 potential risk factors were identified from literature-review or based on clinical opinion. Bootstrapping using 200 samples and backward selection was used to identify factors for inclusion in the multiple logistic regression model. Factors selected in less than 1/3 of samples were omitted and cross-validation was used to assess the predictive ability of those that remained. Factors with poor predictive ability were excluded. Model calibration and discrimination were assessed for the development (D) and validation (V) datasets.

Results: 11 factors were excluded (RECIPIENT: large male, raised PVR, circulatory support prior to transplant, diagnosis, age, gender; DONOR: inotropic support, history of drug abuse, diabetic, gender and size mis-match) and 7 were retained - RECIPIENT peripheral vascular disease (Odds Ratio 3.5 (1.2-10.0)), pre-transplant ventilation (2.6 (1.0-7.1)), diabetes (2.0 (1.1-3.5)), creatinine clearance<50 mls/min at HTx (1.9 (1.2-2.9)), >1 previous open heart operation (1.6 (0.9-2.9)), ORGAN ischaemia time (1.4 (1.1-1.8) per increase in category from <2hrs to 2-3hrs to 3-4 hrs to >4hrs) and DONOR age (1.3 (1.1-1.6) per increase in category from <26yrs to 26-40yrs to 41-55yrs to >55yrs). The model showed good calibration (test for lack of fit: p=0.57 for D and p=0.44 for V), and moderate discrimination (area under ROC curve: 0.67 for D and 0.74 for V).

Conclusion: This model has been used to risk stratify cases in both intra and inter-centre audit and could be used to inform decisions about case selection and also to assist patient consent based on individual risk.

O60

Long-Term Patient Survival For Heart Transplant Recipients In The UK

JC Hussey, ZC Bond, D Collett and J Wallwork

on behalf of UK Transplant Cardiothoracic Advisory Group, UK Transplant, Bristol, BS34 8RR, United Kingdom

Background: The National Heart Transplantation Programme in the UK was established in 1979 and since then nearly 5000 heart transplants have been performed. The aim of this study was to investigate the long-term patient survival of these heart transplant recipients.

Methods: Data on cadaveric and domino heart only transplants performed in the nine designated cardiothoracic centres in the UK from 1 January 1985 to 31 December 2002 were obtained from the National Transplant Database. Multi-organ transplants, urgent transplants and regrafts were excluded from the analyses. Long-term patient survival estimates were obtained using the Kaplan-Meier method. Patient survival time was calculated as time from first graft to patient death with censoring at last follow-up for patients still alive at time of analysis.

Results: Between 1985 and 2002, 4264 cadaveric and domino first heart only transplants were analysed: 3853 (90%) in adult and 411 (10%) in paediatric recipients. Patient survival estimates and corresponding 95% confidence intervals (CI) were 67% (CI 66-68%) at 5 years, 52% (CI 50-54%) at 10 years and 35% (CI 32-38%) at 15 years post-transplant. The median patient survival time was 10.6 years (95% CI 10.1-11.1 years). There was some evidence to suggest better long-term patient survival for paediatric recipients compared with adult recipients: 46% and 34%, respectively, of patients surviving at least 15 years, p=0.12.

Analysing these data by year of transplant (in three-year groups from 1985 to 2002) showed that patient survival was significantly different between the groups, p<0.0001, with recipients of more recent transplants doing better. This was mainly due to a significant improvement in patient survival in the first three months post-transplant over the years analysed. Three month patient survival was 79% (95% CI 75-82%) for recipients of transplants in 1985 to 1987 compared with 86% (95% CI 83-89%) for recipients of transplants in 2000 to 2002, p<0.0001. Analysis of long-term patient survival of those recipients still alive three months post-transplant showed no statistically significant effect of transplant year.

Conclusions: Since 1985, long-term patient survival of heart only transplant recipients has improved in the UK, with 50% of patients surviving at least 10.6 years. This improvement in patient survival has been mainly in the first three months post-transplant.

O61

Initial Experience With Renal Rescue Using Sirolimus And Mycophenolate After Heart Transplantation

H Lyster, G Panicker, P Kaul, A Khaghani and NR Banner

Harefield Hospital, Harefield, UB96jh, United Kingdom

Calcineurin inhibitors (CNIs) have become the cornerstone of immunosuppressive regimens following heart transplantation but their use is associated with chronic nephrotoxicity. Newer immunosuppressive agents may allow CNIs to be eliminated from long term therapy after transplantation. We evaluated a ciclosporin elimination protocol in 14 (13 male) heart transplant recipients with renal impairment. The mean serum creatinine was 321±107μmol/L at a mean of 1166 (30-3880) days. Ciclosporin was discontinued and sirolimus commenced immediately initially aiming for a target trough level of 16 (12-20) ng/ml. Those patients who were receiving mycophenolate continued this medication; those on azathioprine were transferred to mycophenolate at an initial dose of 1g bd. The transfer period was covered with a tapering course of corticosteroids. The patients' clinical status, haematology, biochemistry and sirolimus levels were monitored. Graft function was assessed by echocardiography, ECG and, when indicated, endomyocardial biopsy. Renal function improved in 13 of the patients with 7 having a greater than 40% decrease in serum creatinine (41-76%), while 6 patients had a decrease of 3-27%; 1 patient progressed to need renal dialysis. Two patients who were transferred 414 and 3880 days after transplantation experienced grade 3A rejection following conversion (both after corticosteroids had been withdrawn). One patient experienced a fall in left ventricular ejection fraction (from 50% to 27%) without histological evidence of rejection. Sirolimus was discontinued in 3 patients because of side effects (leucopaenia and thrombocytopenia in 1, presumed lymphocytic pneumonitis in 1 and a generalised acneform rash complicated by an axillary abscess in 1). Seven patients continue on sirolimus (6 of these are receiving mycophenolate and 2 prednisolone). In conclusion withdrawal of ciclosporin after heart transplantation resulted in an improvement in renal function in most patients with 50% of the patients experiencing a substantial improvement. Non-calcineurin inhibitor immunosuppression protocols need to be refined to reduce the risk of breakthrough rejection and to minimise side effects whilst protecting renal function after heart transplantation.

Parallel Session II(b)

Basic Science Symposium

Friday 30 April

12:00 – 13:00

O62

Transplantation Of Metanephroi To The Abdominal Cavity Of Outbred Adult Rats Results In Growth And Histologically Normal Development Followed By Immunolgical Rejection And Transplant Destruction

M.J. Clancy[1], M. Bottomley[2], D.P. Marshall[2], C.A. Bravery[2] and P.E.C. Brenchley[1]

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End-stage renal failure is a growing, worldwide problem. One potential approach is transplantation of foetal kidney rudiments. We have demonstrated successful transplantation of metanephroi to the abdominal cavity of inbred rats. We have further demonstrated glomerular filtration and urine production following microsurgical connection of transplant ureters to the recipient ureter. This study attempted to replicate previously-reported, similar findings in both outbred and congenic, MHC-mismatched rats in the absence of immunosuppression. (Rogers et.al. K.I. vol 54 (1998) p27-37)

We transplanted 96 E14-E15.5 metanephroi into the abdominal cavity of 32 CD rats. Transplants exhibited a growth success rate of 63.5% and grew to a mean mass of 93.7mg(+/-90mg) and mean length of 6.37mm(+/- 1.9mm). This represents growth of more than 1000 fold of the transplanted structure. In contrast to our findings in inbred rats, no transplant exhibited cystic dilatation of the metanephric ureter consistent with urine production nor progression to hydronephrosis if not connected.

On histological examination after 17-21 days, tubules were identified in 77% of the transplants and glomeruli in 29%. All transplants examined exhibited a lymphocyte infiltrate. Damaged tissue architecture consistent with acute rejection was present to a variable extent. 46% of transplants exhibited histologically severe rejection with fibrosis. Immunostaining confirmed high levels of infiltrating αβ T cells with lesser levels of γδ T cells and macrophages.

In rats sacrificed as early as day 7 following transplant, all developing renal structures are present including blood vessels, tubules, mature glomeruli and immature, cortical glomeruli. There is a lymphocyte infiltrate without tissue damage. We interpret these results as normal early transplant development followed by lymphocyte infiltration and progressive tissue destruction.

These findings are in keeping with previous experiments on the transplantation of embryonic tissues but distinct from previous reports of abdominal metanephros transplantation in outbred or non-histocompatible rat strains. These findings suggest that a permissive immunosuppressive regime will be necessary for successful metanephros transplantation between non-identical individuals.

O63

Detection Of Vimentin Specific Autoreactive CD8+ T Cells In Cardiac Transplant Patients

L.D. Barber[1], A. Whitelegg[1], J.A. Madrigal[1], N.R. Banner[2] and M.L. Rose[3]

[1]The Anthony Nolan Research Institute, Royal Free Hospital, Pond Street, Hampstead, London, NW3 2QG, United Kingdom, [2]Royal Brompton and Harefield NHS Trust, Harefield Hospital, Harefield Middlesex, UB9 6JT, United Kingdom and [3]Imperial College School of Medicine, Heart Sciences Centre, Harefield Hospital, Harefield Middlesex, UB9 6JT, United Kingdom

Background: Evidence is emerging that autoimmunity can play a role in allograft rejection. Reports have described presence of autoantibodies in transplant patients and CD4+ autoreactive T cells in rodent models of allograft rejection. We have previously reported presence of autoreactive antibodies to the non-polymorphic cytoskeletal protein vimentin in cardiac transplant patients. The objective of the present study was to seek evidence of CD8+ T cell-mediated autoimmunity to vimentin in these patients.

Methods: Two peptide sequences from vimentin that bound HLA-A*0201 were identified and fluorochrome-labelled A*0201 tetramers with each peptide constructed to screen for vimentin-specific T cells. Peripheral blood lymphocytes were collected from six A*0201 positive heart transplant patients. The patients were 3-6 years post transplant and all had high titres of autoreactive anti-vimentin antibodies.

Results: Tetramer-binding CD8+ T cells were detected in peripheral blood lymphocytes from two of the six patients after expansion by in vitro stimulation with peptide. One patient possessed T cells specific for vimentin peptide residues 226-234 and a second patient had responses to both this peptide and peptide 79-88. The tetramer-binding T cells produced interferon γ in an antigen-specific fashion. No vimentin tetramer binding T cells were detected following peptide stimulation of peripheral blood lymphocytes from eight healthy A*0201 positive volunteers.

Conclusions: This finding is the first evidence of CD8+ T cell-mediated autoimmunity in human transplant patients.

O64

Pre-Transplant sCD30 Levels And HLA-Antibody Status As Indicators Of Cellular And Vascular Rejection

N Jivanji[1], A Gupta[1], R Rajakaria[2], M Varagunam[1], P Sinnott[1] and M Yakoob[2]

[1]Tissue Typing Laboratory, 2nd Floor Medical School, Royal London Hospital, London, E1 1BB, United Kingdom and [2]Department of Renal Medicine and Transplantation, Royal London Hospital, London, E1 1BB, United Kingdom

CD30 is one of the members of the tumour necrosis factor superfamily, originally described as a marker of Reed-Steinberg and Hodgkin's cells in Hodgkin's lymphoma. CD30 is released and preferentially expressed by the CD4+ and CD8+ T cell clones capable of producing T Helper 2 (TH2)-type cytokines. Elevated levels of sCD30 in diseases where TH2 type immune responses predominate are shown to correlate with raised antibody production and increased disease activity, e.g. systemic lupus erythromatosus and atopic dermatitis. A recent study has shown that elevated pre-transplant serum sCD30 levels are indicative of an increased risk in graft loss in renal transplant patients and hence predictors of acute and chronic rejection. In our studies, the sCD30 levels in three patient groups were as follows: Cellular rejection group (50 patients) mean CD30 level was 119.3 U/ml (SE 8.8). Vascular rejection group (17 patients) mean CD30 level was 260.6 U/ml (SE 37.6) No rejection group (41) mean sCD30 was 180.5 U/ml (SE 10.5)

sCD30 levels were found to be significantly elevated in the vascular rejection group compared to the cellular rejection group ($p<0.001$). Increased levels and may be correlated with a TH2 cytokine mediated humoral immune response, whereas in the cellular rejection group (TH1 response) there were significantly lower levels of sCD30 compared to the no-rejection group ($p<0.01$).

No significant difference between the vascular and cellular rejection groups was seen when we compared the presence or otherwise of pre-transplant HLA-antibodies ($p = 0.094$): However, we noted that where antibodies are present, the rejection 'type' is more likely to be cellular. No significant difference was observed for the presence or absence of antibodies between the rejection and no-rejection groups ($p = 0.322$) suggesting that pre transplant antibody status alone is not indicative of graft outcome in this study.

O65

Depletion Of CD25+ T Cells Or Ligation Of GITR Reverses The Induction Of Operational Tolerance

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Nuffield Department of Surgery, University of Oxford, Headington, Oxford, OX3 9DU, United Kingdom

Background and Aims In animal models T cell regulation may be a common feature of tolerant recipients irrespective of the type of induction therapy. Regulatory T cells that arise as a consequence of engraftment almost certainly require encounter with alloantigen, in most cases in the form of the graft itself but this may lead to irreversible graft damage before regulation develops. We have taken the alternative approach of generating CD25+ T-regs *prior* to transplant by antigen challenge combined with anti-CD4 antibody thus affording the graft protection from the outset. These CD25+ cells prevent skin graft rejection in a sensitive adoptive transfer model but the aim of this study was to ask if they play any role in the function or survival of primary cardiac allografts by depleting CD25+ T cells or ligating GITR.

Results H-2^k mice received anti-CD4 antibody on days –28 and –27, H-2^b donor-specific transfusions (DST) on day –27 and H-2^b cardiac allografts on day 0. This leads to long-term allograft survival (MST >100 days) and tolerance. Additional groups also received the depleting anti-CD25 antibody *PC61* on either day -7 or -1, or the GITR-ligating antibody *DTA-1* on days -1, 0, +3, +6. 36% of mice given PC61 at day -1 rejected their grafts (MST 56 days) and although the remainder survived beyond 100 days all had severely compromised function and extensive vasculopathy (mean arterial occlusion, 40%). Similar results were seen in the day
-7 group. In mice given anti-GITR only 1/6 rejected its graft acutely but the remainder had barely detectable graft function after 100 days and severe vasculopathy (mean arterial occlusion, 57%).

Context and Significance These data show firstly that CD25+ T-regs generated by pretreatment with donor alloantigen under anti-CD4 cover play an important role in primary allograft recipients and that their ability to regulate is not confined to adoptive transfer models. Secondly, the fact that signalling through GITR abrogates long-term graft function shows that like their dependence on both CTLA-4 and IL-10, regulatory T cells generated by alloantigen exposure share common mechanisms with naturally occurring T-regs. Thus, observations made in non-transplant settings (for example autoimmunity) may have direct relevance to the regulation of transplant rejection. Such parallels may guide the design of tolerance induction protocols, identify mechanisms of action or suggest assays which measure T-reg function in vitro.

O66

Complement Activation On Epithelial Cell Surface Regulates The Capacity Of Epithelial Cells To Stimulate Alloreactive T Cells

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Previous studies have shown that complement regulates the adaptive arm of the immune response, in both B cell and T cell functions. However, little is known whether the complement is also able to regulate alloimmune response. In this study we examine the effect of complement on the capacity of proximal tubular epithelial cells (PTEC), which may function as antigen presenting cells, to stimulate primed alloreactive T cells. In particular, we examine the specific components in the complement cascade which mediate these effects. In vitro studies utilised complement-treated mouse PTEC to stimulate primed alloreactive T cells, whose proliferation and cytokine production were examined. The PTEC were derived by primary cell culture from the kidneys of C57BL/6 mice. Primed T cells were from the spleens of BALB/c mice that had received a C57BL/6 skin graft 14 days previously. PTEC were co-cultured with T cells after treatment with different mouse sera (normal, heat inactivated, C3-, C5- or C6-deficient), and the T cell proliferation and cytokine production were assessed over 96 h. In this study, we provide evidence that primary cultures of murine PTEC have the capacity to stimulate alloreactive T cells. We also show that complement activation and subsequent deposition of split complement fragments on the PTEC enhances the alloreactive T cell response. Furthermore, we dissected the level of the complement cascade (C3/C5/C6) involved and found that complement exerts its effects to enhance the alloreactive T cell response through the binding of the early complement component C3 to the PTEC, and does not involve the terminal pathway components.

O67

The Roles Of Donor Leukocytes And Soluble MHC Class I In Transplantation Tolerance

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Induction of tolerance without immunosuppression occurs spontaneously in the DA to PVG rat model following liver transplantation, while heart grafts are acutely rejected in 8 days. The liver, unlike the heart allograft, is a source of both secreted soluble MHC molecules and resident leukocytes. This study has quantified the levels of soluble MHC following liver transplantation. We have further investigated the effect of both soluble MHC and donor leukocytes on the survival of DA cardiac allografts in PVG recipients. We have used continuous perfusion of sol MHC at monitored concentrations and given donor leukocytes intravenously, thus mimicking the phenomenon seen after liver grafting.
Recombinant class I MHC heavy chains and β2m were produced using a prokaryotic expression system, refolded around single peptides and purified by HPLC. These recombinant molecules were used as standards in allotype specific ELISA assays to permit the quantification of sol MHC levels in the sera of rats. Following liver allografting, sol donor MHC levels (RT1Aa) rise rapidly, peaking around 400ng/ml before returning to a plateau of ~200ng/ml two weeks after the transplant. With perfusion of sol Aa alone, prolongation of cardiac allografts was not demonstrated, as determined by the duration of palpable beating (median survival time, mst 8d). This was despite achieving sera levels as high as 2000ng/ml. Administration, at the time of transplantation, of donor leukocytes, either lymph node spleen cells, also failed to give tolerance (mst 6.5d). However, a combination of both donor cells and donor sol MHC class I (sera levels >1000ng/ml), did influence cardiac allograft survival, with hearts continuing to beat for up to 13 days (mst 12d). This effect was donor MHC specific since it was not observed unless both the cells and the sol MHC were of donor haplotype. An effect of the sol MHC alone was observed when infusion commenced prior to the cardiac transplant (mst 12d). Thus, despite presenting a single peptide in the groove, sol MHC produced using a prokaryotic expression system could modulate allograft rejection. We are investigating further the role of MHC-bound peptide in blocking an allo-response and in allo-tolerance. Eukaryotically-derived class I, bearing heterogenous peptides in the groove and glycosylation, is currently being prepared to assist with these studies.

Poster Session I

Wednesday 28 April

P1

Low Dose Mycophenolate Mofetil Is A Safe, Effective And Well-Tolerated Way To Reduce Exposure To Calcineurin Inhibitors In Chronic Allograft Nephropathy

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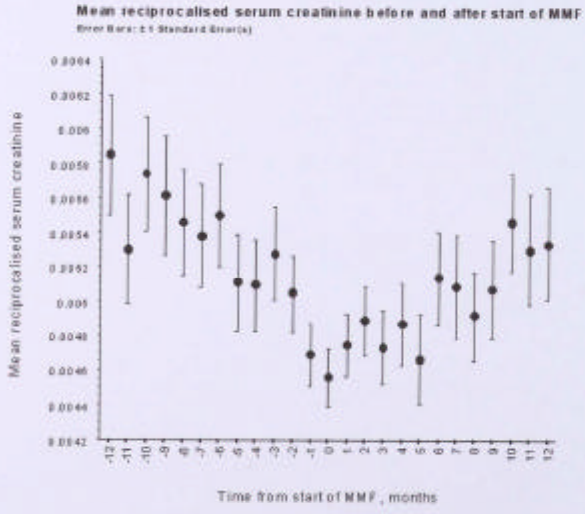
With the success of strategies to combat acute rejection, increasingly attention has turned to therapies for chronic rejection and chronic allograft nephropathy-graft fibrosis (CAN). It is known that calcineurin inhibitors (CNI) can accelerate allograft fibrosis in experimental and clinical situations. It is now commonplace to use mycophenolate mofetil (MMF) or sirolimus to facilitate reduction or withdrawal of CNIs in chronic "switch" patients, but significant side-effects have been noted.

We embarked on a strategy of gradual CNI reduction under the cover of low-dose (~ 1 gram / day) MMF. We present the findings of an intention to treat analysis of 89 patients selected by virtue of declining allograft function, and a biopsy showing CAN (56 patients). We used the slope of the reciprocated plasma creatinine values for 12 months prior to, and after the start of MMF therapy, to evaluate response. Side-effects and discontinuations were noted.

We found that at 6 months 89% of patients were still on MMF, and at 12 months this was 79%. Only 5 patients ceased MMF due to side-effects (diarrhoea, leucopoenia, infection), while 10 ceased due to death (2) and return to dialysis (8). GFR at -12 months was 47.1 mls/min, at t=0 37.2 mls/min and at +12 months was 41.6 mls/min ($p < 0.05$). See Figure 1 for reciprocalised creatinine values with time. There were 4 episodes of acute cellular and two of acute vascular rejection. BP did not fall significantly.

We conclude that the use of low dose MMF is a safe, effective and well-tolerated means of reducing CNI to improve outlook in CAN



P2

C2 Monitoring Is Of No Value In Stable Renal Transplant Patients

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C2 monitoring has been advocated in the early period after renal transplantation, but few data exist regarding its value in long-term, stable patients. Since C2 levels are said to be a more accurate assessment of cyclosporin exposure, measurement in stable patients might allow dose reduction to reduce the incidence of calcineurin-inhibitor related chronic allograft nephropathy.

The aim of this study was to determine whether routine measurement of C2 levels in stable patients would offer any advantage over trough levels.

Patients who had undergone a renal transplant more than twelve months previously with serum creatinine levels which had varied by less than 20% over the last two clinic visits were included. Those who had undergone recent changes in immunotherapy were excluded.

Target trough cyclosporin levels were 100-200ng/ml, and target C2 levels were 800ng/ml.

26 patients were included in the study. Mean time after transplant was 9.6 years (s.e. 1.3). All but 2 patients received a cadaveric kidney. Mean serum creatinine was 132 umol/l (range 81-285).

Two patients had a high C0 level (250 and 209 ng/ml) and C2 levels reflected this (984 and 840 ng/ml). Only 3 other patients had a high C2 level with normal C0 levels (942 v 156, 1020 v 156 and 810 v 135 ng/ml). Serum creatinine in these patients was 169, 95 and 125umol/l respectively.

C2 measurement in stable patients does not have a significant advantage over conventional C0 monitoring, particularly given the additional demand this would make on patients, staff and facilities.

P3

Early Withdrawal Of Calcineurin Inhibitors (Cnis) At 6 Months Post Kidney Transplant In The Presence Of Mycophenolate Mofetil (MMF): Safety And Efficacy.

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Recently, a number of immunosuppressive regimens have been suggested with the aim of avoiding or minimising CNIs. We have reported that withdrawal of CNIs in patients with chronic allograft nephropathy in the presence of MMF is not only safe but it improves long-term kidney allograft function.

The aim of this study was to evaluate the safety and efficacy of early withdrawal of CNIs, at 6 months post kidney transplant using MMF as a maintenance therapy. The primary objective was incidences of biopsy proven acute rejection (AR). Composite secondary objectives included delta creatinine 3-6 months and 9-12 months, graft or patient loss, incidence of infection and changes in cardiovascular risk factors.

Patients (n=15) having received first or second transplants and were maintained on a CNIs based therapy, with no history of irreversible acute or vascular rejection were included in the study. MMF was commenced at 6 months post-transplant and CNIs was withdrawn over a period of 3 months.

Two patients (13%) had vascular rejection, one during and one at end of the withdrawal period. Both were reversible, but required switch of immunosuppression. There was no graft or patient loss after a mean follow-up period of 6 months post withdrawal. There was no significant difference (p=0.06) in mean delta creatinine between 3-6 months (+9.9) and 9-12 months (-7.6).

Infection was documented in 5 patients (38%), 2 patients had CMV , 2 had one or more UTI's and one had a chest infection. Withdrawal of CNIs did not confer any improvement in cardiovascular risk factors such as blood pressure and serum cholesterol. One patient (cyclosporin based) developed glucose intolerance and required an oral hypoglycaemic, following withdrawal no further treatment was required.

In conclusion, withdrawal of CNIs, at 6 month following kidney transplant is associated with increased risk of rejection but overall improvement in renal function was observed. The increased risk of acute rejection may be reduced further by defining patients who are at high risk (second graft and patients with prolonged delayed graft function). Further follow up is needed to establish the long term results of calcineurin sparing regimen.

P4

Evaluation Of Cyclosporine Peak Level (C2) Target Range In Stable Paediatric Liver Transplant Recipients.

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Introduction: Traditionally Cyclosporine (Neoral) dose monitoring has been based on trough levels, despite poor correlation with overall drug exposure (AUC). Recent adult studies have suggested that the 2-hour cyclosporine level (C2) is a surrogate for AUC (0-4 hours.

Aim: To establish an optimal therapeutic range for C2 levels in stable children post liver transplantation.

Methods: 41 (23M:18F) children, median (range) age at transplant 17.9 months, (0.5-117) and median (range) [table nn here]time post transplant 99 months (32-196) were enrolled. All were stable with normal graft function. Paired sample monthly for C0 and C2 measurements in whole blood (Abbott axsym analyzer, FPIA technique) were taken on two occasions. Statistical analysis: minimum, median, maximum and quartile.

Results: Median (range) initial trough level (n=41) 61 mg/L (25-132) and subsequent visit (n=34) 62.5 mg/L (25-192). Comparison of peak and trough levels are shown in Table 1 [table nn here]

1 child developed abnormal transaminases and was found to have chronic hepatitis on liver biopsy and was treated with steroids. 3 patients required adjusting Cyclosporine dosage to maintain trough levels in the identified range (range 65-90 mg/L as per protocol).

Summary: There was good correlation between the trough levels taken on 2 occasions in the stable paediatric post liver transplant group of patients. The range of C2 peak levels were also similar suggesting good bioavailability.

Conclusion: This preliminary study on long term post transplant recipients suggests that a peak C2 level is within the range of 230-470 mg/L. This needs further prospective validation for routine therapeutic drug monitoring in this patient group

	1st visit Trough (n=41)	2nd visit Trough (n=34)	1st visit Peak (n=41)	2nd visit Peak (n=34)
Minimum (µg/L)	25	25	121	126
Lower quartile (µ	39	45	236	241
Median (µg/L)	61	62.5	310	350
Upper quartile (µ	80	92	434	489
Maximum (µg/L)	132	192	706	800

P5

C2 Monitoring In The UK: Hitting The Target

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Neoral dose adjustment in response to C2 serum levels reduces the incidence of acute rejection (AR) in de novo renal transplant recipients receiving MMF and steroids. We report our experience of introducing C2 monitoring in patients receiving azathioprine, steroids, and basiliximab when considered at high immunological risk.

Between Nov 2002 and Oct 2003, 108 consecutive patients were C2-monitored; these fall into three chronological groups reflecting a changing protocol driven by sequential audits of efficacy and toxicity.

Group 1: 11 patients; 368 days median follow-up (range 8-389); Neoral induction at 4mg/kg bd and target serum levels of 1300-1700ng/ml at day 3 in accordance with published protocols. No patients achieved target levels on time and subsequent overshooting of targets occurred. There was one graft failure due to AR and no deaths. The incidence of AR was 33%

Group 2: 24 patients; 318days median follow-up (range 2-353); Neoral induction at 7 mg/kg/bd, target levels, as per Group 1, were achieved in 21%. 84% of patients subsequently overshot levels and significant cyclosporin toxicity, particularly heptotoxicity, was seen. The mean Group 2 billirubin was 51 (±43) at day 7. One graft was lost due to AR and there were 4 deaths. The incidence AR was 12.5%

Group 3: 73 patients; 186 days median follow up (31-296); induction at 4mg/kg/bd, target levels reduced to 1100-1400ng/ml at day 5, which were achieved in 41%. There was little evidence of toxicity. Three grafts were lost, two due to primary non-function and one from CMV. There were two deaths. The incidence of AR was 14.5%. The group 3 protocol has been adopted as unit standard practice.

Acute rejection: In 65% of cases of AR, vascular rejection was present. The acute rejection rate in group 3 (14.5%) was significantly lower than in a historical pre-C2 control group (AR = rate of 37% (p=0.029)).
Three month creatinine (µmol/l), mean (±) values were: Group 1, 224 (±191.8); Group 2, 166 (± 61.6); Group 3, 195 (±149.5).

The introduction of C2 monitoring of Neoral presents challenges. In particular, recommended target levels and dose changing protocols lead to reduced efficacy and increased toxicity. By contrast, target level reduction and less aggressive dose alteration minimised toxicity and reduced acute rejection to very acceptable levels. A prospective evaluation of C2-monitoring, including its impact on renal function, is still required.

P6

Intracellular Cytokine Response To Immunosuppressive Agents In Patients With End Stage Renal Failure On Haemodialysis Awaiting Transplantation

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Introduction:Immunosuppressive drugs used in transplantation may polarize the T-cell response by altering the balance of cytokine expression.
The aim of this study was to investigate the intracellular cytokine response to cyclosporine A (CsA), sirolimus (Sir), tacrolimus (Tac) and mycophenolate mofetil (MMF) in 15 haemodialysis patients awaiting transplantation. Each patient was given a single immunosuppressive drug sequentially for two weeks at a standard dose used for renal transplantation, followed by a two-week washout period before the introduction of the next immunosuppressive drug.
Methods:T-cells in peripheral blood were phenotyped and analysed for the ability to produce the Th1 cytokines IL-2, IFN-gamma, TNF-alpha, the Th2 cytokines IL-4, IL-10 and the Th3 cytokine TGF-beta before, during and after the administration of each drug. The percentage of CD3+ cells producing intra-cytoplasmic cytokines were determined by flow cytometry analysis before and after 6 hours mitogen stimulation (PMA + ionomycin).
Results:Changes in the percentage of cytokine producing cells before (non) and after (st) mitogen stimulation are compared to baseline (wt – without drug). After performing analysis of variance we observed that Tac and Sir differ in their effect on mitogen stimulated TGFb (2.59±0.59; vs. 15.51±11.13; p=0.04) and spontaneous TNFa (0.57±0.07; 1.49±0.60; p=0.02) production. Spontaneous IL-10 production was significantly higher in patients treated with CsA (2.17±0.40) when compared to Tac (1.19±0.21; p= 0.02), Rap (1.29±0.33; p=0.47) and MMF (0.79±0.34; p=0.003). TNFa production on MMF (23.12± 3.13; p=0.0002) was higher compared to CsA (7.95±1.39; p=0.0002) and on Tac (14.18±1.94 ; p=0.021).
Conclusions:These results suggest that immunosuppressive drugs differ in their effect on T-cell cytokine production and may differentially polarise the immune response. This might contribute to understanding of side effects such as nephrotoxicity, which in the long term is partly related to the up-regulation of IL-10. Of interest, although there is evidence that sirolimus reduces fibrosis, it was associated with increased production of TGF-beta – this observation requires further investigation.

P7

Ethnic Variations In Polymorphisms Within Genes Involved In Response To Immunosuppressive Agents

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

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

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

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

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

P8

Underestimation Of The Risk And Severity Of Anaemia Associated With Mycophenolate Mofetil (MMF) When Introduced Late In Renal Transplantation

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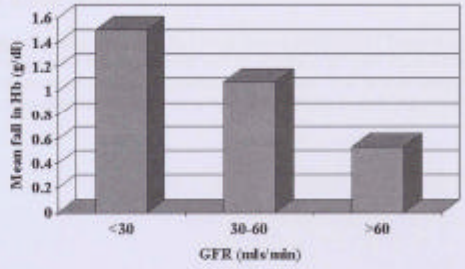
Anaemia is a recognised side-effect of mycophenolate mofetil when used de novo in renal transplantation. However, there is little information on the incidence, severity and risk of developing anaemia when MMF is introduced later in renal transplantation either to increase overall immunosuppression or to spare/withdraw calcineurin inhibitors. We have performed a retrospective study to examine the incidence, severity, time course and risk factors for the development of anaemia when MMF is added late in transplantation.

80 patients (65% male, mean age 45.2 yrs) who were started on MMF at least 3 months after transplantation and who remained on this agent for at least 1 week were identified. At the time of starting MMF, 50 patients were taking CsA, 25 patients were taking tacrolimus and 5 patients were on no CnI. The mean decrease in haemoglobin (Hb) within 3 months of starting MMF was 1.13 g/dl when compared with Hb at start of MMF ($p<0.001$). 69.7% of patients starting MMF experienced a fall of Hb by at least 0.5 g/dl. In 50% of patients the fall was by at least 1 g/dl. A fall in Hb by more than 3g/dl was observed in 8.7% of patients. 7 patients were commenced on erythropoietin therapy within 3 months of starting MMF. GFR (Cockcroft and Gault formula) at the time of starting MMF was the best predictive factor for fall in haemoglobin (Figure 1). Haemoglobin fell by at least 1 g/dl in 65.2% of patients who started MMF with a GFR of less than 30 mls/min and in 42.5% of patients whose GFR was 30-59 mls/min. The incidence and severity of fall in Hb when MMF is used late in transplantation should not be underestimated.

Mean fall in Hb and graft function at time of starting MMF



P9

A Single Centre Audit Of The Side-Effect Profile Of Sirolimus In Renal Transplant Recipients

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Sirolimus (a fermentation product of Streptomyces hygroscopicus) is a novel immunosuppressive agent which has excited interest in the fields of organ transplantation, interventional cardiology, and oncology. In the setting of organ transplantation sirolimus has been used acutely to prevent rejection, and also in the chronic setting to facilitate removal of calcineurin inhibitors (CNI).

At our unit between 2000 and 2003 we have used sirolimus in 79 patients. Of these 6 died, and 12 were transferred out. We present a detailed side-effects audit of 61 patients who were exposed to sirolimus and remained under active follow-up. There were 37 men, 24 women, mean age 45 years (range 20 - 69). The range of exposure was 7-1010 (mean 431) days, and we report a total of 843.5 patient months. 19 patients started de novo, 31 for chronic allograft nephropathy, and the others for diverse reasons. Of the 61 patients, 44 remain on sirolimus, but 17 discontinued - 6 due to return to dialysis, 3 patients were stopped pre-elective surgery, and 8 due to severe side effects (including pneumonitis (3), HUS (1), mouth ulcers (1), oedema (1)), rash (1)). 40 out of 61 patients experienced mild-moderate side effects. Overall, the five commonest side-effects were dyslipidaemia 27 patients), oedema (16), thrombocytopoenia (14), lymphocoele (12) and skin reactions (10). Only dyslipidaemia was more common and more severe with elevated sirolimus levels (> 10) compared to low levels (< 10). Statin use increased from 23% pre-sirolimus to 63% after sirolimus was started. Side effects were no more common in "early" than "late - switch" patients. Most side-effects were reversible if the drug was discontinued (eg pneumonitis in all 3 cases).

We conclude that sirolimus has a wide side-effect profile, and that with steroids / CNIs, dyslipidaemia requiring statins is the norm. The lipid elevating effects of sirolimus seem to be related to blood levels. Cosmetic problems (oedema, skin reactions) were common, as were early post transplant lymphocoeles, and mouth ulcers.

It remains to be seen how tolerable sirolimus will be in the long-term, and whether its use will change as more information accrues about the correct blood level targets.

P10

Late Steroid Withdrawal For Renal Transplant Recipients On Tacrolimus And MMF Is Safe And Not Associated With Rejection

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Background: Following successful experience with a short-course (7 day) steroid avoidance regime for renal transplantation under combined Tacrolimus and Mycophenolate Mofetil (MMF) immunosuppression, we have undertaken a cohort study to assess the safety of staged, late steroid withdrawal in kidney or kidney/pancreas transplant recipients on steroids, Tacrolimus, and MMF.

Methods/Patients: We studied 50 patients. Inclusion criteria specified stable kidney or kidney/pancreas transplant recipients with no rejection in the previous six months, no previous history of vascular rejection, and no history of disease requiring steroid treatment at the time of transplantation. Mean time post-transplantation was 5.1 years (range 2.1-7.9 years) The group comprised 33 cadaveric renal transplant recipients, 8 live donor kidney recipients, and 9 kidney/pancreas recipients.

All patients were on prednisolone (mean dose 8.3mg, range 5-10mg) Tacrolimus (mean trough level at the time of study entry 8.8ng/ml) and MMF (mean daily dose at time of study entry 1.46gm). Steroids were withdrawn over 5-6 months by 20% monthly dose reduction until cessation. The rate of steroid reduction was reduced in the face of typical steroid withdrawal symptoms (limb-girdle arthralgia/myalgia). Patients were reviewed monthly until 4 months after steroid withdrawal.

Results: No rejection episodes occurred during steroid withdrawal and no patients have required transplant biopsy for graft dysfunction. 6 patients failed steroid withdrawal (5 due to arthralgia/myalgia and 1 due to recurrence of pulmonary sarcoidosis which had been quiescent off corticosteroids at the time of transplantation). The unexplained rise in serum creatinine following steroid withdrawal described in several other steroid withdrawal studies was not observed in this patient cohort (mean serum creatinine 137 mmol/L with Δcreatinine –6.8 mmol/year in year prior to steroid cessation: 138 mmol/L with Δcreatinine –5.9 mmol/year in year post steroid cessation).

Conclusions: Careful steroid withdrawal from a platform of Tacrolimus and MMF is safe and is not associated with the significant risk of rejection or graft dysfunction seen in steroid withdrawal from other immunosuppressive regimes. The rate of failure of steroid withdrawal due to musculo-skeletal symptoms is however similar to that reported from other studies.

P11

The Successful Re-Use of a Transplanted Kidney - A First for the UK

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In the presence of the continuing gap between the supply and demand in organ transplantation, utilisation of donors with extended criteria must be maximised.

A 46 year old man who had been on the waiting list for 20 months, received a well matched cadaveric transplant. Within 12 hours of the surgery, the recipient developed a large ischaemic stroke and was intubated, ventilated and transferred to Intensive Care. The transplanted kidney functioned immediately. On the third post transplant day, his death was confirmed using brain stem death criteria. His family were approached by the transplant team and were keen to agree to organ donation.

Liver donation and subsequent transplantation occurred uneventfully. Cardiopulmonary donation was not considered due to the recipient's history of hypertension and left ventricular hypertrophy. Tissues were not suitable for donation as per current Microbiological Safety of Blood and Tissue for Transplantation guidelines.

There was debate in our centre regarding the ethical, surgical and immunological suitability of re-transplanting the kidney. After much discussion, the kidney was retrieved and offered via UK Transplant. Seven UK centres rejected the offer, citing immunological reasons, but the eighth centre accepted. The kidney was transplanted into an elderly recipient (who was well matched against both the original donor and our recipient), and although it's function was delayed initially, the gentleman was discharged home on day ten. 23 months later, the recipient still has normal graft function. This was the first report of the re-use of a previously transplanted kidney in the UK.

This isolated case illustrates the importance of identifying and procuring organs from all donors, even though at first they may seem inappropriate. The quality and continuity of care given to the recipient / donor family, by the transplant team, may also have contributed to the positive outcome in this case.

P12

Renal Transplantation After Cardiac Or Liver Transplantation: Excellent Long Term Function Of Both Grafts Can Be Anticipated

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Renal transplantation after the transplantation of another organ remains relatively rare and outcomes sparsely reported.

Aim:
We have retrospectively reviewed the outcomes of patients who have received a renal transplant after either cardiac or liver graft.

Methods:
Patients receiving a renal transplant after a liver or heart graft during the period 1993 -2003 were identified retrospectively from unit and UKT records. Follow-up data was obtained from individual patient's notes and hospital databases.

Results:
12 patients underwent the transplantation of a kidney at varying times after having received either a heart or liver graft. Mean age was 48 years (range 37-56). There were 10 males and 2 females.

The cause of renal failure causes were: cyclosporine toxicity 8, renal artery stenosis 1, hyperoxaluria 1, polycystic disease liver and kidney disease 1, Wilson's disease

Five patients had previously undergone a liver transplant, 3 of which occurred in excess of 1 year prior to the renal transplant (mean time 5 years). 2 received kidneys immediately after liver transplants using organs from the same donor.

7 received kidneys after a heart transplant, 6 of which occurred in excess of 1 year prior to the renal transplant (mean time 9 years). 1 patient received a kidney immediately after heart transplants using organs from the same donor.

The mean cold ischemia time for kidneys transplanted immediately after other organs was less than the mean CIT for the unit overall. Patients with working transplant were independent of dialysis within two weeks.

All remain alive at 1 year. 11 still have initial cardiac or hepatic graft function. One patient required an early liver re-transplant, in course of which a renal vein thrombosis sustained and the kidney lost. The overall renal graft survival was 83%

The mean serum creatinine at 1 and 5years was 156 and 186 respectively.

A renal transplant after either a cardiac or liver transplant remains relatively unusual. However, in most instances any increased complexity is manageable and long term graft function can be anticipated. Accordingly, the allocation of scarce cadaver kidneys would appear entirely appropriate in this situation as would live donation if a suitable donor was available.

P13

Post-Transplant Lymphoproliferative Disorder In Renal Transplant: The Manchester Experience

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Post-transplant lymphoproliferative disorder (PTLD) complicates 1 to 10% of all transplantations. The intensity of immunosuppression is thought to be a crucial factor in the development of these tumours. Our Centre has a policy of ¡§minimal¡¨ immunosuppression and this is made possible due to our organ allocation policy on the basis of least mismatches. In this study we review all cases of PTLD in renal transplant recipients in Manchester between 1975-2000.

During the period of study 2678 kidneys were transplanted into 2273 recipients. PTLD developed in 36 (1.6 %) patients (34 adults and 2 children). The median time to onset of PTLD was 59 months post-transplantation (range 10 weeks-15 years).

Twenty patients had multiorgan disease, 13 patients single organ, and in 3 extent was unknown. Pathological classification was early (infectious mononucleosis-like) lesions (8%), polymorphic PTLD (13.8%), monomorphic B-cell PTLD (58.3%), monomorphic T-cell PTLD (8%) and Hodgkin¡¦s disease (2.7%).

Of the 36 PTLD patients, 23 (63%) died with disease. All early lesions and polymorphic PTLD, excluding 2 cases of fulminant post-ATG PTLD, regressed with immunosuppression dose reduction (ISDR) alone. Of the 21 patients with monomorphic PTLD, 19 were treated with chemotherapy in addition to ISDR (¿b surgery) and all of them died of the disease

In our unit there is a higher proportion of monomorphic PTLD, with a poor prognosis, than reported in most series and presentation is much later. We hypothesise that the later onset of PTLD seen in our patients is due to our policy tending to lower levels of immunosuppression. This may allow less florid, clinically undetectable proliferation of Epstein Barr Virus-infected B-cells in the early post-transplant period.

P14

Hypertension Following Renal Transplantation- Potential Cardiovascular Benefits Of Beta Blockers On Patient Survival

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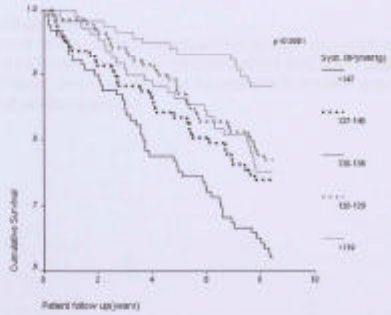
Background Hypertension is common following renal transplantation and adversely affects graft and patient survival. The impact of the use of targets for therapy with individual antihypertensive agents on graft and patient survival has not been clearly described.

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 with drug therapy recorded. We analyzed patient and graft survival after 102 months follow up to assess the influence of hypertension and drug therapy on these outcomes.

Results There were 158(2.9% per annum) deaths and 115(2.2% per annum) graft failures. Univariate analysis showed 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) to be 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. Kaplan-Meier survival analysis demonstrated patient survival to be reduced with increasing quintile of systolic blood pressure as is shown in the figure below, and with increasing number of antihypertensives (p<0.05). Graft survival was also reduced with increasing number of antihypertensives (p<0.05). Reduced patient and graft survival was seen in patients prescribed calcium channel antagonists (p<0.01). No increased patient mortality was seen in 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 protective cardiovascular effect and therefore reduce patient mortality.



P15

Lipoprotein Profiles After Renal Transplantation: A Five-Year Prospective Study

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Background: Various dyslipidaemias in the normal population predict an increased risk of cardiovascular disease (CVD). Similar changes in lipoprotein profiles are found in renal transplant recipients (RTRs) and this group of patients also have an increased incidence of CVD, where it is one of the leading causes of death. Therefore, appropriate modification of the lipoprotein profile in RTRs may prove to be beneficial. The causes of these changes in lipid metabolism may include the immunosuppressive agents used to prevent graft rejection.

Methods: In an observational study total cholesterol (TC), triglyceride (TG) and HDL-cholesterol (HDL-C) levels were monitored prospectively for five consecutive years in 101 patients from years 1 to 5 post-transplantion (Group 1) and in 70 patients from years 6 to 10 post-transplantion (Group 2). Serum LDL-cholesterol (LDL-C) values were estimated using the Friedewald formula. Individual lipid profiles were assessed using a linear regression model. Mean lipid levels were compared with reference to the immunosuppressive drug used and whether or not patients were taking oral hypolipidaemic agents.

Results: TC, TG and LDL-cholesterol levels all decreased in both patient groups over the five-year period (mean regression coefficients Group 1: -0.155, -0.092 and -0.179; Group 2: -0.164, -0.135 and -0.148, respectively). HDL-C readings for all patients increased moderately over the follow-up period (mean regression coefficients Group 1: 0.033; Group 2: 0.019). Patients taking a cyclosporine-based regimen demonstrated higher TC, TG and estimated LDL-C, and lower HDL-C levels, than those patients taking tacrolimus-based treatment. The use of hypolipidaemic drugs increased over the five-years, but TC did not appreciably differ in those patients taking hypolipidaemic therapy from those who were not.

Discussion: These data demonstrate an overall downward trend in TC, TG and estimated LDL-C levels, with moderate increases in HDL-C, over the five year period. This may be explained partially by an increased use of hypolipidaemic therapy and the low prevalence of CVD in this group of patients may be a reflection of the fact that the patients studied are "survivors". Our findings confirm the observation that the use of tacrolimus-based regimens appears to maintain lipid values nearer to the desired levels when compared to cyclosporine based protocols in RTRs.

P16

Determination Of Risk Factors In Immediate And Delayed Graft Failure In Renal Allograft Recipients

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Introduction

Early graft loss and death with a functioning graft remain a difficult problem in renal transplantation. The factors that may contribute to these are not well understood. The aim of the current study is to identify donor, recipient, and peri-operative factors that may be potentially associated with graft failure and with patients who die with functioning graft.

Methods

Of the 444 cadaveric allografts performed between 1996-2000 in our unit, 30 were lost within 1 month, 54 grafts failed within 5 years. During the same period 67 patients died with a functioning graft. 293 grafts remain functional. A retrospective review was carried out to evaluate the risk factors for graft loss and patients who die with a functioning graft. These were categorised into donor factors (age, duration on ventilator and the use of ≥2 ionotropes), recipient factors (age, duration on dialysis, previous transplant, presence of hypertension, DM, CAD, peripheral vessel disease (PVD) and HLA mismatch), peri-operative factors (warm ischaemia time, cold ischaemia time (CIT), presence of multiple arteries, delayed graft function (DGF), number of clinical (CR) and biopsy proven rejections and steroid resistant rejections (SRR). Serum creatinine (S. Cr) was determined for all functioning grafts up to 7 years.

Results

Among patients whose grafts were lost within 1 month, 27 had PNF. No statistically significant difference was found in any of the risk factors between this group and the patients whose grafts are functioning.

Among the patients who lost their grafts from 30 days to 5 years after transplantation, donor age ($p < 0.0003$), CIT ($p < 0.02$), presence ($p < 0.05$) and duration of DGF ($p < 0.0001$), number of CR ($p < 0.0005$), biopsy proven ($p < 0.005$) and SRR ($p < 0.01$). Elevated S. Cr at 3 months ($p < 0.0001$) and 1 year ($p < 0.0001$) correlate with poor outcome.

Factors significantly associated with patients who died with a functioning allograft were donor age ($p < 0.01$), recipient age ($p < 0.0001$), presence of CAD ($p < 0.002$) and PVD ($p < 0.002$) and DGF ($p < 0.01$).

Conclusion

In our study, patient death with a functioning graft was the commonest cause of graft loss and it was mainly recipient related factors that contributed to this. Patients who lost grafts between 30 days and 5 years after transplantation were significantly affected by donor age, CIT, DGF, and acute rejection

P17

Assessing Perioperative Cardiovascular Mortality In Renal Transplant Patients

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Introduction

It is well known that the greatest mortality post renal transplant is from cardiovascular death. In the Northern region we devised a modified risk assessment system for renal transplant patients based on the American college of cardiology/American Heart Association guidelines on perioperative cardiovascular evaluation for non-cardiac surgery. To validate this as a useful tool we performed a retrospective case control review of our patients.

Methods

All patients who died post renal transplantation from 01.01.1996 to 31.10.2002 were included for the study (Group B). The patients were identified, their notes retrieved and the CVS risk score at the time of operation was calculated. The next consecutive transplant to be performed that survived was taken as the control (Group A).
CVS risk was based on age at transplantation, the presence of diabetes, ischaemic heart disease, peripheral vascular disease, cerebrovascular disease, hyper or hypotension, exercise tolerance and BMI (29 points). The patients were scored into low risk (0-3), medium risk (4-8), high risk (9-12) or very high-risk groups (>12).
Of the total 146 deaths 101 scores were available and these were compared with 101 survivors and these were used as controls.

Results

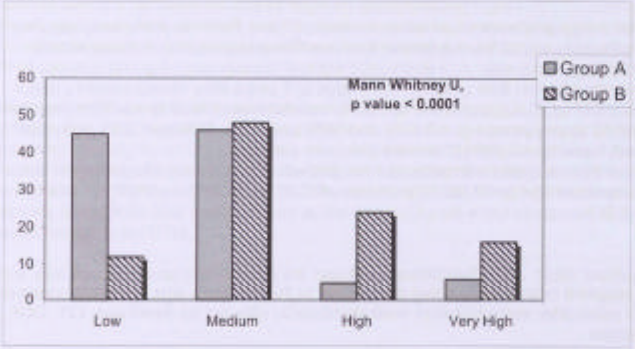

Fig 1

Conclusion

Simple cardiovascular risk score has benefit in predicting poor recipient outcome. The score is currently being evaluated prospectively for selecting suitable recipients.

P18

Pre-Emptive Therapy Is Effective In The Prevention Of CMV Disease Following Renal Transplantation

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Introduction

CMV disease following renal transplantation is a recognised cause of morbidity and mortality. In order to prevent CMV, high risk patients, who are CMV seronegative and are receiving organs from donors who are CMV seropositive, can be given prophylaxis with oral valacyclovir or valganciclovir for 3 months after transplantation. However, it is known from our own series that only 50% of such patients will develop CMV infection and so considerable over treatment will result. Also, it has been shown that CMV disease may be deferred until months later when prophylaxis has been implemented. Pre-emptive therapy on the other hand targets treatment only in those patients at risk of disease by early diagnosis of CMV transmission using PCR, which has been shown to be sensitive and specific. We analyse the results of our policy.

Method

High risk patients were monitored by twice weekly PCR for 3 months following renal transplantation. Those patients who developed two consecutive positive CMV PCRs were treated by intravenous ganciclovir for at least 14 days. The results of graft and patient survival and renal function were monitored and compared.Results57 consecutive patients were donor positive/recipient negative for CMV. 29 patients were treated for CMV transmission and hence 28 did not require treatment. No patient developed CMV disease in either group. Two patients required a further course of treatment with ganciclovir for persistant CMV. Patients received a variety of immunosuppression including cyclosporin alone, cyclosporin with mycophenylate mofetil, or tacrolimus. There was no difference in the number and type of acute rejection episodes in the two groups.S erum creatinine, however, was elevated in the treated group at 3 months (199 v 150 micromol/l; $p<0.01$) , 1 yr (173 v 147;$p>0.05$) , 2 yr (190 v 152; $p<0.02$) and 3 yr (182 v 162;$p>0.05$). Patient and graft survival were not statistically different in the two groups.

Conclusions

Pre-emptive therapy, using PCR to diagnose CMV transmission, is an effective method for the prevention of CMV disease. However, patients may have a slightly elevated serum creatinine compared to those without CMV infection. Whilst there was no difference in graft survival, a longer period of time is required to assess whether the elevated creatinine will lead to a greater incidence of chronic allograft nephropathy.

P19

Pre-Emptive Antiviral Therapy Against CMV Infection With Valganciclovir As An Alternative To Iv Ganciclovir In Solid Organ Transplant Patients

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Background: Cytomegalovirus (CMV) endorgan disease in solid organ transplant patients has a high mortality. Close virological monitoring for CMV infection and prompt pre-emptive antiviral therapy with iv Ganciclovir (GCV) or Foscarnet is highly effective in preventing CMV disease in solid organ transplant patients. However, antiviral treatment with ganciclovir or foscarnet requires intravenous infusion and frequently hospitalisation. Valganciclovir (VGCV) a pro-drug of GCV gives similar GCV plasma levels to iv GCV when given 900 daily. We compared in a retrospective case control study the virological response to oral VGCV compared to iv GCV in the pre-emptive antiviral therapy setting in adult solid organ transplant patients (liver and renal).

Methods: Transplant patients were monitored for CMV viremia by whole blood PCR (sensitivity 200 genome copies/mL). Patients with two consecutive positive blood PCR results received either iv GCV (5 mg/kg twice daily) or VGCV (900 mg bd) at the descretion of the physician. Antiviral therapy was stopped after two consecutive negative PCR results. The primary endpoint was defined days to become CMV PCR negative, secondary study outcomes were decline in viral load within 7 and 14 days and viral load decline rate 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 with VGCV. 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 duration 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 iv GCV group compared to 15.2 days within the VGCV group (p=0.88). Median viral load decline after 7 days was -0.58 log10 in the iv GCV compared to -0.94 log10 in the VGCV arm (p=0.09) and -1.167 log10 (GCV) vs -0.98 log10 (VGCV) after 14 days (p=0.92). The half-life of decline in the iv GCV 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). Pre-emptive antiviral therapy with valganciclovir is a viable option to iv ganciclovir avoiding hospitalisation of the asymptomatic patient.

P20

Predictors Of Long-Term Live Donor Outcomes In A Single Transplant Unit

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There has been a 30% increase in the kidney transplant waiting list in the last 10 years coupled with a 14% reduction in the number of cadaveric kidney transplants. Concerted efforts have been made to expand living donor programs in an attempt to address this problem.

We undertook a study to examine the association between the characteristics of living donors and donor and recipient outcomes. A retrospective case note review was completed on all live adult donor and recipient pairs from a single transplant centre between 1996 and 2001. Forty-five donors with follow-up for greater than 1 year post-donation were included in this analysis to investigate factors adversely affecting donor outcome. The donor characteristics collected are displayed in the table.

There was a significant positive association between pre-live donation (pre-LD) renal function as determined by creatinine and MDRD calculated glomerular filtration rate (GFR) and follow-up renal function ($p<0.001$, $p<0.001$ respectively) but no association with the degree of change in renal function per year. Systolic blood pressure (SBP) pre-LD had a significant positive association with follow-up SBP and diastolic blood pressure (DBP) and significant negative association with GFR ($p<0.05$, $p<0.001$, $p<0.05$ respectively). DBP had a significant positive association with follow-up BP but no association with renal function. Neither SBP nor DBP had an association with actual change in blood pressure or renal function with time. A lower GFR pre-LD predicted the presence of hypertension at follow-up ($p<0.05$, OR 0.93) however this effect was lost when pre-LD SBP and DBP were included in the multivariate model. No factors were identified that would predict those donors with the largest change in creatinine or GFR per year.

This study shows that although renal function following live donor nephrectomy is associated with renal function pre-LD, those donors with the lowest GFR pre-
LD do not have an accelerated decline in renal function. Similarly older donors or those with the highest quartile for blood pressure do not have an accelerated change in blood pressure or renal function. We have previously shown donor age is the most valuable predictor of recipient outcomes. Larger studies are indicated to investigate whether the criteria for LD could be widened.

	Pre-LD	Post-LD
Creatinine (μmol/l)	94.4	110.7
GFR (ml/min)	71.6	59.2
Change in creat/yr		7
Change in GFR/yr		-5.2
SBP (mmHg)	131.2	141.1
DBP (mmHg)	77.7	80.8
Age (yrs)	42.7	
Follow-up (yrs)		3.82

P21

Senescence In Chronic Allograft Nephropathy

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Chronic allograft nephropathy (CAN) is a factor in renal transplant loss. The falling cadaveric donor rates means that a better understanding of its mechanisms is essential. Senescence may play an intrinsic role in its development. We have demonstrated, in a rat transplant model, that accelerated senescence due to oxidant stress at the time of transplantation predisposes to CAN. Calcineurin inhibitors (CNI) have also been implicated in CAN, however, animal models have highlighted their usefulness in ischaemia/reperfusion injury (I/R). Consequently, we have been able to demonstrate preservation of telomere length in cyclosporin treated transplanted rat kidneys.

We have furthered our investigations through an analysis of the response of human tubular epithelial cells to oxidative damage, both with and without CNI, to relate this to the in vivo situation in both a rat model of I/R and in human time zero biopsies.

Human tubular epithelial cells exposed to oxidant stress show evidence of accelerated ageing. Lipofuscin and senescence-associated b galactosidase (SA b gal) expression is increased, as are SAGs. Both cyclosporin and tacrolimus are successful in ameliorating the increased gene expression, and tacrolimus also reduces SA b gal and lipofuscin levels.

These data have facilitated investigations in a rat model of I/R and in human renal transplant biopsies. Preliminary data confirm that one gene involved in telomere dynamics and DNA repair is altered in both systems.

In the rat model of I/R, XRCC5 expression increases with age, and is higher after ischaemia. Interestingly, XRCC5 baseline expression is also greater in diabetic as opposed to non-diabetic kidneys. Analysis of human cadaveric renal transplant biopsies is still ongoing, although it appears that, as in the rat, XRCC5 expression increases with donor age. P16 gene expression, a classic marker of senescence, also increases with donor age, although there seems to be no relationship between SAG expression and cold ischaemia time in pre-transplant biopsies. It will be of interest to compare these parameters in live donor transplant biopsies and CAN specimens.

In summary, oxidative damage affects cellular ageing mechanisms of human tubular epithelial cells in vitro, and of renal biopsies in rat and human in vivo. The amelioration of the oxidative damage by CNI seen in the in vitro situation raises interesting questions about their mechanisms of action.

P22

Histopathological Diagnosis Of Antibody Mediated Rejection Using C4d Staining In Renal Biopsies

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Background

Antibody mediated rejection(AMR)is a very serious type of rejection that is becoming an increasing problem. AMR is difficult to recognize histologically. It is associated with accelerated graft failure. New diagnostic criteria are now being developed for the identification of AMR. Histological features, such as polymorphs in peritubular capillaries and/or glomeruli, fibrinoid necrosis and glomerular thrombosis, are suggestive of AMR. C4d, a split product of the classical complement pathway, can be detected immunohistochemically and is a recognised marker of humoral rejection in tissue samples. In addition,there is a significant association between the production of donor HLA specific antibodies and positive C4d staining.

How to Achieve Diagnosis of AMR

The Banff recommendation is that C4d staining should be performed on all transplant biopsies. C4d can be detected either in frozen tissue (monoclonal antibody) or paraffin embedded tissue (polyclonal antibody). Criteria for positivity are as follows: diffuse(greater than 50% of area) strong linear circumferential, peritubular capillary wall staining in cortex or medulla. The diagnosis of AMR requires at least two of the following:

· Detection of donor specific antibodies(DSA) serologically
· Positive C4d staining in tissue
· Histological features of polymorphs in peritubular capillaries and/or fibrinoid necrosis in vessels or glomeruli or thrombi, or acute tubular injury

Pilot Study

To date we have piloted the staining for C4d in thirty recent transplant cases using the polyclonal antibody on paraffin sections.There were five clear diffusely positive cases, six focally positive cases and nineteen negative cases. The histological features in the diffusely positive cases varied with three showing Type I acute rejection, one showing Type II rejection and one showing acute tubular necrosis. Four of these cases showed lymphocytic glomerulitis. In the positive group two patients had donor specific antibodies in the serum. None were detected in the C4d negative cases.

These preliminary results suggest that positive C4d staining is present in around 15% of cases and classical histological features of AMR are not usually present. 6% of cases had criteria of AMR on combined C4d and DSA presence. Further work is in progress using C4d on all transplant biopsies correlating positivity with DSA presence and graft outcome.

P23

Simvastatin Inhibits Natural Killer Cell Cytotoxicity In Normal Subjects

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Statins (HMG-CoA reductase inhibitors) block the mevalonate pathway, preventing biosynthesis of cholesterol and isoprenoids. We investigated the effect of simvastatin on lymphocytes from normal human subjects in order to provide a model for the in vivo actions of statins. Thirteen healthy volunteers were treated with 40mg per day of simvastatin following which mean total cholesterol was reduced by 23% and mean LDL-cholesterol by 39%. Lymphocyte lipid raft are cholesterol rich membrane domains where isoprenylated signalling proteins are concentrated are represented by Lyn and Fyn. These levels were also reduced by simvastatin. Treatment with simvastatin did not alter ex vivo T cell proliferation. However, the in vitro addition of 1μM simvastatin reduced T cell proliferation by 38% and a combination of prenyl transferase inhibitors reduced proliferation by 17%. NK cell cytotoxicity ex vivo was reduced by 37% following oral simvastatin treatment and by 52% after the in vitro addition of 1 μM simvastatin. The differences between the effects of simvastatin and prenyl transferase inhibitors suggests that statins exert immunomodulatory effects by isoprenoid dependent and independent mechanisms that are likely to involve direct effects on cell membrane structure and function.
These immunosuppresive actions therefore make simvastatin a possible treatment for allograft rejection.

P24

Hyperacute Rejection, A New Model in the Pig.

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Introduction: Hyperacute rejection (HAR) caused by preformed antibodies remains a major obstacle to clinical xenotransplantation and can also complicate human allotransplantation. We have developed a model of this process using a porcine allotransplantation system.

Methods: Inbred Yucatan minipigs of either c/c or d/d phenotype were sensitised by subcutaneous injection of MHC mismatched allogeneic peripheral blood mononuclear cells. Into these primed animals we then transplanted carotid arteries from donors syngeic to the priming cells. These vessels were then harvested after 48 hours and examined histologically for inflammatory infiltrate, fibrin deposition, luminal occlusion with thrombus and antibody deposition.

Results: High titres of allospecific antibodies were generated by the approach. The carotid artery allografts transplanted into sensitised minipigs(n=3) primed with cells identical to the donor animals phenotype underwent a process with features comparable with HAR: inflammatory infiltrate, immunoglobulin deposition and fibrin deposition on the luminal aspect of the endothelium. This was absent from all controls. We measured annular luminal fibrin deposition in the sensitised animals ((39.1% +/- 12.6%), (52.5% +/-27%), (86% +/-12%)), compared to zero luminal occusion in any of the controls (c/c into c/c (n=1), d/d into d/d (n=1), c/c (n=1), and c/c into unsensitised d/d (n=3)).

Conclusions: This model is relatively simple to perform technically with few post-operative complications. It provides a good model of HAR for future work in this area of transplant rejection such as testing interventional methods without the need to set up expensive lines of transgenic animals, or perform relatively complex solid organ transplants.

P25

The Role Of Polymorphonuclear Granulocytes In The Recognition Of Xenogeneic Endothelium

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The polymorphonuclear (PMN) granulocyte is an important phagocyte of the innate immune system which has non-adaptive recognition systems for non-self. We hypothesised that xenogeneic endothelium may be recognised as non-self by PMNs and, as such, even if the effect of the adaptive immune response is circumvented, recognition of endothelium by these cells may yet prove to be a hurdle in xenotransplantation.

We used isolated PMNs in both static and flow adhesion assays and demonstrated a higher background adhesion of human PMNs to porcine than to human endothelium. While resting PMNs were not activated by this interaction, if it occurred in the presence of sub-optimal concentrations of phorbol myristate acetate (PMA), then exposure of human PMNs to porcine endothelium produced a much higher respiratory burst than was seen when they were exposed to human endothelium. Similarly, human PMNs underwent diapedesis through porcine endothelium to a much greater extent than was seen through human endothelium. From transwell assays, we determined that if the PMNs were separated by a semi-permeable membrane from porcine endothelial cells (ECs), a much greater percentage of human PMNs underwent chemotaxis across that membrane than if the ECs in the lower chamber were human. We could reproduce this chemotactic effect using culture supernatant, confirming that porcine endothelium secretes a factor that is chemotactic for human PMNs. We are currently attempting to characterize that factor.

We believe these data support the hypothesis that direct recognition of porcine endothelium by human PMNs might yet prove an additional hurdle to successful xenotransplantation.

P26

Protection Of Pig Endothelial Cells From Human Complement-Mediated Cytotoxicity By Small Interfering RNA

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Introduce: Human immunity to the carbohydrate antigen galactose (¦Á-1,3)galactose (¦Á-Gal), synthesized by the enzyme 1,3-galactosyltransferase (¦Á1,3GT), mediates rigorous rejection of porcine xenografts and represent a major immunological obstacle to successful discordant xenotransplantation. Therefore, targeting ¦Á1,3GT with an alternative approach for the elimination of the ¦Á-Gal epitope is of great interest. The use of small interfering RNAs (siRNAs) as genetic inhibitors of gene expression has been shown to be an effective way of studying gene function in mammalian cells. The purpose of this study was to demonstrate the feasibility of RNA interference (RNAi) mediated by siRNA in porcine aortic endothelial cells (PAECs) in vitro.

Methods: Three short (21 nucleotide) duplexes of RNA specific to the ¦Á1,3GT gene (siRNA-GT) were designed and synthesized. siRNA-GT was then transfected using RiboJuice™ into PAECs. 48 hours after transduction, ¦Á1,3GT transcriptional down regulation were analyzed by RT-PCR. Surface expression of ¦Á-Gal in transfected PAECs was assessed by Western blots, flow cytometry and immunofluorescense staining with FITC-Griffonia simplicifolia IB(4) (GSIB4) lectin. Protection from human complement-mediated cytotoxicity was evaluated by standard 51Cr-release assays after incubation of PAECs with normal human serum (NHS).

Results: A SiRNA was proved to be more effective than the other two for ¦Á1,3GT gene knockdown, resulting in an approximate 60% decrease in ¦Á-Gal epitope expression in the SiRNA transfected over control cells. Flow cytometry and western bolt analysis further confirmed that the transfectant reduces its antigenicity to NHS and GSIB4. In addition, PAECs transfeced with siRNA-GT markedly increased their resistance to NHS in complement-mediated cell lysis.

Conclusions: Our data are the first to demonstrate that RNAi is a specific and potent tool to down modulate the expression of ¦Á-Gal epitope. Gene silencing by RNAi may become a valid alternative for gene intervention in xenotransplantation.

P27

A Statsical Model To Predict Survival After Elective First Liver Transplantation

on behalf of the UK & Ireland Liver Transplant Audit
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Background: Patient survival after liver transplantation depends on patient and donor characteristics as well as on peri-operative events.There have been previous attempts to develop models to predict post-transplant survival, but the discriminatory ability was always poor.Our objective was to develop a new risk model that predicts 90-day survival after an elective transplantation based on patient and donor characteristics known immediately before transplantation for adult patients who underwent an elective transplantation in the UK and Ireland.

Methods: We included all 3800 adult patients who underwent a first elective liver transplantation in the UK and Ireland between 1 March 1994 and 30 September 2002.Risk factors found to be significant predictors of survival in univariate analyses were candidates to be included in a multivariate logistic regression model.The selection of risk factors was based on backward stepwise selection and bootstrapping. The discriminatory ability of the final risk model was expressed as the area under the receiver operating characteristic curve(c-statistic).The calibration of the model was evaluated with the Hosmer-Lemeshow goodness-of-fit test. The model was also cross-validated using subsets of the data according to year of transplantation and transplant centre.

Results: The overall 90-day mortality was 9.7% (95% confidence interval 8.8% to 10.7%). The factors that were included in the final risk model were patient age and sex, indication for transplantation, pre-operative ventilatory status, ascites, serum creatinine, serum bilirubin, serum sodium, donor organ appearance, type of graft (whole/partial), and cold ischaemia time. The overall c-statistic was 0.67 and the goodness- of-fit was reasonable (p=0.10). The c-statistic for model performance according to the results of the cross-validation study varied between 0.54 and 0.72.

Conclusions: The performance of our risk model of post-transplant survival developed in patients transplanted in the UK and Ireland was as unsatisfactory as that of the risk models developed in patients transplanted elsewhere. This indicates that we may have to accept that the ability to predict post-transplant survival based on patient and donor characteristics is limited given the large influence of events that occur during and immediately after the surgical procedure.

P28

Predicting Mortality After Liver Transplantation In Super-Urgent Patients

On Behalf of the UK & Ireland Liver Transplant Audit
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Background: The prognosis of fulminant hepatic failure is relatively poor compared to chronic liver disease. Likewise, post-transplant survival of patients listed for a super-urgent transplantation on the waiting list of UK Transplant is worse than that of patients who undergo an elective transplantation. A better understanding of the risk factors in this context may help improve patient management. Our objective was to develop a risk model that predicts 90-day mortality after a super-urgent transplantation based on patient and donor characteristics.

Methods: We included all 639 adult patients who were listed as super-urgent on the waiting list and who underwent a first liver transplantation in the UK and Ireland between 1 March 1994 and 30 September 2002. Risk factors found to be significant predictors of mortality in univariate analyses were included in a multivariate logistic regression model. The selection of the risk factors was based on backward stepwise selection and bootstrapping. The discriminatory ability of the final model was expressed as the area under the receiver operating characteristic curve (c-statistic). The calibration of the model was evaluated using the Hosmer-Lemeshow test. The risk model was also cross-validated using subsets of the data according to year of transplant and transplant centre.

Results: The overall 90-day patient mortality was 25.9% (95% confidence interval 22.7% to 29.5%). The factors that were included in the final risk model were patient age, pre-operative ventilatory status, serum creatinine, INR, donor organ appearance, type of graft (whole/partial) and cold ischaemia time. The overall c-statistic was 0.69 and the goodness of fit was satisfactory (p=0.66). The c-statistic for model performance according to the results of the cross-validation exercise varied between 0.56 and 0.74.

Conclusions: These results suggest that abnormal donor organs and prolonged cold ischaemia time significantly affect the post-transplant prognosis in super-urgent patients. However, the ability of risk models to predict post-transplant survival in super-urgent patients is as poor as in patients undergoing elective transplantations, which limits the applicability of these models.

P29

A Prognostic Model To Predict Survival In Patients Undergoing Liver Re-Transplantation

on behalf of the UK Ireland Liver Transplant Audit
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Background: Patient survival after liver re-transplantation is inferior when compared with that after primary transplantation. Moreover, patient selection criteria are less well defined for retransplantation. Our objective was to develop a risk model that predicts 90-day survival after re-transplantation for patients in the UK and Ireland based on patient and donor characteristics known immediately before re-transplantation.

Methods: We included all 420 patients who underwent a first liver re-transplantation between 1 March 1994 and 28 February 2002. Risk factors found to be significant predictors of survival in univariate analyses were candidates to be included in a multivariate logistic regression model. The selection of risk factors was based on backward stepwise selection and bootstrapping. The discriminatory ability of the final risk model was expressed as the area under the receiver operating characteristic curve (c-statistic). The calibration of the model was evaluated with the Hosmer-Lemeshow goodness-of-fit test.

Results: The overall 90-day patient mortality was 25.5% (95% confidence interval 21.4% to 29.9%). The factors that were included in the final risk model were patient age, hospitalisation and ventilation status, serum creatinine, bilirubin, donor age, blood group matching and cold ischaemia time. Indication for retransplantation was not found to be a predictor of post transplant mortality after adjustment for other risk factors. The overall c-statistic was 0.78 and the goodness- of-fit was satisfactory (p=0.46).

Conclusions: The risk factors for survival after retransplantation are similar to those for primary transplants. The predictive ability of our final risk model was satisfactory for our cohort of patients and may inform clinical decision making. However, the model has yet to be validated on an external dataset.

P30

Immediate Extubation of Children following Liver Transplantation

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Background
Immediate extubation of adults after liver transplantation is considered safe and is normal practice in our unit unless there are specific contraindications. Currently no data is available on immediate extubation of children following liver transplantation. We believe that immediate extubation of children may be particularly advantageous because they frequently require deep sedation to tolerate the tracheal tube. This can cause haemodynamic instability and delayed recovery.

Method
Between June 2002 and October 2003, we conservatively introduced a practice of immediate extubation unless there were specific contraindications (redo transplant, rising lactate, on going requirement for inotropes, FiO^2 greater than 0.4, blood transfusion of greater than 3 circulating blood volumes, evidence of ongoing bleeding).

Anaesthesia was induced with Propofol and maintained with Remifentanil and Sevoflurane. A bolus of morphine was administered at time of closure of the peritoneum. Following extubation all patients were transferred to PICU and commenced on a Morphine infusion at 0-40 mcg/kg /hour.

Results
Seven out of twelve patients were considered suitable for immediate extubation. The indications for liver transplantation were: biliary atresia (2), Wilson's disease, alpha-1-antitrypsin deficiency, cystic fibrosis, hepatopulmonary syndrome (2). Six were successfully extubated and had an uneventful recovery. One patient with hepatopulmonary syndrome and Alagille's syndrome demonstrated a rise in CVP and a decrease in lung compliance at time of closure of the abdomen and required reintubation. She subsequently responded to diuretics and was successfully extubated later. Characteristics of the seven patients were as shown:

Age: 13 months - 16 years (mean 7 years)
Weight: 8 - 61 kg (mean 38 kg)
Transfused packed cells: 0 – 90 ml / kg (mean 20 ml / kg)
Grafts: whole 3, split (left lateral segment) 3, reduced left lobe 1.
Duration of PICU stay: 1-10 days (median 2 days)

Conclusions
Immediate extubation of children following liver transplantation is practical and safe. Whilst consideration must be given to the primary pathology, particular attention must be applied to their physiological status at the end of the procedure. These early results suggest that small size and split liver transplantation do not represent contraindications.

P31

Biliary Complications Following Right Lobe Split Liver Transplantation

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Introduction:
Biliary complications continue to be the Achilles' heel of partial liver grafts. Right lobe ex-vivo split liver grafts are often considered marginal with frequent border-line size matching, longer ischaemic time and rewarming at the bench therefore external biliary drainage might be beneficial. Biliary reconstruction may be by duct-to-duct anastomosis (DD) or Roux-en-Y hepaticojejunostomy (RYHJ).

Methods:
From August 1994 to April 2003, 54 adult recipients received right lobe split liver transplants with RYHJ in 12 (22 %), DD without T-tube in 27 (50 %), and DD over a T-tube in 15 (28 %) most recently transplanted patients. We retrospectively analyzed the incidence, treatment and outcome of biliary tract complications.

Results:
The incidence of ischaemic biliary complications from hepatic artery thrombosis was 3.7 % (n=2); there was one death from biliary peritonitis and one successful retransplant for biliary necrosis. In the DD/non-T-tube group 5 (18.5%) patients had anastomotic leaks. Treatment was by immediate RYHJ (1), endoscopic stenting (2) and delayed RYHJ for subsequent stricture formation after endoscopic failure (2). DD/T-tube anastomosis resulted in 2 strictures (13%) that were managed endoscopically and 1 leak (6.7%) which resolved spontaneously. There were no complications noted from primary RYHJ. One patient with DD/non-T-tube anastomosis died from a bile leak at the cut liver surface and one DD/T-tube recipient died from biliary peritonitis following T-tube removal.

Conclusions:
(i) Biliary peritonitis is associated with high mortality (ii) Biliary complications requiring surgical repair may be more common when no T-tube is used
(iii) Hepaticojejunostomy results in fewest biliary complications in right lobe split liver transplantation.

P32

Cardiovascular Mortality And Morbidity Post Liver Transplantation

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The incidence of coronary artery disease in patients undergoing liver transplantation suspected to be between 5-10%. The American College of Cardiology (ACC) have issued guidelines aimed at identifying patients at risk of coronary artery disease. The aims of our study were
a) To determine the incidence of cardiovascular (CV) mortality and morbidity in patients undergoing Orthotopic Liver Transplantation (OLTx) and b) To evaluate the potential use of ACC clinical predictors as a guide for further cardiac investigation. Methods: We studied retrospectively 111 consecutive patients who had a liver transplant for chronic liver disease and a post operative follow up of 6 months between 13/07/2000 and 31/12/2002. Their cardiac risk factors were identified at assessment. Predictors of cardiac risk were defined as two or more of the following (obesity, hypertension, smoking, increased cholesterol, cardiac family history, age >50) or one of the following (abnormal echo, LBBB, ST, T wave changes or a rhythm other than sinus, previous MI or CVA). A CV event was defined as a myocardial Infarction, angina, unexplained pulmonary oedema, arrhythmia, cardiac failure and cardiac arrest.

Results: The majority of those transplanted had alcoholic liver disease (25%) or primary biliary cirrhosis (16%). Mean age (54.5±1.1), mean BMI (26.8 ±0.6), 21% of patients had a BMI >30, 21% were smokers, 19% had type II DM and 12% were hypertensive. Twelve patients (10.8%) died during follow-up, two (16.7%) deaths were due to CV events (MI and CCF). Non-fatal CV events occurred in 15 (13.5%) patients during follow-up. Pre-operatively 60% (67/111 patients) were at high risk of CV events, but only 65% of CV events (11/17) during 6 months occurred in this group and 35% (6/17) in the low risk group (p>0.5).

Conclusions: CV events are surprisingly uncommon within 6 months of liver transplantation considering the predicted high risk of our population. In addition the proposed American College of Cardiology clinical predictors of cardiovascular risk do not identify a population at higher risk of CV events following liver transplantation and if applied would result in a large number of unnecessary invasive investigations.

P33

Liver Transplantation Without Extra-Corporeal Veno-Venous Bypass; A Comparison Of Conventional Versus Piggyback Techniques In Adult Orthotopic Liver Transplantation

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Background The use of the piggyback cavo-cavoplasty technique of IVC reconstruction at liver transplantation (CCP-LT) has the advantage over conventional caval replacement (CON-LT) since extra-corporeal veno-venous bypass is not required. In February 2002, we changed to this technique. This study analyses the impact of this change on peri-operative parameters, renal support, complications and outcome.

Methods All data were prospectively collected on a dedicated data-base. A consecutive series of 384 primary liver transplants (2000-2003) were analysed. There were 138 CON-LT and 246 CCP-LT transplants. Two hundred and twenty were male (55%), median age 52 years (16-73). Indications and disease severity as judged by MELD scores were comparable in both groups. The two groups were compared with respect to patient and disease characteristics, ischaemia times, operating time, use of blood products, in-patient stay and morbidity and survival. Chi-squared test was utilised to study the differences between the two groups, $p<0.05$ was considered statistically significant.

Results (Table) The requirement of respiratory support was higher (P=0.03) in the CON-LT group. There was no difference in the requirement of renal support. 30 day patient survival was 89% for CON-LT and 93% for CCP-LT (P=1.4;NS). Temporary porto-caval shunts (PCS) were used in 192 (78%) patients who underwent CCP-LT. The outcome parameters between those who had PCS and those who did not were not significantly different. However the group without a temporary PCS had a significantly lower warm ischemia time (P=0.03).

Conclusion Conversion to cavo-cavoplasty has not had an adverse impact on surgery, complications and patient survival. We observed a reduction in requirement for FFP and platelet support in the CCP-LT group. CON-LT group had a higher requirement for respiratory support and a longer ITU stay.

Characteristics	CCP-LT		CON-LT		χ^2 p value
	Median	Range	Median	Range	
Operating time (hrs)	5.3	3-15	5.3	3-13	0.49
Cold ischaemia time (hrs)	10.7	3-19	12	5-20	0.013*
Warm ischemia time (min)	43	22-56	44	25-69	0.182
RBC use (units)	4	0-36	5	0- 36	0.16
Platelets (units)	8	0-35	10	0- 30	0.01*
Fresh frozen plasma (units)	9	0-36	10	0- 52	0.03*
Peak post op AST (IU)	641	76-9750	691	105-9850	0.96
ITU stay (days)	3	1-97	4	1-53	0.005*
Total hospital stay (days)	11	5-115	13	5-142	0.15

P34

Safety And Efficacy Of Sirolimus In Children Following Chronic Rejection And/Or Nephrotoxicity Post Combined Liver Small Bowel (Sbtx) And Liver Transplantation (Ltx)

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Chronic rejection after transplantation and drug induced nephrotoxicity continue to be major clinical problems. Sirolimus is a potent immunosuppressant acting on the mTOR receptor with no adverse effects on renal function.

AIM: To evaluate the safety and efficacy of Sirolimus in combined small bowel and liver (CSBLTx) and liver (LTx) transplant recipients in children.

METHODS: Retrospective case notes analysis from January 2001 to December 2002

SUBJECTS: 14 children (9M:5F) who showed renal toxicity (n=5) or recurrent rejection after SBTx and/or LTx (n=9) were included. These included: LTx =9; combined SBTx/LTx = 4; isolated SBTx = 1. Indications were: metabolic (6); cholestasis (3); intestinal failure (5)

RESULTS: Median (range) age: - at transplant: 34 months (6-131). - at transfer was 41.8 months (1-140) and median days on Sirolimus was 277 (range 100 – 500). Median Sirolimus level was 7.1ng/ml. 12 patients are alive; 1 died after bowel perforation and another after fungal endocarditis. The following table shows the data on efficacy and safety of Sirolimus.[table nn here]

SIDE EFFECTS: Infection was the main adverse effect but graft function remained normal. Infections included: Non specific pneumonia (7 episodes), adenovirus (1), Influenza (1), EBV viraemia (4) and staphylococcus (2). 2 children developed self limiting oesophageal and buccal mucosal lesions. 3 patients showed high triglycerides not requiring treatment. 5 children showed transient thrombocytopaenia. Sirolimus was temporarily withheld in 1 for EBV viraemia

CONCLUSION: We conclude Sirolimus as a concomitant immunosuppressant is effective in acute steroid resistant and chronic rejection for CSBTx/LTx recipients and require careful monitoring.

	CSBLTx (n=5)	CSBLTx (n=5)	LTx (n=9	LTx (n=9
	Pre Sirolimus	Post Sirolimus	Pre Sirolimus	Post sirolimus
ALT (IU/L) (n=4)	129 (42-662)	43 (25-292)	141 (19-565)	86 (32-377)
GGT (IU/L) (n=4)	93.5 (26-350)	49 (28-403)	251 (27-1004)	128 (25 – 1818)
Creatinine (µmol/L)	52 (36-115)	61 (39-113)	71 (21-142)	68 (32-108)
eGFR* (n=4)	n/a	n/a	52 (34.7- 70.6)	73 (62.4-81.9)
WCC x 109/L	7.3 (4.7-13.8)	7.45 (3-16.5)	7.3 (1-22.2)	6.2 (2.3-20.6)
Platelet x10^9/L	188(120-1119)	210 (141-538)	166 (27-558)	142 (33-546)
Tacrolimus (ng/ml)	14.5 (3-22.4)	9.7 (2-28)	9.9 (6.2-26.2)	7.8 (2-26.5)
Sirolimus (ng/ml)	n/a	5.85 (2-20.2)	n/a	7.6 (2.4-31)

P35

Biliary Reconstructions In Isolated Liver Transplant (ILTx) For Intestinal Failure Associated Liver Disease (IFALD)

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Background: Infants with IFALD may develop rapidly progressively liver disease on parenteral nutrition (PN) some of who have the potential for gut adaptation. In these children isolated liver transplant (ILTx) may be life saving but biliary reconstruction may be technically challenging, because of the size and limited length of intestine.

Aim: To review outcome of duct to duct and duct to intestine biliary anastomosis in children who underwent ILTx.

Subjects & Methods: Retrospective review of all children with IFALD who underwent ILTx in a single centre between 1998 to 2003. Patients & grafts surviving more than 1 month were included in the study.

Results: 9 children (6 male) median age (11.8) range 5.5-14.7 months with residual bowel length 30-80 cm underwent ILTx. Biliary reconstruction, Duct to Duct (Group I, n=4); duct to intestine without Roux loop (Group-II, n=5). Surgical complications, biochemistry, radiology, histology, patients and graft survival were compared and summarised in table.[table nn here] In group II, children with duct to duodenum anastomosis (2/5), showed air in the biliary tree; One required re-exploration for bile leak and one had cholangitis with mild fibrosis at 5 year with normal liver function tests. Patient and graft survival in both groups was 100% with normalization of liver function test within 3 months and similar incidence of complications. The median time to discharge was 71 days with 7/9 patients requiring partial PN at the time of discharge.

Conclusion. Both types of biliary reconstruction are technically possible in children with IFALD undergoing ITLx with good medium term outcome.

	Group I (n=4) Median (range)	Group II (n=5) Median (range)
Recipient Age (months)	9.6 (9.4-14.7)	12.2 (5.5-14.6)
Recipient Weight (kg)	8 (5.8-8.9)	7.5 (5-9)
Donor weight (kg)	25 (10-60)	65 (62-80)
Type of grafts	Reduced 3, Full	Split 4, Reduced 1
Cholangitis (liver biopsy)	Nil	One
Transient duct dilatation	3/4	2/5
Interventions for biliary complications	nil	1/5, re-exploration for bile leak
Acute rejection < 6 months	1/4	1/5
Follow –Up (months)	9.5 (2-30)	21 (35-65)

P36

Roux-En-Y Choledochojejunostomy Is The Method Of Choice For Biliary Reconstruction In Liver Transplantation For Primary Sclerosing Cholangitis.

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Background Opinion is divided regarding the optimal method of biliary reconstruction following orthotopic liver transplantation (OLT) for primary sclerosing cholangitis (PSC). To reduce the risk of recurrent disease affecting the distal bile duct, the biliary anastomosis has conventionally been performed using a Roux loop (RL) rather than a duct-to-duct (D2D) anastomosis. However with improved surgical techniques this dogma has been challenged.

Aims of Study To analyse the UK experience of OLT for patients with PSC, specifically asking if the type of biliary reconstruction influenced the incidence of biliary complications and impacted on graft/patient survival.

Methods All patients with PSC who underwent 1st OLT in the 7 UK centres between May 1994 and April 2003 were identified from the prospective UK Transplant database. Details including type of biliary anastomosis, post-operative complications, date and cause of graft failure and death were collated. Categorical data were analysed using a 2-tailed Fisher's Exact Test. Survival analysis was performed using the Kaplan-Meier method and Log Rank test.

Results 377 patients underwent 1st OLT for PSC during the study period. 15 patients were excluded (anastomotic type not recorded). 264 patients (73%) underwent biliary reconstruction using a RL and 98 (27%) had a D2D. The median follow-up was 52 months (range 1-108). Overall 17 patients (5%) developed a bile leak and 13 (4%) a biliary stricture. There was no significant difference in biliary leak rates between the 2 anastomotic types, however biliary stricture occurred more frequently in patients undergoing a D2D anastomosis (8% vs 2% for RL, p=0.05). The mean graft survival of patients with a RL was 85(80-91 95%CI)months, significantly higher than those with a D2D [74(64-84 95%CI)months, p=0.034]. Mean patient survival in those with a RL was 95(90-99 95%CI)months, significantly higher than patients with a D2D [76(67-86 95%CI)months, p=0.0001].

Conclusions The UK Transplant data suggest an increased incidence of biliary complications and reduced graft and patient survival in patients with PSC undergoing OLT with a D2D anastomosis compared to those with a RL. These data support the traditional stance that patients with PSC treated by OLT should undergo biliary reconstruction using a Roux loop.

P37

Exchanging Livers From Non Heart Beating Donors: A Report Of 2 Cases.

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BACKGROUND- Shortage of livers for transplantation has prompted the use of non heart beating donors. Livers from such donors demand a short cold ischaemic time. Therefore when a suitable recipient cannot be found locally, exchanging organs is difficult.
We present two cases where the liver was used successfully in such circumstances.

Case 1
Newcastle donor: 28 year old Maastricht category III donor. Liver placed before withdrawal and surgeon to surgeon communication with Leeds unit. Withdrawal in ICU and femoral canulation. Patient transferred to theatre where liver removed and portal flush on back table. Liver transported to Leeds where transplanted immediately. Cold ischaemic time 8 hours 43 minutes. Primary function and recipient left hospital.

Case 2
Leeds donor: 28 year old Maastricht category III donor. Liver placed before withdrawal and surgeon to surgeon communication. Withdrawal in theatre, laparotomy after death confirmed and normal cannulation. Liver transported to Newcastle where transplanted immediately.Cold ischaemic time 7 hours 37 minutes. Primary function and recipient left hospital

CONCLUSION- Livers from NHBD can be used in centres other than the retrieval unit. Placement of the liver and communication between the donor and recipient surgeons before retrieval is essential. A short cold ischaemic time is mandatory.

P38

Quality Of Life Of Patients Undergoing Auxiliary Liver Transplant And Standard Orthotopic Liver Tansplant For Paracetamol Induced Acute Liver Failure – A Pilot Study

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Introduction

Attempted suicide by paracetamol overdose (POD) is the commonest cause of acute liver failure (ALF) in the UK. Superurgent liver transplantation is a well recognized form of treatment. But long term immunosuppression gives rise to significant morbidity. These patients often have serious psychosocial problems and non compliance with treatment can be an issue. Auxiliary liver transplantation (ALT), with the potential for native liver regeneration and withdrawal of immunosuppression, is an attractive option. Health deliverers talk of outcome measures other than survival, like quality of life (QOL) and cost effectiveness. We hypothesize that the QOL of POD ALF patients who undergo ALT is better than those who undergo standard orthotopic liver transplantation (OLT).

Materials and Method

To assess QOL we used a well validated generic QOL questionnaire, the Short Form 36 Version 2 (SF 36 V2). We sent this questionnaire to all the patients who had undergone ALT and standard OLT for POD ALF since 1998 when the ALT programme started. For each domain of the SF36 V2 the raw scores were transformed into scores on a scale of 0 (worst)-100 (best). An algorithm was then used to calculate Norm Based Scores (NBS). This score allows easy comparison and interpretation with the general population. 50 is considered to be the mean NBS score for the general population with any score above or below 50 being above or below the mean. The mean NBS of each domain for the general US population, ALT and standard OLT were compared using the nonparametric Mann Whitney U test.

Results

Since 1998 there have been 12 ALT and 13 standard OLT for POD ALF. 7 patients in each group are alive. Six out of seven patients are off immunosuppression. In each domain of the SF36 V2, ALT fared better than standard OLT and compared favourably with the general population. The Mann Whitney U test did not show the difference ALT and standard OLT mean NBS to be statistically significant. This is due to the small number of patients.

Conclusion

ALT for POD ALF achieves the objectives of native liver regeneration and withdrawal of immunosuppression. It gives a better QOL than standard OLT though statistical significance could not be shown. A formal cost effectiveness study should be done.

P39

Quality of Life and Relationship Issues in Living Kidney Donor Transplantation

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Aim: The clinical aspects of living donor transplantation have been well-documented. We explored the effect on quality of life and relationships for both the donor and recipient before and after living donor kidney transplant.

Method: A prospective longitudinal study was performed in two UK Transplant Centres. Both donor and recipient were asked to complete the World Health Organisation Quality of Life (WHOQOL) questionnaire plus additional questions before; six weeks and one year after living donor transplant.

Results: Fifty-two pairs participated. Complete data were obtained from 40 donors and 35 recipients. The WHOQOL physical and psychological domain scores are summarised in Table 1. There were no significant changes in the social or environmental domains for either group. Responses to additional questions (Table 2) were scored on a linear scale of 1-10 (1: not at all; 10: an extreme amount).

Discussion: The physical QOL score for the recipient steadily improves over time, while that of the donor, although reduced post-operatively returns to normal in the longer term. Live kidney donation improves the relationship between donor and recipient. The level of concern of the recipient for the donor before donation may reflect apprehension of the peri-operative risks, but is reduced at six weeks. The intense evaluation and follow-up for donors appears to minimise concerns about living with a solitary kidney. The donors would donate again if possible, providing reassuring information for the future.

Table 1 WHOQOL domain scores (median)

	Donor			Recipient		
	Pre	6 wks	1 year	Pre	6 wks	1 year
Physical	18.8 (17.6-19.4)	16.6* (14.2-17.7)	17.7 (16.0-18.9)	11.4 (9.7-13.7)	14.9* (13.1-17.1)	16 (13.7-18.3)
Psychological	16.7 (16.0-18.0)	16.0 ** (14.7-16.7)	16 (14.0-17.2)	15.3 (12.7-16.0)	16 (14.7-16.7)	16 (15.3-16.7)

* p<0.001 ** p=0.012

Table 2 Additional questions (mean)

	Donor			Recipient		
	Pre	6 wks	1 year	Pre	6 wks	1 year
Has the issue of live kidney donation improved your relationship?	3.5	4.9	5.4	4	5.7	5.8
Do you have concerns about the donor?				7.6	5.5	5.8
Do you worry about the remaining kidney?	2.4	2.1	2			
If it were possible, would you donate again?		8.9	9.3			

P40

A Decade Of Unrelated Living Kidney Donor Transplantation In Bristol

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Background

A living kidney donor (LKD) transplant service was established in Bristol in 1993. The aim of this study is to assess the outcome of transplants between unrelated individuals since the start of the programme.

Method

All patients receiving LKD transplants from 1993 to November 2003 are included. Patient records were used to retrieve demographics, length of hospital stay, post transplant renal function and rejection rates. Data was obtained for analysis of graft and rejection-free survival (length of time from transplantation to the first histological diagnosis of any grade of rejection).

Results

We have performed 126 LKD transplants, of which 31 were between genetically unrelated donors and recipients. A median follow-up of 20.7 months (IQR 6.8-51.5) was obtained. There was a preponderance of male recipients and female donors overall but no difference between the unrelated and related groups.

The recipients unrelated group were significantly older than the related group (p<0.001 Mann-Whitney). The total mismatches for the unrelated group (median 4, IQR 3-5) was significantly greater (p<0.001 Mann-Whitney) than the related group (median 2, IQR1-3). There was no difference in post operative hospital stay (median 11 days IQR 9-4).

By life table analysis the overall actual 1 and 5 year graft survival was 96.0% (SE±1.7) and 90.9% (SE±4.0). There was no difference in graft survival between the unrelated LKD and related LKD transplants (Log rank p=0.391). Rejection free survival was 58.6% at 30 days overall, with no statistical difference between the related (59.1%) and unrelated (53.5%) groups (Log rank p=0.726). However compared to the related group, a greater proportion of patients in the unrelated group received either IL-2 receptor antibody or ATG therapy during the peri and post operative period (Chi sq. p=0.009).

Conclusion

In our series, LKD transplantation between unrelated individuals has as good outcomes as transplantation between related individuals. The only barriers to live donation are now positive crossmatch and blood group incompatibility. The irrelevance of MHC mismatching suggests that in such situations the concept of 'paired exchange' is a viable way of addressing the transplant waiting list.

P41

Comparison Of Multi-Slice Spiral CT And Magnetic Resonance Imaging In Assessment Of Renal Vascular Anatomy For Live Donors - A Prospective Study

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Objectives: This study was designed to assess the role of multislice CT angiography and magnetic resonance angoigraphy (MRA) in evaluation of renal vascular anatomy for laparoscopic and open live donor nephrectomy.

Material and methods: Twenty consecutive donors underwent both CT and gandolinium enhanced MR angiography on separate occasions before surgery. In addition to axial images. Multiplanar reconstruction and Maximum intensity projections were used to display renal vascular anatomy.
Out of twenty donors, nine underwent laparoscopic surgery. Laparoscopic donor surgery was only considered if the renal vascular anatomy was favourable on the left side otherwise right sided open procedure was performed. CT and MRI images were analysed by two radiologists independently. Radiological and surgical correlation was made after donor surgery.

Results
please refer to the table below.

Conclusion: CT angiography was found to superior to MR angiography in this study for both arterial and venous anatomy and therefore was more useful to plan donor surgery especially laparoscopic donor nephrectomy.

	CT (n=20)	MRA (n=20)	Surgical findings
Accessory renal arties	8	6	8
Accessory renal veins	1	0	1
Lumbar veins	6	2	6
Adrenal vein	13	1	13
Lt-Gonadal vein (Lap. Group)	9	5	9

P42

Trans-Atlantic Telementored Hand-Assisted Laparoscopic Live Donor Nephrectomy

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Objectives
Hand-assisted laparoscopic (HAL) live donor nephrectomy is routinely used in many centres worldwide. It is a major advance in the recruitment of live donors due to reduced hospital stay, post-operative pain and scarring, and an earlier return to work. It is however a stressful and technically challenging procedure to learn even following laparoscopic training. Telementoring has already been shown to aid inexperienced surgeons and reduce the laparoscopic learning curve in other fields. Of six cases of hand-assisted laparoscopic live donor nephrectomy, an on-site mentor supervised the initial two. We present the subsequent four cases as the first documented examples of telementored HAL live donor nephrectomy.

Methods
Details of the donor pairs are shown in the results table below. The connection was made using a Comstation (Zydacron) incorporating a Z360 telementoring codec and employed 4 ISDN lines (512kb/s) and a time delay of 500ms supplying both audio and video. The remote surgeon (experience of over 200 cases) in Minnesota, USA watched the laparoscopic view and an external overhead view and could change independently between them. The operating surgeons were able to look at the mentor and converse with him throughout.

Results
See Table

Conclusion
Telementoring for donor nephrectomy is a feasible and effective technique that has the potential to aid in the development of a HAL donor nephrectomy programme. It appears to significantly reduce the learning curve and may have implications for living donor transplant programmes and other surgical specialities.

Results: Trans-Atlantic telementored hand-assisted laparoscopic live donor nephrectomy

Donor-Recipient	Op time (mins)	Warm ischaemic time (sec)	Estimated blood loss (ml)	Donor Total morphine dose (mg)	Donor Creat pre op	Donor Creat day 1	Length of stay (days)
Son-Mother	125	146	28	4	94	165	2
Wife-Husband	170	185	287	1	66	120	3
Father- Son	296	245	135	5	83	136	4
Father- Son	240	178	100	6	118	176	3
Mean	**208**	**189**	**171**	**4**	**90**	**149**	**3**

P43

Factors Influencing the Rate of Live Donor Transplantation

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The Transplant Framework imposes considerable responsibility on Transplant Units to increase transplant rates. UKT have funded specific co-ordinators to improve live donor transplantation (LDT) and there has been a gratifying rise in the number of such transplant performed in the past few years. We have analysed our performance over the past 5 years to evaluate progress and highlight problems, which impact on the programme.

Whilst gratifyingly the number of referrals has increased, the number of actual LD transplants performed has not increased commensurately. The reasons for this are multifactorial and include unsuitability of donors for a wide variety of reasons some of which are included in the table, delay in medical assessment, delays in ULTRA referral, and surgeon's views on donor acceptability. The processing time for LDT ranged from 6 months to 2 years with an average time of 9 months. Other issues have surfaced-what is an acceptable GFR? Should a history of renal calculus prevent donation? Should patients who have a suitable donor remain on the cadaveric waiting list? Should immunoadsorption be used to manage ABO incompatibility? Should we accept the well controlled mild hypertensive donor?

The figures do not reflect the actual workload since each donation involves multiple face-to-face interactions and telephone calls. Too many potential donors failed the screening process. In conclusion, there are many factors which delay and prevent live donation taking place. Each unit should audit its own performance in order to circumvent obstacles to improve the live donor transplant rate.

	1998	1999	2000	2001	2002	2003	TOTAL
No. Recipients	30	32	64	69	61	85	314
Donors contacted	39	52	96	137	107	120	551
LDT performed	14	14	18	19	19	18	102
Received cad. Tx	3	5	13	9	10	6	46
Factors preventing donation							
No. unsuitable	10	18	27	35	46	53	189
ABO incomp.	1	4	9	10	20	25	69
Pos xmatch	3	2	11	12	10	9	47
Received cad. Tx	3	5	13	9	10	6	46

P44

"I Want To Become An Organ Donor – Can You Help?"

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Overview. The Organ Donor Register(ODR)was set up to coordinate transplant services nationally. There are now over 9 million donors on it. Most of the UK public(80%) support the principle of organ donation; but less than 20% are registered on the ODR.

Aims The aim of the study was to look at GP surgeries and Pharmacies within Manchester to assess the information that they provided to the public regarding organ donation.

Methods110 GP surgeries and 112 Pharmacies were surveyed. Posing as an 'uninformed' member of the public, all venues were asked for information regarding access to the old donor card, the ODR, as well as Internet access to information. A standardised series of questions (& supplementary questions) were asked throughout, and all responses recorded.

Results In GP surgeries, all initial respondents (n=110) were receptionists. In 49 cases (45%) , the receptionist asked for advice from another staff member. Only 33% of GP surgeries were able to offer the new information leaflet for the National ODR. 26% could only offer the old (out of date) Organ Donor Card, and 41% could offer nothing. Within pharmacies, the results were even more worrying. The first contact was 'counter staff' in 73%, or pharmacist in 27%. 10 pharmacists (9%) offered correct advice, 17 (15%) offered incorrect advice, and in 85 cases (76%) staff were unable to give any advice. The results showed a worrying gap in the knowledge of both GP Surgery and Pharmacy staff. In the majority of cases they were either unable to provide any information or the information they provided was incorrect. Some sites had information about the donor card but only a small number could provide information about the ODR. Only a minority had any leaflets available and old style donor cards were still available from a number of surgeries and pharmacies. Very few had any insight into registering on-line.

Conclusion GP surgeries and Pharmacies are seen, by the public, as the first point of contact to receive donor information. This study shows that the information available was, in the majority of cases was either incorrect or they had no information available. Clearly education of the clerical as well as the medical staff is essential in order to provide the public with more opportunities to become organ donors. The misinformation and ignorance demonstrated must be seen as a barrier to the recruitment of new donors to the NHS organ donor register.

P45

Cellular Infiltrates After Blocking Co-Stimulation Pathways With CTLA4-Ig And/Or Anti-CD154 Antibody In Corneal Allotransplantation

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Background: Blockade of co-stimulation pathways via CD28 and CD154 is known to prolong corneal allograft survival. In this study we investigated the cellular infiltrate pattern of CTLA4-Ig and anti-CD154 antibody treated recipients in a corneal allograft mouse model.

Material and Methods: BALB/c or BALB/c background CD28 KO mice received orthotopic corneal allografts from C3H donors. Following graft recipient groups were investigated: (i) untreated BALB/c, (ii) BALB/c treated with CTLA4-Ig, (iii) BALB/c treated with anti-CD154 mAb, (iv) BALB/c treated with a combination of CTLA4-Ig and anti-CD154 mAb, (v) untreated CD28 KO, (vi) CTLA4-Ig treated CD28 KO, and (vii) anti-CD154 treated CD28 KO mice. Immunohistochemical staining was performed for following antigens: CD45, CD4, CD8, CD40, CD80, CD86, CD154 and MHC class II.

Results: CTLA4-Ig or anti-CD154 treated recipients, and untreated or anti-CD154 treated CD28 KO mice had a significantly lower number of graft-infiltrating cells and all the above-mentioned antigens. This was in parallel with extended graft survival in these recipient groups. No change in the relative proportions of cell markers was found compared to allografts in untreated BALB/c controls.

Conclusion: Blockade of the CD28 and/or the CD154 co-stimulation pathways results delayed corneal allograft rejection, characterised by immunohistochemistry by less cellular infiltration. The relative proportion of T-cell and dendritic cell markers is unchanged compared to untreated allograft recipients.

P46

Newcastle Corneal Transplant Registry: Five-Year Clinical Outcomes

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Purpose: To evaluate the current management and clinical outcomes of consecutive corneal transplant patients in a single centre in the UK.

Methods: This was a prospective, single-centre, consecutively enrolled registry of all patients receiving penetrating keratoplasty (PKP) at the Royal Victoria Infirmary, Newcastle upon Tyne, February 1997 - August 2002. Pre-operative recipient information and annual follow-up data were analysed. Improvement in visual acuity was analysed by logistic regression. Unadjusted graft survival rates were obtained from Kaplan-Meier estimates.

Results: 267 PKPs were reported in the analysis, including 34 bilateral transplants. 124 (53%) recipients were male and 105 (39%) grafts were regarded as high risk. The main desired outcome in 83% of grafts was to improve visual acuity. 93% of the grafts were performed as elective surgery. The major diagnostic categories were Fuchs' endothelial dystrophy and stromal dystrophies (21%), bullous keratopathy (19%), keratoconus (13%) and regraft (13%). 78% of grafts improved by at least one line of visual acuity post-operatively. Three-year graft survival was 88% (95% CI 82-93) and in total 27 (10%) grafts failed, 10 (37%) due to irreversible rejection and 12 (45%) due to endothelial decompensation. Multiple logistic regression analysis of corneal graft success (at least 3 lines of improvement using Snellen acuity recordings after 12 months) of 149 grafts (38% success) found a statistically significant effect of two pre-operative factors. Odds of success were greater for size-matched donor and recipient (3.4, 95% CI 1.1-10.4) than for size mismatched corneas (>0.25mm), p=0.03. The presence of an intra ocular lens was also associated with greater success (odds ratio 2.5, 95% CI 1.2 – 5.1), p=0.01.

Conclusions: The best improvement in visual acuity after corneal grafting is obtained in keratoconus and bullous keratopathy; and when the difference between donor and recipient size is small (0.25mm). This registry offers a unique opportunity to analyse long-term outcomes of corneal transplantation in a UK ophthalmic unit. It also provides the opportunity to analyse relationships between different outcome measures and to observe how these evolve as a result of an established post-operative management protocol, but also to refine the criteria for patient selection and to guide clinical practice.

P47

The Impact Of Primary Disease On Two Year Graft Survival Of Corneal Transplants

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On behalf of the UK Transplant Ocular Tissue Advisory Group, UK Transplant, Bristol, BS34 8RR, United Kingdom

BACKGROUND: UK Ocular Tissue Transplant Audit data collection forms were introduced in April 1999. This study summarised indications for graft and investigated graft survival, using data collected on the Audit forms. Completed forms are collected by UK Transplant and currently consist of a Transplant Record Form and an Annual Follow-up Form issued at one and two years post-transplant. In addition, the new Five Year Follow-up Form will be issued from April 2004. The data requested on these forms are more detailed than data collected for transplants undertaken prior to April 1999.

METHODS: Unadjusted survival rates were obtained from Kaplan-Meier estimates. Two-year graft survival was analysed for first Penetrating Keratoplasty (PKP) grafts undertaken between 1 April 1999 and 31 August 2001. There were 3100 first PKP grafts in this time period, of which 2418 (78%) had a complete record of survival at two-year follow-up. The only patients considered were those receiving a first PKP graft (90.5% of all first grafts in the time period analysed) and the graft indications considered were Keratoconus, Fuchs' Dystrophy, Pseudophakic Corneal Oedema, Aphakic Corneal Oedema, Viral Infection and Bacterial Infection. All other graft indications were grouped together to provide an additional category.

RESULTS: The main indications reported for the 3100 first PKP grafts undertaken between 1 April 1999 and 31 August 2001 were Keratoconus (26%), Fuchs' Dystrophy (20%) and Pseudophakic Corneal Oedema (20%). Two-year graft survival estimates for first PKP grafts ranged from 96% (95% CI 94-97%) for recipients with Keratoconus to 64% (95% CI 53-75%) for those who required a graft following a bacterial infection. There was significant variation in survival according to indication ($p < 0.0001$).

CONCLUSIONS: There are now sufficient data collected at time of transplant and at follow-up to enable meaningful analysis of the Audit data. The quality and completeness of the data are of a high standard. Preliminary investigation of first PKP grafts shows significant differences in two-year graft survival according to indication for graft.

P48

Ten Year Review Of Cornea Donor Rates In The Uk

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BACKGROUND: In addition to information on solid organ donation and transplantation, the National Transplant Database also contains a complete record of cornea donations and transplants in the UK. This study reviewed cornea donor rates in the UK over the 10 years 1993 to 2002.

METHODS: Data relating to cornea donors in the UK from 1 January 1993 to 31 December 2002 were analysed. Number of donors per year and the donor rates per million population (pmp) in each country within the UK were investigated. Donor causes of death and age of donors were summarised separately for "cornea only" and "solid organ and cornea" donors.

RESULTS: Between 1993 and 2001 there was an 18% decline in number of cornea donors reported in the UK, from 3991 donors in 1993 to 3287 donors in 2001. However, it appears that the downward trend in cornea donation may have been arrested, as the figures for 2002 showed a 4% increase to 3431 donors. Data for the first ten months of 2003 suggest that this small increase has been sustained this year.

There were differences in the donor rates pmp for each of the countries in the UK; the donor rates pmp per year over the three-year period 2000-2002 were 32.1 in England, 41.6 in Wales, 14.6 in Scotland and 9.2 in Northern Ireland.

Only a minority of cornea donors also donated a solid organ (15%). The majority of both cornea only donors and solid organ and cornea donors died from natural causes (94% and 89% respectively). 31% of cornea only donors died from a cardiovascular event compared with 4% of solid organ and cornea donors. The majority (79%) of solid organ and cornea donors died from an intracerebral event compared with 13% of cornea only donors. As expected, cornea only donors are generally older than solid organ and cornea donors. In 2002, the mean age of cornea only donors was 64 years (s.d. 18) compared with 49 years (s.d. 15) for solid organ and cornea donors ($p < 0.0001$).

CONCLUSIONS: Following an 18% decline in the number of cornea donors over nine years, the number increased by 4% to 3431 in 2002 and this upward trend is being sustained in 2003. Investigation of cornea donor rates pmp shows variation across the countries within the UK.

P49

Inflammation-Induced Apoptosis of Corneal Endothelium

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Background: Although corneas are the most commonly transplanted tissue worldwide, graft survival rate is low (50% at one year) in patients at a high risk of graft failure. Apoptosis plays a key role in the deterioration of corneal transplants *in vivo*, primarily as a mediator of immunological rejection. Using whole tissue, a Corneal Endothelial Cell line, and primary corneal endothelial cells, we are characterising apoptotic signalling activated by apoptotic agents known to participate in corneal allograft rejection. Our aim is to identify important rejection-specific apoptogens in order to develop targeted strategies to prevent endothelial cell death occurring during an allograft rejection episode.

Methods: Apoptosis was detected using a flow cytometry based TUNEL assay. We have investigated the signalling involved in cytokine-induced apoptosis of CEC, examining the roles of specific caspase proteins by flow cytometry; the activation status of NFκB and its regulated pro- and anti-apoptotic genes by western blotting; expression of inducible nitric oxide synthase (iNOS) by Reverse Transcriptase-PCR; and generation of nitric oxide using the Greiss Reaction.

Results: Although CEC are largely resilient to insult by each of the pro-inflammatory cytokines IL-1α, IFNγ and TNFα alone (100ng/ml each), we have observed a synergistic apoptotic effect of sustained exposure to a combination of all three pro-inflammatory cytokines in both primary and transformed corneal endothelial cells. An upregulation of iNOS, increased and sustained activation of NFκB, and high levels of NO generation (up to 80μM) were all correlated with inflammatory cytokine-induced apoptosis of CEC. Using pharmacological signalling inhibitors of specific cytokine signalling pathways we were able to elicit full cytoprotection from inflammatory insult, only on inhibition of both NFκB and iNOS activity.

Conclusions: Based on our results, we propose the specific release of pro-inflammatory cytokines from activated cellular infiltrates, in combination with the inflamed environment of a corneal allograft, results in the apoptosis of CEC which is mediated by the *de novo* generation of NO and sustained NFκB activation. Inflammatory cytokine-induced apoptosis may be an important factor contributing to the destruction of corneal endothelial cells during corneal allograft rejection, and presents a new target for the development of anti-rejection therapies.

P50

Differential Gene Expression in Blood During Human Corneal Transplant Rejection

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Purpose: To characterize large-scale gene expression patterns in blood from patients undergoing corneal transplantation and extend our understanding of the molecular changes underlying corneal graft rejection, using oligonucleotide microarrays.
Methods: Atlas Plastic Human 12K Microarray (BD Biosciences) containing 12,000 duplicate-spotted gene-specific oligonucleotides fragments were used. First strand 33P-labeled cDNA generated from total RNA extracted from blood from corneal graft patients undergoing graft rejection and patients whose grafts were not rejected. The data were analysed for differential gene expression, which will be confirmed by real-time RT-PCR and Northern blotting for selected genes.
Results: A total of one hundred and forty-eight genes showed at least a two-fold change in expression. Among them, 100 genes were up-regulated, whereas 48 genes were down-regulated in the blood samples from rejecting patients compared with non-rejecting patients. Approximately 25% of these genes are currently functionally unclassified. The identified and differentially expressed genes with a known function included cytokines and inflammatory mediators, transcription related proteins, extracellular transport proteins, intracellular transducers, and cell surface antigens.
Conclusion: The cDNA Microarray technology has been demonstrated to be a useful tool to monitor the expression of a large number of genes simultaneously. Most results are considered to be preliminary as there is a need to develop an accepted approach to validate the obtained mass of data. However, our data imply that multiple pathways might be involved in the graft rejection of corneal transplantation, including immune and non-immune responses.

Poster Session II

Thursday 29 April

P51

CMV PCR Surveillance In Renal Transplant Patients

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PURPOSE

To assess the impact of routine CMV PCR surveillance on the incidence of CMV infection/disease and the efficacy of aciclovir prophylaxis in the high risk CMV mismatch (Donor +, Recipient -) renal transplant patients in Freeman Hospital Newcastle-upon-Tyne.

METHODS

Retrospective analysis of case notes of high-risk CMV mismatched renal transplant patients who had CMV PCR surveillance between January 2000-March 2002.

RESULTS

249 renal transplants were performed between January 2000-March 2002.
40 patients were in the high-risk (D+, R-) group.
34 of these were available for surveillance out of which only 2 patients had full surveillance, 4 had partial surveillance and 28 had significant gaps in surveillance.
20 of the 34 patients were CMV PCR positive.
Out of the 20 who were PCR positive, 14 had CMV disease and 6 had infection (of which 4 received pre-emptive treatment).
24 of the 34 patients under PCR surveillance received aciclovir prophylaxis however 10 of these patients developed CMV disease.
The mean interval between transplant and CMV PCR positivity was 77 days in the aciclovir group and 44.6 days in the patients who did not receive prophylaxis.
The cost of managing patients with CMV according to current protocol was £46884 as compared to £51015 if all the high-risk patients were to receive ganciclovir prophylaxis with the caveat that 15% of these patients will still end up with CMV disease.

CONCLUSIONS

No significant difference was noted between the cost of managing high-risk patients as per the current protocol and if all these patients were to receive ganciclovir prophylaxis instead.
Prophylactic aciclovir does not prevent CMV disease/infection but may delay it.
Incidence of CMV infection/disease and rate of adverse outcome was higher than expected in the study group.
As a result of this study,we have now introduced universal prophylaxis with 90 days of oral ganciclovir for the high risk CMV mismatched renal transplant patients and plan to reaudit in the future.

P52

Cholestasis In Pregnancy Associated With Cyclosporin Therapy In Renal Transplant Recipients

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For most women with end stage renal disease, transplantation is their only chance for successful pregnancy but it carries various risks. Obstetric cholestasis (OC) presents in the 2nd or 3rd trimester with pruritis and is both distressing for the mother and linked to adverse foetal outcomes: premature birth (≤60%), foetal distress (33%) and intra-uterine death (2%). In Europe the incidence of OC is between 0.1% & 1.5% of all pregnancies. We report 5 cases of OC in renal transplant recipients on cyclosporin that occurred within a cohort of 20 transplant recipient-pregnancies and denote a greatly increased incidence of this complication, which has not been previously reported. All 5 women required prolonged in-patient antenatal care and early delivery (table). OC is associated with an elevated 3α:3β hydroxysteroid ratio together with large amounts of sulphated progesterone metabolites in urine. Under normal circumstances biliary canalicular transporters, such as the bile salt export pump, excrete hydroxysteroids into bile, suggesting a defect in such transporters in OC. Indeed, recently OC has been shown to be more common in women heterozygous for mutations in transporter proteins. Mild canalicular pump dysfunction due to heterozygosity, may lead to OC when the system is overwhelmed by sex hormones. Recent studies have identified effects of cyclosporin on canalicular transport. In rat hepatocytes, cyclosporin disrupted both bile salt export pump localisation and canalicular vacuole accumulation. In contrast, tacrolimus had no such effects. Additionally, cyclosporin is a competitive inhibitor of the human bile salt export pump expressed in insect cells. We speculate that cholestasis may arise in women on cyclosporin who become pregnant, due to the competing influences of increased sex hormones in an environment of relative inhibition of canalicular pump function by cyclosporin. Increased awareness of this problem is needed amongst transplanted women and those caring for them.

P53

FSGS and Renal Transplantation – A Review Of Outcome In A Single Centre

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Background

The shortage of donor kidneys has led to concern about the transplantation of those recipients where there is a possibility of recurrence of primary kidney disease. One of these diseases is focal segmental glomerulosclerosis (FSGS).
A retrospective audit was undertaken in our centre to identify the outcome of renal grafts in this group of patients.

Method

Our transplant databases were searched for these patients. Thirty five recipients were identified as having 44 grafts. Data on graft outcome in terms of function, recipient age, HLA matching, immunosuppression regimen, and disease recurrence was collected for all. A comparison was made between those cases of recurrence and other grafts.

Results

Number of patients = 35
Total number of grafts = 44 (1 graft = 29 patients, 2 grafts = 3, 3 grafts = 3)
Grafts loss = 11 (2 had disease recurrence)
Died with functioning graft= 8
Grafts still functioning = 25 (2 have disease recurrence)
Mean age of recipient = 39.6 yrs
- non recurrence = 40.8 yrs
- with recurrence = 25.0 yrs
Mean HLA mismatch A,B,DR.
grafts with no recurrence (40)= 0.83, 1.05, 0.5
grafts with recurrence (4) = 0.75, 1.5, 0.75
Immunosuppression
Cyclosporin based = 26 (2 recurrence)
Tacrolimus based = 12 (2 recurrence)
Azathioprine/Prednisolone = 6 (0 recurrence)
Four grafts had recurrence of disease at < 1 week, 4 weeks, 19 months and 4 years post transplant. Two have been rescued and are currently functioning, and two were lost.
The proportion of grafts suffering recurrent disease was 9.1% (4) with 4.6% (2) being lost.

Discussion

In this small group of patients graft loss to recurrent disease was <5%. Recurrence of disease occurred both in the early post operative phase and later. Recipients should be carefully observed for signs of recurrence usually manifesting initially as proteinuria. Appropriate immunosuppressive protocols including strategies for rescue should be developed for this recipient group.

P54

Revival Of A Whole Organ Pancreas Transplantation Programme, Early Results And Lessons Learnt

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Introduction: Despite major advances in pancreatic islet transplanation,whole organ pancreas transplatation remains the only widely reproducible method of inducing insulin independence in type I diabetes. Pancreas transplanation does have a significant morbidity and mortality. In June 2001, we revived the pancreas transplatation programme in our unit.

Aim: This study is a prospective audit of our first 25 whole organ pancreatic transplants.

Results: Between June 2001 September 2003 we performed 19 simultaneous kidney and pancreas transplants and 6 pancreas after kidney transplants. The median age of donors was 30(range14-45).The median HLA mismatch was 5(range1-6). The median cold ishaemic time was 14 hours. The median recipient age was 45(range26-59);20 males and 5 females. Recipients had been on insulin for a median of 25 years(range 2-49 years).
Patients received Basiliximab at induction and on the 4th post-operative day. Tacrolimus, Mycophenoite Mofetil and Steroids were used to maintain immunospuression. Steriods were tapered down to 5mg by 3 months with an aim to discontiue them completely.
Early surgical complications included wound infections (6%), peritonitis (12%), minor fistula(12%), major enteric fistula (8%), bleed (8%) and haematoma (4%). Other complications included respiratory 28%), excessive bicarbonate loss(12%), chronic native pancreatis in one patient(4%) and post-transplant lymphoproliferative disease in one patient(4%).A long cold ischaemic times was associated with major complications. Eight patients (32%) had no complications. There were 5 patients(20%)who had a single episodes of acute rejection, all responded to methyl prednisolone. We had two post-operative deaths, both patients had a body mass index >30.
At the time of audit the patient survival was 92% and pancreas graft survival was 8O%. One patient died with a functioning graft and we had 4 grafts that have thrombosed. All patients with functioning grafts are insulin free, have a normal glycosalted haemoglobin and have a good quality of life.

Conclusion: Our early results in pancreas transplantation mirror that reported in the literature. We have identified a body mass index of >30 was associated with mortality in our series. Longer than average cold ischaemic times were associated with major complications. Paying attention to these risk factors may further improve our results.

P55

Pregnancy Outcomes Post Kidney Transplantation: 5 Year Single Centre Experience

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105 female renal allograft recipients between the ages of 16 and 50 receive routine follow up at our centre. Between January 1998 and Oct 2003 15 women had 21 pregnancies. In 10 women this resulted in at least one live birth, 3 women had one or more miscarriages, 2 women went on to have a successful pregnancy following a previous miscarriage and 1 chose termination. Of the 21 pregnancies 13 (62%) had successful outcomes and 8 (38%) were unsuccessful (7 lost in the first trimester). Mean serum creatinine at the time of conception was 139 umol/L (range 103 – 184). The 13 live births included one set of twins and 12 singletons (14 babies), 10 were premature and 3 went to full term. 2(15%) were delivered vaginally and 11 (85%) were delivered via caesarean section. There was no significant difference in age, vintage of transplant, type of immunosuppression, blood pressure control or number of antihypertensive drugs used between those with successful and unsuccessful pregnancy outcomes. Outcomes of kidney function postpartum in those with a successful delivery could be divided into 2 groups. In 3 (43%) there was no change in renal function whereas 4 (57%) had a decline in renal function in the months following delivery (graph below). 3 patients were excluded due to rapid recurrence of additional pregnancy. Those patients in whom renal function remained unchanged post pregnancy had a significantly lower creatinine at estimated time of conception (mean creatinine umol/L 115 v 157 p= 0.03). They also demonstrated a greater fall in serum creatinine during pregnancy (60%) compared to those whose renal function deteriorated (30%)(p = <0.01). In conclusion pregnancy is common in female renal allograft recipients of child-bearing age. Outcome is good once past the first trimester in those patients with a mean serum creatinine of 139 umol/L at the time of conception. A fall in creatinine during pregnancy indicates good renal functional reserve and predicts better renal function post partum.

P56

Renal Transplantation For Patients From Ethnic Minorities

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Background: Kidney allocation in the UK is based on matching two principal biological characteristics between donor and recipient: blood group and HLA phenotype. It is recognised that these characteristics differ according to ethnicity which has implications for allocation to ethnic minority patients as organ donors are almost exclusively White. This study examines the differences between White and ethnic minority patients in relation to access to and outcome of kidney transplantation in the UK.

Methods: The ethnicity of adult cadaveric solid organ donors (2000-02), kidney waiting list patients (Jan 2003) and kidney transplant recipients (2000-02) was compared. The groups considered were White, Asian, Black and 'other'. For patients awaiting transplant, ethnic differences with regard to blood group, HLA 'matchability' and geographical area were analysed. Median waiting times were also compared. For transplanted patients, HLA matchgrades and transplant survival were investigated.

Results: Compared with the normal UK population (8% ethnic minorities), ethnic minority patients are under-represented among donors (2%) and over-represented among patients awaiting transplant (22%). Investigation of the waiting list showed significant ethnic differences according to blood group ($p<0.0001$), geographical area ($p<0.0001$) and patient 'matchability' ($p<0.0001$). Median waiting times of patients listed for transplant in 1998-2000 showed that ethnic minority patients wait longer for transplant (1333 days, 95% CI 1183-1612) than White patients (722 days, 95% CI 685-762). HLA matchgrades of transplants in ethnic minority patients were significantly inferior ($p<0.0001$). Analysis of transplant survival showed no difference between White and Asian patients. More detailed analyses are ongoing.

Conclusion: The study showed that ethnic minority groups are under-represented among donors and that potential recipients from minority groups wait longer for transplant. Once transplanted, ethnic minority patients were found to have inferior HLA matchgrades. There are major initiatives to increase organ donation in ethnic minority groups. In addition, the national Kidney Allocation Scheme is under review and any proposed new scheme will seek to increase the availability of cadaveric kidneys for patients from the ethnic minorities.

P57

Pre-Emptive Living Donor Transplantation; The Primary Objective In Chronic Renal Failure?

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Introduction. The practice in our unit has been to develop pre-emptive living donor transplantation (LDT). The total LDT rate in our unit is 20pmp/yr in 2003, compared to 15 in 2002 and 13.8 in 2001. This increase is associated with the proportion of pre-emptive transplants rising from about 10% of activity to about 50%.

Aim. To examine the potential to perform pre-emptive LDT in 50% of medically fit patients approaching end stage renal failure.

Methods. All patients starting treatment for end-stage renal failure (ESRF) between 1/6/2001 and 1/11/2003 were analysed, according to their fitness for transplantation and LDT status.

Results. A total of 205 patients started ESRF treatment, or developed transplant failure. 56/66 (85%) patients aged <50 years were listed for transplantation; 5 received cadaveric (CAD) transplants, 9 pre-emptive LDT and 9 LDT after starting dialysis. 25/128 (20%) patients aged 50-69 years were listed for transplantation; 2 received CAD transplants, 5 pre-emptive LDT and 0 LDT after starting dialysis. 0/63 aged >70 years was listed for transplantation.

Of transplant-eligible patients not transplanted, 9 are having conventional LDT work-up and another 4 are identified for transplantation across antibody barriers. Patients aged under 50 had a higher LDT rate (52% transplanted or in work-up) compared to those over 50 (28% transplanted or in work-up).

Of the 9 patients who received LDT after starting dialysis, 4 had been on dialysis for <9 months and might have had pre-emptive LDT with more efficient work-up.

The results of pre-emptive LDT were compared to other LDT performed between 1/6/01 and 1/12/03, mean recipient age 41.9 (SEM 3.2) vs 35.2(2.6) years, respectively; DR mismatch 0.86 (0.18) vs 0.72 (0.19); graft survival 14/14 (100%) vs 17/18 (89%); acute rejection in first 3 months 29% vs 22%; estimated creatinine clearance at 3 months 58.4 (6.7) vs 58.7 (3.2) ml/min.

Comment. Pre-emptive LDT was achieved in 18% of patients, but another 27% had received LDT or are in work-up. There is the potential to increase the pre-emptive transplant rate towards 50% by more efficient work-up and by exploring further the potential for LDT in patients aged over 50 years. The results of pre-emptive LDT were excellent.

P58

Laparoscopic Live Donor Nephrectomy: A Case-Control Study

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Introduction

Since the introduction of laparoscopic live donor nephrectomy in 1995, many centers have reported excellent results. Since 2002 we have offered transperitoneal laparoscopic donor nephrectomy in our department. This has been done on the background of considerable experience of laparoscopy in general urology.

Methods

All donors with a single left renal artery and a normal size left kidney were assessed by the operating surgeon for the laparoscopic procedure (n=20). Donors with complex left renal vascular anatomy were offered open right sided nephrectomy and formed the control group (n=20).

Results

At the time of writing data is available on 17 patients in each group. There have been no peri-operative deaths in either group. The early results show significant differences between the two procedures (Table 1: figures are medians with range). 53% of open donors reported wound problems at 3-month follow-up. In the laparoscopic arm no operation, to date, has been converted to the open technique and there has been no reported donor morbidity at 3 months.

Conclusions

The early results show significant advantages in the laparoscopic group. Although the graft warm ischaemia time is significantly lengthened this does not appear to affect early graft function. Full data will be available at the time of conference.

	Laparoscopic (n=17)	*Open (n=17)*	*Significance (p value)*
Operating time	160 mins (110-190)	170 mins (105-240)	0.519 (MW)
Blood loss	200 mls (100-600)	350 mls (100-1700)	**0.032**
Warm ischaemia	5 mins (2-7)	2 mins (1-4)	**<0.001**
Recipient Cr at disch.	126 µmol/l (90-268)	122 µmol/l (50-203)	0.590
Donor Hospital Stay	3 days (2-7)	5 days (2-10)	**0.01**
Delayed Graft Function	6% (1/17)	6% (1/17)	NS
Ureteric Complications	0	6% (1/17)	NS

P59

Outcomes Of Pregnancies After Renal Transplantation: A Report Of The UK Transplant Pregnancy Registry

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Background: For most women of childbearing age, successful transplantation following end-stage renal failure allows an opportunity to start a family. There are, however, increased incidences of maternal and fetal complications for pregnancies in such patients.

Methods: The UK Transplant Pregnancy Registry was set up in March 1997 to study post-transplant pregnancy outcomes. Data concerning pregnancies in all solid organ transplant recipients were collected through a follow-up form. However, due to there being very few pregnancies in cardiothoracic and liver transplant recipients, only the results for renal transplant recipients are presented. To date, data have been collected from 35 renal transplant follow-up and 41 obstetric units in the UK for pregnancies between 1994 and 2001.

Results: A total of 193 pregnancies in 176 kidney transplant recipients were reported. Outcomes were reported for 188 pregnancies as follows: 149 (79%) live births, 21 (11%) miscarriages, 11 (6%) therapeutic terminations, three (2%) intra-uterine fetal deaths, three (2%) stillbirths and one (<1%) ectopic pregnancy. Of the 121 live births with reported gestational age, 61 (50%) had a preterm delivery.
Logistic regression results suggest that the presence of drug-treated hypertension during pregnancy ($p = 0.006$) and a high (>150 µmol/l) serum creatinine before pregnancy ($p = 0.05$) increase the risk of a preterm birth.
A comparison of serum creatinine levels showed a statistically significant ($p = 0.025$) increase in the median serum creatinine level from 125µmol/l pre-pregnancy to 131µmol/l after pregnancy, with greater increases for those with poorer graft function before pregnancy ($p = 0.05$). A matched case-control study was used to investigate the effect of pregnancy on graft function, with matching on age, transplant date, graft number, HLA mismatches and serum creatinine level prior to the pregnancy. Preliminary results from this study suggest that pregnancy has no significant effect on long-term graft outcome.

Discussion: These results are consistent with published literature in that if renal function before pregnancy is good and hypertension is absent, the pregnancy can have a favourable outcome. Although there are conflicting opinions about the effect of pregnancy on graft function, our preliminary analyses suggest that pregnancy does not compromise graft survival.

P60

Impact Of A 'Direct Approach' On Live Kidney Donation In The Indo-Asian Community

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Whilst there is a general imbalance between the demand for renal transplants and the supply of suitable organs, the shortfall is particularly severe for patients from the Indo-Asian (I-A) community. It seems unlikely that this will be remedied by any increase in cadaveric donation.

Aim: To increase the rate of live donor transplantation in the I-A population through a direct approach to patients and their families.

Methods: All I-A patients on the renal transplant waiting list were visited at home, in the presence of their families, by the Asian transplant co-ordinator. Information was given regarding the difficulties in obtaining suitable kidneys alongside the potential benefits of live donor transplantation. Following all visits, potential donors, their relationship to the patient and other demographic information was entered in a prospective database. Individuals expressing a desire to donate were referred into the unit's live donor programme where they were assessed in accordance with the British Transplantation Society guidelines on Live Donor Transplantation (LDT). The progress of potential donors through the assessment process was included on the database. Reasons for any "drop-out" were recorded.

Results: A total of 248 potential donors were identified and interviewed between June 1997-May 2003. Of these, 152 expressed a strong desire to donate and were referred for donor assessment. However, significant attrition occurred, only 15 (9.9%) eventually coming to donation.
When attrition within the assessment process was analysed, 57 (37.5%) potential donors withdrew early before any specific investigation. Thereafter, further attrition occurred, the principle reasons being donor unsuitability (29%), donor withdrawal (23.2%), and ABO/cross-matching issues (13%). 23 donors remained, all of whom were deemed suitable for transplantation. Of these 15 came to donation, four potential donors withdrawing late in the process whilst two patients received cadaveric transplants and two recipients became unsuitable.

Conclusion. A pro-active approach to LDT in the I-A community can increase the rate of donation but the attrition rate is high and many potential donors ultimately fail to come to donation.

P61

Contrast Enhanced Cardiac MRI- A Novel Technique For Cardiovascular Assessment Of Potential Renal Transplant Recipients

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Background Potential renal transplant recipients have a very high risk of cardiovascular disease. Noninvasive assessment in this population is difficult with a high incidence of asymptomatic coronary artery disease. Contrast enhanced cardiac MRI (ce-CMR) has been validated for identifying ischaemic myocardial damage.

Methods We studied 76 potential renal transplant recipients (52 male, median (range) age =52(27-72)). We performed ce-CMR using a 1.5T MRI scanner (Siemens Sonata). Left ventricular(LV) dimensions were assessed by cine stack. Further images were acquired 10 minutes after injection of 0.2 mmol/kg gadolinium-DTPA.

Results 21(27.6%) patients had late gadolinium hyper enhancement, suggesting ischaemic myocardial damage. Gadolinium hyper enhancement correlates with LV abnormalities (LV mass(R=0.38, p<0.01), LV dilatation(R=0.3, p<0.05)) and presence of coronary artery disease at angiography(R=0.70, p<0.01), with increased infarcts seen in diabetics(p<0.05). 15(19.7%) patients had subendocardial enhancement consistent with myocardial infarction.10 patients had a diffuse pattern of gadolinium hyper enhancement, not previously described, associated with increased duration of RRT(p<0.05). This may represent small vessel ischaemia and related myocardial fibrosis. The areas of diffuse hyper enhancement are arrowed in the image below.

Conclusions Potential renal transplant recipients have a high incidence of asymptomatic ischaemic myocardial damage. ce-CMR is a novel noninvasive method for identifying patients with large vessel coronary artery(subendocardial enhancement) disease requiring coronary angiography as part of transplant assessment, as well as demonstrating those with possible small vessel ischaemia(diffuse enhancement). These patients may benefit from intensive risk factor modification with established therapeutic agents pre- and post-transplantation.

P62

Kidney Allocation Scheme Success In Improving HLA Matching

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Introduction: A revised Kidney Allocation Scheme was introduced in the UK in July 1998 and modified in July 2002 to improve access to well matched kidneys for DR homozygous patients and children. This study reviewed the effectiveness of the scheme in improving HLA matchgrades.

Methods: Comparisons were made between transplants under the revised scheme (July 1998 - June 2003) and transplants under the last 18 months of the previous scheme (January 1997 - June 1998). HLA matchgrades achieved in the latest three years of the revised scheme were investigated according to different patient groups.

Results: There have been significant improvements in HLA matchgrade for adult and paediatric transplant recipients in comparison with the previous scheme (p<0.0001) and over the five years of the revised scheme (adults p<0.0001, paediatric patients p<0.03). For adults, the proportion of 000 HLA-A, B, DR mismatched transplants was 7% under the previous scheme and increased from 13% to 19% over the five years of the revised scheme, while the proportion in paediatric recipients was 5% previously and has increased from 11% to 16% in the revised scheme, with a substantial fall in non-favourably matched grafts from 64% previously to 32% last year.
There were significant differences in HLA matchgrade between different patient groups in the latest three years. The proportion of 000 mismatched grafts differed significantly according to 'matchability' (26% of 'easy to match' recipients compared to 8% of 'hard to match' recipients, p<0.0001), blood group (20% of group O patients compared with 10% of blood group B patients, p<0.0001) and ethnicity (18% of White patients compared with 4% of Asian and 5% of Black patients, p<0.0001). HLA-DR homozygous recipients received a similar proportion (18%) to HLA-DR heterozygous recipients (16%), but a much smaller proportion of favourably matched grafts (29% compared with 53%).

Conclusion: Overall, 66% of patients received 000 or favourably matched kidneys in the latest three years of the current allocation scheme, compared with 38% under the previous scheme, but there were significant differences in HLA matchgrades achieved in different patient groups. The national Kidney Allocation Scheme is currently under review and the accessibility of different patient groups to well-matched kidneys will be taken into account.

P63

Role Of Angioplasty In The Management Of Renal Artery Stenosis In Renal Transplantation.

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Introduction:
Renal artery stenosis (RAS) is a rare complication leading to allograft dysfunction and failure. Radiological salvage procedures include angioplasty and intra-vascular stents. This audit was designed to assess outcomes for both percutaneous transluminal angioplasty (PTA) and endo-vascular stenting in the management of renal transplant artery stenosis in a single centre.

Patient and methods:
Of 1351 transplant procedures (Jan 1991 – June 2003) there was a clinical stenosis rate of 2.5 % (n =34). These were predominantly cadaveric renal transplantation: HBD 94.1 % and 5.9 % NHBD. The mean interval between transplantation and diagnosis of stenosis was 17 (2-84) months.

RAS presented with renal impairment 79.4% (n = 27), refractory hypertension 55.9% (n = 19), peripheral oedema 23.5 % (n = 8) and post ACE inhibitor induction renal impairment 14.7% (n = 5). Mode of diagnosis was angiography 58% (n=20), doppler ultrasound 32% (n=11) or MRA 2.9% (n=1). Stent was only used for resistant stenosis. The mean duration of follow up after PTA was 26(2-112) months.

Results:
PTA had a clinical success rate of 73.5 % (n = 25), and resulted in a mean reduction in serum creatinine level of 141umol/l (range 17-438), and improvement in blood pressure control in 35 % (n = 12). Angioplasty failed in 3 cases resulting in thrombosis necessitating transplant nephrectomy (8.8 %). Contrast toxicity occurred in 3 patients, and groin haematoma in 3 patients, which resolved subsequently. One patient developed pseudo- aneurysm, 3 patients had recurrent stenosis needing repeat procedure, and one needed stenting after initial angioplasty for complex recurrent stricture.

Conclusions:
A clinical success rate of 73.5% was achieved using primary angioplasty alone. Stent was not required in the majority of the patients, it was used in resistant stenosis. However allograft loss can be major complication in severe stenosis.

P64

Urinary Monocyte Chemoattractant Protein-1 (UMCP-1) & Transforming Growth Factor-β1 (UTGF-β1) In Proteinuric Renal Transplant Patients

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In diseased kidneys (native/failing transplants with chronic allograft nephropathy, CAN), proteinuria and progression to renal failure are linked. Also, tubular injury and interstitial fibrosis determine the rate of progression to dialysis. In transplanted patients (TP) calcineurin inhibitors (CI) might contribute to this injury. MCP-1 is a powerful chemokine produced by tubular epithelial cells and important in tubulo-interstitial injury associated with proteinuria. TGF-β1 is also key in promoting tubulo-interstitial fibrosis. Data remain limited in proteinuric transplanted (TP) patients. In this study, we measured 24h UMCP-1 and UTGF-β1 and used regression analyses of these parameters against urinary protein (UProt), to define the separate effects of CI from those of proteinuria.

UMCP-1 and UTGF-β1 were measured in 88 TP patients (31 had biopsy diagnosis of CAN), 8.0(1-18) (median and range) years after transplantation. CrCl was 30.5(10 -111) ml/min, UProt 0.7 (0.2-6.9) g/24h. 46 patients were taking cyclosporin (CyA), 24 were on tacrolimus (Tac) and 18 were on neither CI. A group of 80 Non-TP patients with native nephropathies [GN (47), DM (7), PKD (6), HT(5) and CPN (5), CrCl 33(11-152) ml/min, UProt 2.5(0.1-12.2) g/24h] and 20 normal volunteers were also studied.

In TP & Non-TP patients, UMCP-1 levels were higher [253(1.1) pg/24h and 438(1.1)], than normal [140(1.3) pg/24h, $p<0.05$, Geometric means(SE)]. Values rose in all patients with increasing UProt ($r = 0.65$ TP & 0.47 Non-TP, $p<0.001$). In TP patients on CI, the regression slopes UMCP-1/Upr were similar 3.8(1.21) pg/g (CyA) and 5.7(1.27) (Tac) but greater than in TP(Non-CI) [1.8 (1.34) pg/g, $p<0.01$]. The intercept for UMCP-1 at UProt 1.0g/24h for TP(CyA) was higher [386 (1.13) pg/24h] than in TP(Non-CI) [212 (1.24), $p<0.001$]. UTGF-β1 levels were surprisingly similar in TP and Non-TP patients and controls [6.9 (1.1), 7.0 (1.1), and 7.2 (1.4) ng/24h respectively]. However, UTGF-β1 did increase with proteinuria in both TP and in Non-TP patients ($r = 0.50$ and 0.38 $p<0.001$).

Patterns of UMCP-1 and UTGF-β 1 mirrored those seen in diseased native kidneys but MCP-1 was significantly lower in TP than Non TP patients. UMCP-1 was more sensitive in predicting additional injury related to CI treatment (with Tac as toxic to the tubules as CyA).

P65

Renal Transplant Of Pre-Dialysis Patients Does Have Better Long Term Survival And Function: Is It Time To Settle The Record Forever?

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Introduction: Renal transplantation is regarded as the treatment of choice for patients who require renal replacement therapy. Dialysis is a life saving alternative for patients with ESRF waiting for the appropriate graft but is costly and associated with formidable morbidity and mortality. Preemptive Kidney Transplantation (PKT) is the attractive alternative.

Aim: To assess the impact of pre-emptive renal transplantation on long-term graft survival and renal function and to establish the impact of the length of time spent on dialysis on the same outcomes.

Methods: 769 renal transplants performed between 1/1/1990 and 31/12/2001 (11-year period). The 33 pre-dialysis patients (4%) were compared with the 736 patients who were on dialysis before transplant. The dialysis patients were subsequently divided in those dialysing less and longer than 12 months. The pre-dialysis and dialysis groups were well-matched for CIT (22h), Donor age (Mean: 38.1 v 41.5 yrs), Donor sex(M:F 1.7:1 v 1.2:1), Recipient sex(M:F 1.7:1 v 1.5:1), warm ischemic time(38.8 v 36.6 minutes), and HLA mismatch.

Results: The actuarial graft survival of the pre-dialysis group was superior to the dialysis group at 3 years (95.8% v 85.1%) and 5 years (92% v 60.2%) and statistically significant using Logrank Test ($P<0.05$). The 5 years survival was lower in those dialysing over a year as compared to those dialysing less than a year (80.3% v 81.2%)
The Creatinine Clearance was better in the pre-dialysis group than the dialysis group at 1 year, 3 years, and 5 years (mean: 61.6 v 53.6, 65.8 v 51.2, and 62.7 v 52.3) respectively. The Cr Cl was lower in the long dialysers at 1 year, 3 years, and 5 years (mean: 52.9 v 54.8, 50.7 v 53.5, and 49.5 v 56) respectively.

Conclusion: Pre-emptive renal transplant has better long term graft survival, and offers superior long-term graft function. The length on dialysis adversely affects transplant outcome.

P66

Laparoscopic Right Live Donor Nephrectomy

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Introduction
The left kidney is usually chosen for laparoscopic live donor nephrectomy principally because of its longer vessels. Laparoscopic right nephrectomy is technically easier, although the right kidney has relatively shorter vessels. In 2001, having previously used open clamping, we adopted the technique of purely laparoscopic right donor nephrectomy, and describe our experience.

Operative method
Donor position is 45° left decubitus. Pneumoperitoneum is established with Verres needle unless contraindicated. The right colon/hepatic flexure are mobilised. The right lobe of liver is retracted using a Nathanson retractor. The duodenum is Kocherised to display the entire width of IVC exposing its 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 lateral border of the vena cava displays the renal artery and allows comfortable deployment of an endoscopic stapler. We retrieve kidneys via a 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.

P67

A Comparison Of Longterm Graft Survival Rates Between The First And Second Donor Kidney Transplanted – The Effect Of Longer Cold Ischaemic Time In The Second Kidney

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Prolonged cold ischaemic time is a well known risk factor for delayed initial graft function but may also have a negative impact on long-term graft outcome. We carried out a study comparing graft survival rates between those recipients who received the first of a pair of donor kidneys versus the recipient of the second graft.

All kidney transplant recipients who received one of a pair of donor kidneys at our institution between the years 1989-1995 were included. We did not include any patients in whom the transplanted organ was a single donor kidney. Graft survival rates were compared between the 2 groups at 1, 3, 5 and 10-year intervals.

There were a total of 520 renal transplant grafts included in the analysis (260 in each group). Mean donor age was 35.4 years. Mean recipient age was 42.6 years in the first kidney group versus 43.3 years in the second group. Both groups were almost identical for recipient sex (first group 165 male: 95 female, second group 170 male: 90 female), number of HLA mismatches (first group mean 2.43 versus second group mean of 2.59), transplant number for that patient; 1.2 for both groups and percentage PRA (16.4% in the first kidney group versus 18.5% in the second group). The cold ischaemic time was the only variable that was significantly different between the 2 groups. This was 19.93 hours in the first group versus 25.65 hours in the second group giving an average of 6 hours delay for the second kidney. Delayed graft function occurred in 4% of first kidneys versus 7% of the second kidneys. Acute rejection rates were 24% in the first group compared with 28%in the second group. Longterm graft survival rates for the first kidney were significantly better than the second kidney group – graft survival at 1 year 88.5% versus 84.7%, at 3 years 81.8% versus 76.7%, at 5 years 72.2% versus 64.9% and at 10 years 55.2% versus 40%(p=0.012). The patient survival rates were similar in both groups.

In our experience the long-term graft survival rates are significantly better for the first kidney transplanted compared to the second kidney.

P68

Geldanamycin Protects Renal Cells From Oxidative Damage

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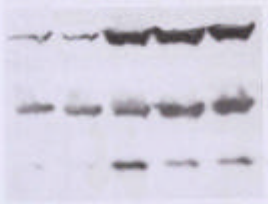
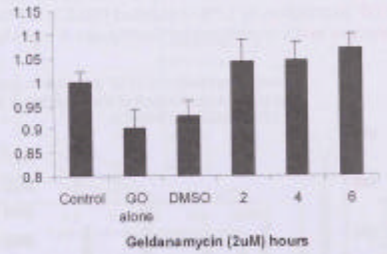
Aims Stress proteins have cytoprotective effects through their chaperone functions and may be useful in limiting the ischemia/reperfusion injury associated with transplantation. Geldanamycin, a benzoquinone ansamycin, is a potent inhibitor of heat shock protein 90 (HSP90), which represses heat shock transcription factor-1 (HSF-1). We hypothesised that geldanamycin would stimulate the stress response through the release of HSF-1 from its complex with HSP90, prompting transcription of stress proteins facilitiating cellular protection.

Methods ACHN (renal) cells were treated with geldanamycin (2μM) for 8, 24 and 32 hours. Western blots were performed for glucose-regulated protein 94 (GRP94), HSP70 and heme-oxygenase-1 (HO-1). Cell survival assays using glucose oxidase were also performed at 2, 4 and 6 hours.

Results Treatment with geldanamycin resulted in increased expression of GRP94, HSP70 and HO-1 (all $p<0.05$). HO-1 was significantly raised after 8 hours but decreased over the following 16 hours. HSP70 became significantly increased at 24 hours. GRP94 was found to be induced at 8 hours and this level was maintained over the course of the experiment (see figure 1 below). Glucose oxidase caused significant cell death compared with untreated control (see figure 2 below). Pre-treatment with geldanamycin protected against glucose oxidase mediated injury ($p<0.05$ at 6 hours).

Conclusions Geldanamycin significantly increased the levels of stress proteins in renal cells. The pattern of expression varied with the greatest effect being apparent in the endoplasmic reticulum chaperone GRP94. This may reflect a direct action of geldanamycin on this HSP90 related protein. Geldanamycin treatment was associated with significant cellular protection against oxidative damage. This work will form the basis of a pharmacological preconditioning strategy in a mouse model of ischemia/reperfusion.





P69

A Functional Association Study Of VEGF Gene Promoter Polymorphisms With Suppressive Effect Of Ciclosporin A And Mycophenolic Acid On VEGF Expression In Stimulated Pbmcs By LPS

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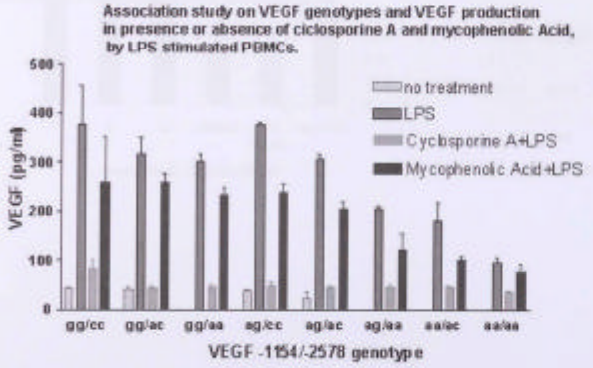
Introduction: One important growth factor for endothelium is vascular endothelial growth factor (VEGF). VEGF induces endothelial cell proliferation and increases vascular permeability. It also has a critical role in graft rejection. The promoter region of VEGF is highly polymorphic and two of these polymorphic sites (G-1154A and C-2578A) were associated with heart and kidney transplant rejection. We have demonstrated a significant relationship between VEGF –1154 G or –2578 C alleles and high VEGF production by LPS stimulated human PBMCs in vitro. Ciclosporin A and Mycophenolic Acid are immunosupressants widely used to prevent graft rejection.

Objective: To ascertain whether Ciclosporin A and Mycophenolic Acid inhibit VEGF production and the influence of VEGF genotype?

Methods: PBMCs were stimulated for two days with LPS (2 mg/ml) in the presence or absence of Ciclosporin A (2 mg/ml) and Mycophenolic Acid (2 mg/ml). VEGF in cultured PBMCs supernatant was measured by ELISA. The VEGF promoter polymorphisms were assessed by ARMS-PCR.
Results: Ciclosporin A strongly inhibited VEGF production. Mycophenolic Acid did too but not as much as Ciclosporin A. Suppression of VEGF production by these drugs was not influenced by VEGF genotype (please refer to figure below).

Conclusion: This study showed there is a significant suppressive effect for Ciclosporin A on VEGF production. Mycophenolic Acid could reduce VEGF production but not as much as Ciclosporin A. This in vitro study demonstrates that VEGF –1154 G and –2578 C can regulate VEGF expression by LPS stimulated PBMCs but there is no association between VEGF genotype and suppression by Ciclosporin A and Mycophenolic Acid.



P70

Effects Of Immunosuppressants On NK Cells

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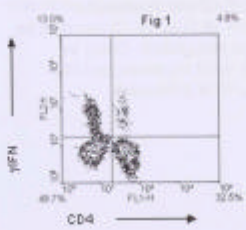
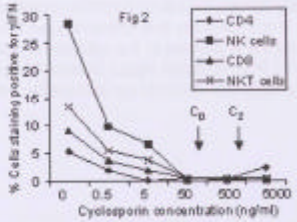
Introduction: The success of organ transplantation depends on effective immunosuppression. Current immunosuppressants are mainly tailored towards T and B cells. Their effects on Natural Killer (NK) cells which have been implicated in the rejection process, has not been well characterised. We have previously shown that in vivo Cyclosporin does not affect NK cell cytotoxicity in renal transplant recipients. However, NK cell cytotoxicity is not the only mechanism of killing target cells. Activated NK cells are known to produce a number of interleukins and chemokines able to trigger the secondary immune response. While there are some studies on the effect of immunosuppressants on NK cell cytotoxicity, little is known about their effect on cytokine production. We therefore investigated the effect of several commonly used immunosuppressants on γIFN production by NK cells.

Methods: PBMC were stimulated with PMA and Ionomycin. Intracellular cytokine staining and FACS analysis was performed to measure γIFN production by NK, NKT cells as well as $CD4^+$ and $CD8^+$ T cells (Fig 1).

Results: In the absence of immunosuppressants, cells staining positive for intracellular γIFN are mostly NK cells (27% staining positive), followed by NKT cells (13%), CD4+ T cells (4.6%) and CD8+ T cells (7.8%). Cyclosporin at therapeutic levels completely inhibited the production of γIFN by all 4 cell types, although levels 10 times below the therapeutic range have less effect (Fig 2). Tacrolimus, however completely inhibited γIFN production by all 4 cell types at concentrations 10 times less than the therapeutic level. Sirolimus also inhibited γIFN production, but only at a concentration 60 times higher than the therapeutic range.

Conclusions: NK and NKT cells are major producers of gIFN following stimulation. Calcinuerin inhibitors are effective in blocking γIFN production by all 4 cell types, presumably by interfering with the action of IL-2, which is known to be a potent stimulator of NK cells.





P71

Lentivirus-Mediated Gene Transfer Of Viral Interleukin 10 Prolongs Survival Of Cardiac Allografts

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Introduction: Lentiviral vectors are increasingly used for *in vitro* and *in vivo* gene therapy due to their efficiency in transducing non-dividing cells, their long term gene expression and the lack of host immune response. We examined transfer of the viral interleukin 10 (vIL-10) gene into rat cardiac grafts using lentiviral vectors by direct intramyocardial injection *in vivo* and investigated its effect on T cell proliferation at different stages *in vitro*.

Methods: Lentiviral vectors containing vIL-10 were generated based on HIV-1. vIL-10 expression in transduced hearts was examined by RT-PCR. Cardiac transplants were performed in allogeneic rat strains (Lewis to DA). Mixed lymphocyte reaction assays (MLR) were used to determine the influence of vIL-10 on T cell proliferation. DA T lymphocytes were used as responder cells and γ-irradiated Lewis monocytes as stimulator. DA rat aorta endothelium cells (RAEC) transduced with HIV-PGK-vIL-10 produced bioactive vIL-10 *in vitro*. MLR were performed comparing MLR co-culture with vIL-10 transduced RAEC at day 1 to MLR co-culture with vIL-10 transduced RAEC at day 3.

Results: vIL-10 was detected in heart isografts at 28 days after transduction. Animals transduced by vIL-10 showed prolonged allograft survival without conventional immunosuppression (14.5±1.0 days vs 8.0±0.7 days for control, $p<0.001$). Up to 30 ng/ml of bioactive vIL-10 was produced by RAEC transduced with HIV-PGK-vIL-10 at day 3. T cell proliferation was inhibited by 50.4% when MLR was co-cultured with RAEC transduced with vIL-10 at day 1. No inhibition was observed in MLR co-cultured with transduced RAEC at day 3. The effect was alloantigen-specific, as vIL-10 had little effect on Con A-stimulated T cell proliferation.

Conclusion: vIL-10 expression in rat heart using lentiviral vectors prolongs allograft survival. The survival time is comparable to that using adenoviral vectors delivering vIL-10. The limited survival benefit may be due to lack of inhibition of the early phase of the alloimmune response. *In vitro* study confirms that efficient suppression of the MLR by vIl-10 can only be achieved if the cytokine is present at the initiation of alloimmune recognition. Delay in expression of vIL-10 from lentiviral vector means that protocols must be developed to suppress the early stages of alloimmune stimulation before vIL-10 is produced.

P72

From Detecting The Alternatively Spliced Variants Of Interleukin-2 mRNA To Detecting Corresponding Translated Protein: Step Forward In Understanding The True Role

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Background: When first reports showed that recombinant human truncated IL-2 proteins, so called rhIL-2δ2 and rhIL-2δ3 proteins, inhibited IL-2 induced proliferation of T cells in vitro it was speculated that truncated proteins, coded by alternative splicing, provide natural receptor blocking and therefore inhibitory feed-back mechanism. To test hypothesis we initially investigated the phenomena of alternative splicing of IL-2 mRNA by looking at the mRNA level. In kidney transplant biopsy samples and in vitro using two-way MLR we were able to show that peripheral blood T lymphocytes use alternative splicing but predominantly express normal form of IL-2 mRNA. Kidney tissue infiltrating T cell predominantly express alternatively spliced variants of IL-2 mRNA. Aim of this study was to explore how accurately detection and quantification of mRNA reflects cell activation and to establish if alternatively spliced forms of mRNA (IL-2δ2 and IL-2δ3 mRNA) are really translated into functional proteins.

Methods and results: By using a two-way MLR and ELISA detection system (R&D) we were able to show that higher IL-2 protein levels in culture supernatants correlated with higher IL-2 mRNA levels. Immunosuppression induced lower mRNA levels were reflected in lower protein levels. To verify if truncated IL-2 proteins coded by alternatively spliced variants of IL-2 mRNA are secreted, we had to produce our own antibodies. To induce antibodies, which would be specific for IL-2δ2 protein, rabbits were repeatedly immunised with small peptide linked to a carrier KHL. Fifteen amino acids long peptide sequence was determined by nucleotides present at an end of exon 1: and the beginning of exon 3 junction. Antibody specificity, tested by ELISA, showed that only one antibody was peptide specific. Other sera reacted with peptide but also cross-reacted with IL-2. Using peptide specific antibody in ELISA detection system we were able to detect weak positive signal in only 2 of 30 tested supernatants. This correlated with high IL-2δ2 mRNA signal.

Conclusion: Although results indicate that alternatively spliced IL-2δ2 mRNA is translated we are using these antibodies to enrich IL-2δ2 protein and establish whether levels and binding specificity of natural shorter forms of protein have meaningful role in immune responses.

P73

Aspirin-Treated DC Induce Hyporesponsiveness And Regulatory Activity In Responder T Cells

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Dendritic cells (DC) are the main professional antigen presenting cells of the immune system. However, recent studies have shown that immature DC (iDC) can induce tolerance, rather than activation, in responding T cells. As iDC are subject to maturation, we sought to "create" a tolerogenic DC with a greater, or more stable tolerogenic potential than that of iDC. Aspirin-treated monocyte derived human DCs (Asp-DCs) were shown to have a reduced expression of MHC-class II and CD86 compared to iDCs. Treatment with LPS only partially reverses their immature phenotype. As previously shown for endothelial cells, a reduced phosphorylation of IkBa was detected in AspDCs compared to iDCs. Functionally, Asp-DCs have a reduced allostimulatory capacity compared to iDCs, and most significantly were shown to induce the development of regulatory T cells. The mechanisms involved in the regulation is probable a combination of cell-contact-dependent mechanisms and inhibitory cytokines.
These data support the potential use of Aspirin-treated DCs in the induction of transplantation tolerance in human.

P74

Iron Depletion Of Cultured Pancreatic Islets Produces Prolonged Over Expression Of Vascular Endothelial Growth Factor (VEGF)

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Introduction: Up to 60% of islet mass is lost after transplantation due to hypoxia, caused by a lack of functional vasculature post transplantation. Revascularisation of transplanted islets takes up to 14 days and is controlled by a number of angiogenic factors, of which VEGF is probably the most important. Over expression of VEGF by viral transfection techniques has been shown to improve islet revascularisation but only produces over expression of a single factor in a limited number of cells. We investigated whether iron depletion of islets could induce over expression of angiogenic factors by inhibiting the breakdown of hypoxia inducible factor; an important molecule in hypoxia sensing.

Methods: Islets were isolated from Sprague Dawley rats by collagenase digestion and Ficol purification. 50 islets were hand picked and cultured in 2ml of CMRL medium alone or CMRL medium containing increasing concentrations of the iron chelating agent desferrioxamine(DFO10μM, DFO100μM and DFO1000μM). After overnight culture, the supernatants were completely removed and the islets were subsequently incubated in the iron containing medium M199.The cultures were continued for a further 6 days with daily complete harvesting of the culture supernatant. The supernatants were centrifuged, frozen at -80°C and analysed using a mouse specific VEGF ELISA.

Results: Incubation of islets overnight in DFO produced a dose dependent increase in VEGF concentration in supernatants: 30.6pg/ml control, 35.3pg/ml DFO10μM ($p=0.14$), 53.5pg/ml DFO100μM ($p< 0.001$) and 181.5pg/ml DFO1000μM ($p< 0.001$). VEGF protein levels remained significantly elevated in the DFO1000μM and DFO100μM supernatants for 96 and 48 hours respectively after transfer into iron containing media.

Conclusions: We have shown that iron depletion of islets in vitro can induce a prolonged increase in the expression of VEGF. This method provides an attractive technique for pre-treating islets before transplantation that does not require the insertion of new genetic material into the islets and should increase a number of angiogenic factors in a physiological fashion, rather than increasing the expression of only a single factor. It remains to be seen whether the over expression of VEGF by iron depletion will lead to enhanced revascularisation and better survival of transplanted islets in an in vivo model.

P75

LDL In Culture Medium Is Crucial For Survival Of Human Fetal Hepatic Stem Cells In Serum Free Culture

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Background
Hepatic stem cells have been reported to express the haematopoietic marker CD90 as well as CD34. Potential clinical use for transplantation demands expansion of these cell types in vitro. However, present cell culture systems contain animal serum, which may carry infection. Similarly, animal serum may not support optimal growth of human cells. In our study we have compared serum-containing (SCM) and serum-free(SFM) media for the expansion of human fetal hepatic stem cells in vitro.

Methods
Hepatic cells from 2nd trimester aborted human fetuses were extracted with collagenase and cultured in William's medium + calf serum(SCM) or SFM(Iscove's medium, insulin, transferrin, mercaptoethanol, albumin, ± LDL 40 µg/mL) in accordance with ethical guidelines. Assessment was carried out by phase-contrast microscopy, flow cytometry for CD90 and CD34, and immunohistochemistry for CD90, CD34, vimentin, cytokeratins(CK) 18 and 19.

Results
Cells grown in SFM without LDL showed vacuolation by the 7th day, which became pronounced by the 10th day. The cells became rounded and detached from the substrate between days 11-14. Cells cultured with the addition of LDL showed long term survival without losing their normal morphological appearance. Microscopy showed cells in SFM to be similar to cells cultured in SCM. Flow cytometry on day 0 showed that 4.16% were $CD34^+$, 4.89% were $CD90^+$ and 1.11%were $CD90^+34^+$ (n=3). On day 10, there were no $CD34^+$ cells in SFM or SCM, but the percentage of $CD90^+$ cells was significantly higher in SFM (63.9% compared to 29.1%). Cells staining for CD90, vimentin, CK18 and CK19 were also identified by immunohistochemistry.

Conclusions
Serum-free medium containing LDL permitted greater expansion of putative human fetal hepatic stem cells ($CD90^+$). Cells demonstrating hepatic and biliary markers and expressing vimentin, were supported in our serum-free system. These data show that serum-free culture may be superior to conventional culture in expansion of fetal human hepatic stem cells. LDL is an essential additive for the serum free culture of these cells.

P76

Fas-Ligand Expressed On Transplanted Cells Alters There Rejection During The Anti-HY Immune Response

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INTRODUCTION: In several studies Fas-ligand has been shown to be important in down-regulating the immune response. It is the processing and selection of Y chromosome-encoded peptides by the MHC antigen presenting molecules that, in part, determine the magnitude of the anti-HY response. In this study we looked at the effect of FasL on this.

METHODS/RESULTS: Female wild type H-2b mice were inoculated with a 1:1 mixture of female and male cells from FasL-deficient or wild type H-2b mice. The cells were labelled with either high (male) or low (female) levels of CFSE prior to inoculation to enable us to follow their fate. We saw no difference in male cell loss between the two groups. There was also no difference in the HY-restricted Db (MHC class I)-specific CD8+ T cell expansion, as detected using HY peptide specific class I tetramers. However, when we innoculated the recipient mice a second time with an identical 1:1 cell suspension, we demonstrated that the presence of FasL on the male cells protected them from rejection (figure 1). This protection led to a decrease in the tetramer specific CD8+ T cell expansion in female mice innoculated with wild type cells. We hypothesised that the presence or absence of FasL on the antigen presenting cells (APC) determines the nature of the immune response. We predicted that this effect was related to the number of dendritic cells that expressed FasL. To test this we varied the presence of FasL on the dominant source of DCs, the recipient mice. Accordingly, female FasL-deficient and wild type recipient mice were innoculated with male/female cell suspensions from wild type mice. We saw that the presence of FasL on the recipient mice led to a reduction in the rejection of the male cells, which was mirrored by a reduced specific T cell expansion.

CONCLUSION: The presence of FasL reduces the susceptibility of the target cells to rejection and reduces the amplitude of the generated immune response. The importance of FasL in rejection of the minor antigen suggests that manipulating it may potentially be beneficial.

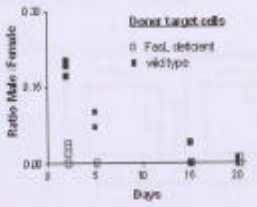

FIGURE 1: FasL protects the male wild type target cells from loss when transplanted onto female wild type recipients.

P77

Fas-Ligand Alters The Nature Of The Induced Immune Response

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BACKGROUND: The best-described receptor-ligand combination that induces apoptosis comprises members of the TNF family, Fas, and its ligand Fas-ligand (FasL). Constitutive expression of FasL is thought to be important at sites of immune privilege. Parenchymal upregulation of FasL is thought to limit local inflammatory damage. We wanted to assess the role of FasL on the afferent limb of the immune response.

METHODS AND RESULTS: To determine if FasL modulated the afferent limb of the immune response, some of the recipient mice also received a second wild type skin graft. Here the presence of FasL on the priming graft reduced the rate of rejection of the second wild type graft ($p = 0.002$). We confirmed this in an in vitro system in which CD4+ cells were purified from these transplanted female recipients and used as responders in a mixed leucocyte reaction (MLR) with FasL-deficient or wild type H-2b dendritic cells as stimulators. The CD4+ cells purified from recipient mice that had been primed with wild type skin grafts showed reduced proliferation (figure 1). We suspected that it was the migration of antigen presenting cells (APCs) from the graft to secondary lymphoid tissue that was modulating the afferent response. To study further, we demonstrated the presence of FasL on stimulated wild type H-2b dendritic cells by flow cytometry. A further MLR was performed using naïve T cells from H-2d (BALB/c) mice as responders mixed with FasL deficient or wild type H2b dendritic cells stimulators. In this MLR we saw that the presence of FasL again reduced the proliferation.

CONCLUSIONS: These results suggest that FasL expression on the donor tissue alters the nature of the induced immune response.

P78

Qualitative And Temporal Differences Between Renal, Cardiac And Skin Allograft Rejection In Mice

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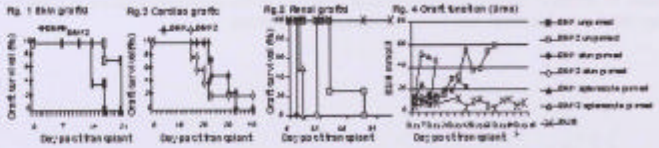
Introduction: Chronic rejection is a major cause of graft loss. Experimental models of skin or heart transplant do not provide quantitative measures of graft function to study chronic rejection. Kidney allografts in some mouse strain combinations are spontaneously accepted while others are acutely rejected. Here, we describe a mouse renal allograft model in which rejection occurs in a delayed fashion with gradual deterioration of renal function and has histological signs of chronic damage.

Methods: BL/6 recipient mice were transplanted with either renal, cardiac or skin allografts from a single class I (BM1) or class II (BM12) MHC mismatched donors. Graft function was monitored by serial blood urea nitrogen (BUN), palpation, and inspection respectively.

Results: Class I and II MHC mismatched skin and cardiac grafts were rejected acutely (Fig 1 & 2). By contrast, renal allografts from class I or II mismatched donors were rejected in a delayed manner. They can sustain life in bilaterally nephrectomised recipeints for more than 70 days with a slow rise in BUN and showed histological signs of chronic damage with glomerulosclerosis, interstitial expansion and evidence of tubule damage (Fig 3 & 4). However, animals primed by donor splenocytes or skin grafts were able to reject kidney allografts acutely (Fig 3 & 4), with florid cellular and vascular rejection with lymphocytic infiltrates and cuffing of small vessels on histological examination, similar to fully MHC mismatched allografts from BALB/c donors transplanted into unprimed recipients.

Discussion: BM1 and BM12 donors differ from recipient BL/6 animals by only 3 amino acids in their MHC class I and II respectively. Nevertheless, this can result in acute rejection of cardiac and skin allografts. Interestingly, these differences are not sufficient to trigger acute rejection of renal allografts but resulted in an insidious loss of graft function. Histological changes in these grafts are more characteristic of chronic allograft nephropathy. The reason for the difference in both nature and tempo of rejection between renal and other organ grafts is unclear but provide further evidence for an immune basis to chronic rejection. This model provides a useful tool to further dissect the role of the immune system in development of chronic allograft nephropathy.



P79

Changes In Vascularity Associated With Chronic Allograft Nephropathy

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Background: A hallmark of chronic allograft nephropathy (CAN) is endothelial cell damage, resulting in hypoxia, providing stimulus for angiogenesis, or new vessel formation. This study aims to compare vascularity of kidneys with end stage CAN versus normal. In addition, validation of renal biopsy samples in the assessment of angiogenesis has been performed.

Methods: Tissue from transplant nephrectomies performed because of CAN (n=29) and normal kidneys (n=61) were studied. Tissue sections were immunostained with the endothelial cell marker, antibody to CD31. Changes in overall vascular patterns were noted and microvessel counts (mvc) performed. Sections from 11 transplant nephrectomies and normal kidneys were stained with antibody to CD31 and the proliferation marker, MIB-1, to determine the presence of proliferating endothelial cells. The percentage of proliferating cells that were endothelial was expressed as the proliferation index. Following staining of endothelial cells with antibody to CD31, microvessel counts were performed on paired samples of core biopsies and cross-section of normal kidney (n=25), thus allowing comparison between the two. Statistical analyses were performed, using Student t test and Pearson correlation as appropriate.

Results: There was loss of regularity of CD31 staining in CAN kidneys compared with normal. Microvessel counts from CAN kidneys were significantly reduced in both cortex and medulla ($p<0.0001$ and $p<0.0001$ respectively) and were accompanied by a significant increase in proliferation index ($p<0.0001$)compared with normal. There was significant correlation in mvc in core biopsies and the corresponding kidney cross-section ($r=0.77$, $p<0.0001$).

Discussion: This study demonstrates reduced density of CD31-positive microvessels in CAN compared with normal. Despite this, evidence of proliferating endothelial cells in the CAN group suggests attempts at endothelial cell repair. These findings support our hypothesis that, early in development of CAN, angiogenesis is stimulated, but that, despite this attempt at tissue repair, progressive microvascular loss results in interstitial fibrosis and eventual organ failure. In addition, further investigation of changes in the microvasculature in CAN by study of sequential core biopsies has been validated in this study.

P80

Manipulation Of The Immune Response To Cardiac Allografts By Donor/Recipient Chimaeric MHC Class I Proteins

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Pretreatment of rodent allograft recipients with donor MHC, either alone or in the presence of immunomodulatory agents such as anti-CD4 or cyclosporin, is a strategy that reliably induces donor-specific tolerance, but the mechanism of tolerance induction is unknown. Kahan et al (1996) have reported prolonged allograft survival by iv treatment of fully allogeneic rat heart graft recipients with recombinant, allochimaeric soluble MHC class I molecules incorporating donor-type amino acid substitutions corresponding to the hypervariable region of the MHC class I alpha1 domain. In a class I-disparate rat model of heart transplantation (PVG.R8 to PVG.RT1u) we have previously identified an immunogenic epitope of the alpha1 hypervariable region that primes T cell help in graft recipients and accelerates antibody-mediated allograft rejection but, when administered iv, diminishes the alloantibody response. This epitope corresponds to the amino acid substitutions in the allochimaeric class I proteins. We explored the applicability of chimaeric class I protein treatment in this high responder transplant model and also in the reciprocal strain combination.

Using a prokaryotic expression system with PCR-induced mutations we synthesised a panel of native and chimaeric MHC class I proteins. ELISAs were developed to detect anti-chimaeric class I antibody responses. Immunising with native donor strain recombinant class I in adjuvant generated an anti-class I protein antibody response that did not cross-react with conformational class I on donor strain targets, as detected by flow cytometry. Similar antibody responses were produced by immunising with chimaeric proteins comprising recipient MHC class I with 4-9 donor-type amino acid substitutions in the alpha1 hypervariable region. However, iv treatment with chimaeric class I failed to prolong heart allograft survival in either the MHC class I-disparate model or in a fully allogeneic strain combination equivalent to the published model. In the class I-disparate reciprocal strain combination heart grafts survived >100 days but demonstrated chronic rejection; this was not influenced by allochimaeric class I treatment.

We conclude that this approach requires careful analysis of TCR-peptide-MHC interactions in order to design effective chimaeric class I treatment strategies.

P81

Thymosin Beta-4 Sulphoxide – A Tolerogenic Peptide?

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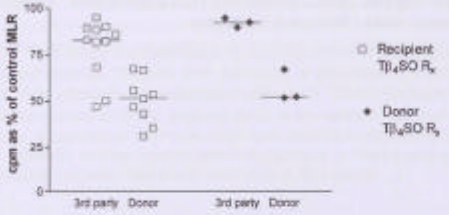
Thymosin beta-4 sulphoxide ($T\beta_4SO$) is a recently characterised peptide with potent anti-inflammatory effects. It was first identified in supernatants elaborated by monocytes exposed to glucocorticoids and is known to inhibit neutrophil chemotaxis and to reduce inflammation in vivo.

Using a mouse skin allotransplantation model we have shown that $T\beta_4SO$ also has hitherto unreported immunosuppressant properties. Mice treated systemically with $T\beta_4SO$ enjoyed prolonged allograft survival. Intriguingly, pre-treating donor mice with $T\beta_4SO$ prolonged allograft survival even where the recipients remained untreated. Prolongation of graft survival was similar, irrespective of whether it was the donor or recipient that received treatment.

Although $T\beta_4SO$ lacked any direct effect on T cell activation or proliferation in vitro, a mixed leucocyte reaction using $CD4^+$ lymphocytes from allograft recipients as responders showed evidence of donor-specific hyporesponsiveness (tolerance)- see figure. We hypothesise that $T\beta_4SO$ exerts its effects by interfering with recipient T cell priming. In support of this, $T\beta_4SO$ inhibits dendritic cell migration in vitro. This effect might impede the afferent limb of the immune response by inhibiting trafficking of passenger APCs from the graft to recipient draining lymph nodes thereby interfering with the direct pathway of allorecognition.

$T\beta_4SO$ may prove to be a useful addition to our immunosuppressive armamentarium with a novel tolerogenic effect that might complement the action of conventional immunosuppressants.



C57BL/6 skin was grafted onto BALB/b recipients treated with either intraperitoneal $T\beta_4SO$ or PBS. Recipient CD4+ cells were isolated at various time points and used as responders in a mixed leucocyte reaction (MLR) with either C57BL/6 (donor) or CBA (third party) dendritic cells as stimulators. Data is presented as cpm relative to the mean cpm in the control MLR (PBS group) expressed as a percentage to facilitate comparison between the responses to donor and 3rd party. Results of MLRs where donor alone received $T\beta_4SO$ treatment also shown for comparison.

P82

Semi-Quantitative Immunohistochemical Analysis Of Il-18 Expression By Tubular Epithelial Cells In Human Renal Transplant Recipients

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Introduction. An important event in solid organ transplantation is the early expression of cytokines at tissue sites. IL-18 is a pro-inflammatory cytokine involved in the innate immune response, and has a key role in the activation of infiltrating immunocompetent cells which direct allograft injury. Animal models suggest that IL-18 may be produced by renal tubular epithelial cells.

Methods. Post-perfusion biopsies from 11 patients (median age 48 years, range 34-65 years) were assessed by 3-stage indirect immunohistochemistry (IHC) using an IgG1 anti-IL-18 ab (R&D systems). The patterns of staining were confirmed by a second anti-IL-18 antibody of an IgG2a isotype. Isotype matched negative controls were performed in parallel. Biopsies were graded 0-3 based on the extent of tubular cell staining by two independent observers; 0 = no staining, 1 = <10% tubular staining, 2 = 10-50% tubular staining, and 3 = >50% tubular staining. Biopsies from 11 patients with acute allograft rejection (median age 35 years, range 24-63 years) were also assessed using identical methods.

Results. In post-perfusion biopsies IL-18 expression was predominantly localised to tubular epithelial cells. The cytokine was variably present in all biopsies (grade 1, n= 2; grade 2, n= 4; grade 3, n= 5). There was a trend towards increased expression in those patients who subsequently developed acute rejection and biopsies from kidneys with long cold ischaemia times (CIT). The expression of IL-18 by tubular epithelial cells was also variably demonstrated in acute rejection (grade 1, n= 2; grade 2, n=5; grade 3, n=4), although in patients with severe rejection expression was also seen by infiltrating mononuclear cells. There was a trend towards increased expression with long CITs.

Conclusions. The expression of IL-18 by tubular cells in both post-perfusion and acute rejection biopsies would support a role for this molecule in the development of immune mediated injury in human renal transplantation. Ischaemic-reperfusion injury may be involved in the induction of this expression.

P83

Are Pre-Transplant Sensitised Kidney Recipients At Higher Risk Of Transplant Failure?

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Introduction: 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. At the end of the follow-up period, 39% were on triple, 45% on double and 16% on mono therapy.

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% (expected 87-92%). Over the five year study period 21.5% of grafts were lost (table 1). In this follow up period, 37 patients had rejection episodes. 34 patients had 1 rejection episode and 3 patients had 2 rejection episodes (table 2). Delayed function, defined as the need for dialysis within the first post transplant week, occurred in 30 (37.9%) transplants (expected 34%). The 1 year graft function is shown in table 3. The mean serum creatinine at one year was 163 µmol/l. 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.

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

Table 1

Rejection	vascular	cellular	combined	classal
Recipients	16	10	8	3

Table 2

serum creatinine µmol/l at 1 year	died	failed	<100	101 -150	151-200	>201
% recipients	2.4	14	2.4	40	30.6	10.6

Table 3

P84

Does Exposure To Swine Leukocyte Antigens After Pig-To-Primate Xenotransplantation Provoke Antibodies That Cross-React With Human Leukocyte Antigens?

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Background: We have previously demonstrated that human leukocyte antigen (HLA) specific antibodies present in the sera of dialysis patients awaiting kidney transplantation cross-react with swine leukocyte antigens (SLA) expressed on porcine tissue. A potential concern of using pig kidney xenografts for human transplantation is that antibodies produced to SLA may cross-react with HLA and thereby limit the scope for future human organ donor transplantation. We therefore chose to investigate whether exposure to SLA after pig-to-primate kidney xenotransplantation gives rise to HLA cross-reactive antibodies.

Methods: Serum samples were obtained from 52 Cynomolgus monkeys that received kidney transplants from human decay accelerating factor (hDAF) transgenic pigs. Samples were collected pre-transplant and at time of autopsy (mean 20 days post-transplantation, range 1-53 days) and analysed for IgG HLA class I and HLA class II specific antibodies by solid phase enzyme-linked immunosorbent assay (ELISA) against pooled purified HLA antigens. To ensure the ability of the HLA ELISA to detect Cynomolgus monkey IgG binding, parallel experiments were performed to detect IgG Gal-α-1,3-Gal specific antibodies.

Results: ELISA analysis of serum samples collected both pre-transplantation and at autopsy was negative for IgG antibody binding to HLA class I and HLA class II antigens. Using the same ELISA antibody detection reagents IgG Gal-α-1,3-Gal specific antibodies were identified in 13 of the 38 (34%) sera obtained before transplantation and 21 of 52 (40%) sera collected post-transplantation, confirming that the negative HLA ELISA results were not due to technical failure.

Conclusion: This study suggests that exposure to SLA following acute vascular rejection of porcine kidneys by primates does not give rise to antibodies that cross-react with HLA.

P85

Probability Of HLA Matching For Human Embryonic Stem (hES) Cell Transplantation

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Introduction:
The use of differentiated human embryonic stem (hES) cells for replacement of diseased or damaged host tissue offers a potential therapy for the treatment of patients with certain metabolic deficiencies, autoimmune conditions and organ failure. The ability to generate an infinite number of differentiated cells from each embryonic stem cell line raises the possibility of creating a hES bank with a view to HLA matching to reduce the risk of graft rejection. We have examined how large a hES cell bank would need to be to make HLA matching a practical approach for minimising HLA disparity between hES donor type and the potential recipient.

Methods:
To generate a data set of blood groups and HLA types representative of a defined population from which hES cell donors might be derived, we used a series of consecutive cadaveric organ donors. ABO blood group and HLA-A, -B, -C, -DR and -DQ type was obtained on 1,500 consecutive cadaveric organ donors reported to UK Transplant during the two-year period from April 1999 to April 2001. A total of 6,577 patients (children and adults) registered on the UK kidney transplant waiting list was used to determine the likelihood of obtaining a blood group and HLA matched stem cell donor.

Results:
Assuming the need for donor blood group compatibility and that each hES cell line can provide donor tissue for an unlimited number of recipients, a donor cohort of 250 provided a 0.0.0 HLA mismatch for 19.8% of potential recipients and a favourable HLA match for 77.5% (Table). Further increases in the size of the donor pool conferred only a small increase in the number of HLA matched recipients.

Conclusion:
Obtaining a 0.0.0 or favourable HLA matched hES cell line can be achieved for the majority of potential recipients with a pool of 250 consecutive donors. Increasing the donor pool size beyond 250 does not confer additional benefits for improved HLA matching.

	HLA match grade			
Donor pool size (n=)	0.0.0	1.0.0/0.0.1	1.1.0	0-DR
50	1.6*	14.6	38.0	63.4
100	7.4	27.3	60.4	87.5
250	19.8	50.7	77.5	92.1
500	21.8	57.9	84.1	94.6
750	24.6	65.3	89.3	97.1
1,000	26.5	69.3	91.1	97.3

*Percentage of recipients within each HLA match grade

P86

DRB3 Allele Specific Antibodies In DRB3 Positive Individuals. A Possible Explanation Of Unexpected Positive Crossmatch In 0-0-0 Mismatched Donor-Recipient Pairs

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INTRODUCTION: Alloantibody results from exposure to mismatched HLA antigen via transfusions, pregnancy or transplantation. The presence of such antibody is often associated with hyperacute rejection and a pre-transplant crossmatch is an absolute requirement. Evidence suggests HLA matching has a beneficial effect on renal allograft survival and in the UK, donor kidneys are allocated preferentially to 0-0-0 mismatch reipients. Despite this high degree of matching, an unexpected positive crossmatch sometimes arises. This has traditionally been explained by the presence of undefined antibody, particularly against HLA-C or HLA-DP antigens. We present evidence suggesting an alternative explanation, namely the production of DRB3 allele specific HLA antibody in DRB3 positive individuals.

METHODS: HLA class II antibody specificity was determined using Luminex microbead arrays in 2 renal allograft recipients both of whom received a DRB3 allelic mismatched kidney and in a potential heart allograft recipient awaiting transplanation. Interpretation of antibody specificity was determined manually without assistance from analytical software.

RESULTS: (see table)

CONCLUSION: Mismatched DRB3 alleles induce an immune response resulting in DRB3 allele specific antibody. Graft failure has occured in both cases but whether this is cause or effect remains unknown. All prospective renal allograft recipients and donors should be typed to the highest resolution for DRB3 and other class II alleles. In the presence of DRB3 allelic antibody, the appropriate DRB3 allele should be defined as an unnaceptible antigen in DRB3 positive recipients on the UKTSSA waiting list and selection for kidneys carrying that allele avoided.

	Patient 1	Patient 2	Patient 3
DRB3 recipient	0301/2	0101-03	0101-03
DRB3 donor	0202/3/5/8	0202/3/5/8	acquired via pregnancy
DRB3 Ab	DRB3-0202	DRB3-0202	DRB3-0202
other class II antibody?	no	no	no
graft failed?	yes	yes	n/a
XM pre transplant	negative	negative	n/a
XM post failure	positive	positive	n/a
comments		Xm +ve DRB3-0202	Xm +ve DRB3-0202
		potential donor	potential donor

P87

Development Of An Improved Preservation Solution To Reduce Ischaemic Damage In The Liver

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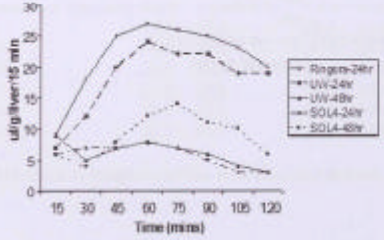
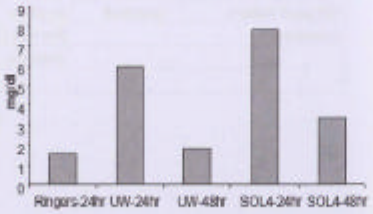
Previous work on isolated rabbit renal tubules showed that phosphate buffered 140mmol sucrose solution (PBS140), produced less tubular swelling in the presence of chemically induced cold and warm ischaemia.These beneficial effects may not only provide an optimal platform for kidney preservation, as already proven, but also produce similar actions in the liver. The aim of this study was to use the PBS140 modifications used in kidney preservation and evaluate their effectiveness in the liver.
Livers from male Wistar rats (n =6/group) were perfused in vivo with experimental flush solutions, removed and stored at 4°C for 24 or 48 hours. The livers were then perfused on an ex-vivo isolated reperfusion circuit. Analysis of functional parameters were used to assess liver recovery from ischaemia. These parameters included bile flow, bilirubin, bile acid content and liver enzymes (LDH, AST, ALT). Analysis of the functional parameters, unexpectedly showed that PBS140 was almost comparable to UW. We therefore made several modifications to PBS140 that led to a new base solution (SOL 4) containing 100mmol sucrose impermeant, alloprinol (antioxidant), lactobionate (impermeant), aspirin (cyclo-oxygenase inhibitor), glutathione (free radical scanenger) and diltiazem (calcium channel blocker), which was comparable to UW at 24 hours, however SOL 4 significantly outperformed UW in terms of bile flow (fig 1a), bile acid content and bilirubin (fig 1b) after 48 hours cold preservation ($P<0.05$).
In the preliminary testing of a modified phosphate buffered sucrose solution, this study has concluded that our new solution may provide more effective preservation than UW. This new solution could find widespread clinical use as it lacks the viscosity of UW. In a clinical setting where non-heart beating organs are required to increase the donor pool, it is hoped that quicker perfusion with a more effective solution could reduce ischaemic damage, with the consequent benefits of reduced primary non-function and reduced immunogenecity, and hence improved long term outcomes.

1a Bile flow following 24 and 48 hour preservation



1b Bilirubin levels following 24 & 48 hour preservation with SOL 4



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P88

Physiological Analyses Of Renal Preservation Solutions For Transplantation

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Common practice in the UK is to flush donor kidneys for transplantation with hyperosmolar citrate buffered Marshall's solution (HOC). HOC has been used for over two decades without further modification despite knowledge of deteriorating preservation over longer periods of cold ischaemic time (CIT). We used an isolated perfused rabbit proximal tubule model to investigate modifications to the core components of HOC to optimise renal preservation. Following organ retrieval and flush with one of the modified preservation solutions, the kidneys were stored at 4°C for 0-72 hours. Single lengths of cortical tubule were microdissected and transferred to an organ bath. The tubules were then subjected to warm ischaemia (37°C) by strophanthidin (5β,20[22]-Cardenoline-19-one-3β,5,14-triol) induced Na^+/K^+ ATPase blockade to biochemically reproduce the effects of ATP deficiency in ischaemia. Such reversible ATP-pump blockade induced cell oedema through intracellular fluid shift. Measurements of tubule cell diameter were made over 75 minutes to assess the degree of oedema in the presence of HOC and modified HOC. By exchanging mannitol with sucrose, a significant reduction in cell swelling was detected ($p<0.05$) after >24 hours CIT. Similarly, by reducing the tonicity of HOC from 400mOsmol to 300mOsmol, significantly less cell swelling occurred ($p<0.05$). Oedema was also significantly reduced by replacing citrate with phosphate ($p<0.05$). The data suggested that using an isosmolar phosphate buffered sucrose solution (PBS140) could improve organ preservation. Analysis comparing PBS140 with HOC and UW, the 'gold standard' organ preservation solution, showed that PBS140 provides better protection from warm ischaemic cell swelling. We then proceeded to develop an isolated rat kidney perfusion model to evaluate the effects of preservation solutions on whole organ function. Rat kidneys were retrieved and stored as described previously and reperfused with a modified 4% albumin ringers solution over a period of 2 hours. The data and histology obtained from these experiments suggested that PBS140 provided better whole organ preservation than HOC. The models developed here have permitted physiological investigations of the response of tubules and whole organs to preservation solutions that may be used to suggest alternative clinical practice to significantly improve outcomes in transplantation surgery in the UK.

P89

Sequence Of Reperfusion Influences Ischaemia/Reperfusion Injury (IRI) In Orthotopic Liver Transplantation (OLT) In The Procine

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Introduction: IRI following organ transplantation is one of the major causes for primary non function of donated organs. In clinical OLT reperfusion is commonly performed via primary portal reperfusion. This study evaluates different reperfusion sequences after OLT.

Material and methods: After premedication (Stresnil 3mg/kg, Dormicum 0.3 mg/kg and Ketamine 2mg/kg i.m.), narcosis (Hypnomidate 1.2mg/kg) was inducted and kept up (volume controlled N2O/O2 ventilation, i.v. ketamie 19mg/kg/h and hypnomidate 1.25 mg/kg/h). No vasoactive substances were applied. After a mean cold ischaemic time of 297 ¡Ó 47min OLT was performed in pigs (German Landrasse, 25-30kg):
Group 1: simultaneous reperfusion, n=8, warm ischaemic time (WIT) 70min
Group 2: primary portal reperfusion, n= 8, WIT 60min, arterial reperfusion 10min later
Group 3: primary arterial reperfusion, n= 8, WIT 60min, portal reperfusion 10min later
Short term survival (STS:> 240 min, MAP>50mmHg, HR < 120bpm), the degree of graft damage and initial graft function were analysed. Haemodynamics (portal venous resistance), laboratory and bile production were analysed 30, 60, 120, 180 and 240min after reperfusion. Histological examination (stainings & immunohistochemistry: HO-1 & HSP 70) examined the degree of graft damage. P- values < 0.05 (Mann-Whitney Test) were regarded to be significant. All animals were sacrificed in narcosis by overdose.

Results: STS results show Group 1:75%; Group 2= 67.5%; Group 3= 37.5%. Group 1 revealed significant better results compared with Group 3, whereas compared with Group 2 it just showed a tendency for better laboratory results. Same tendencies were found in the comparison of Group 2 with Group 3. The histomorphological score was best for Group 1. This was verified by HO-1 and HSP-70 semi quantitative analysis. Differences in haemodynamics compared with physiological data were found in groups 2+3 but not in Group 1. Group 1 produced significant higher amounts of bile compared with both other groups.

Conclusion: Alternations in reperfusion sequence influences graft function and IRI after OLT in pigs. This study supports that liver grafts benefit from a physiological (simultaneous) reperfusion even though warm ischaemic time was 10min longer. The maximum beneficial prolongation of warm ischaemic time for simultaneous reperfusion remains to be studied.

P90

Which Is More Important In Renal Transplantation, Time Or Temperature?

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Introduction
Delayed graft function (DGF) adversely affects the outcome of renal transplants in terms of rejection, allograft and patient survival, and transplant costs. Both donor and recipient factors are implicated in the occurrence of DGF. Ischaemia during allograft preparation and implantation has often been neglected. The aim of the study was to use a non-invasive technique to measure kidney temperature variations during allograft preparation and implantation and its association with DGF.

Methods
25 cadaveric renal transplants performed from May' 2003 to Nov '2003 were assessed. Kidney surface temperature was measured using an infrared thermometer (CHY 6101c) during ex vivo preparation and implantation until reperfusion. The ischaemia period from allograft preparation to reperfusion was quantified using area under temperature - time curve (minute.celsius)

Results (see table)

Conclusion
DGF was associated with older donor age, higher temperature during implantation and higher AUC (minute.celsius). Ex vivo preparation of the allograft should be rapid and adequately performed in an appropriately cold phase.

	IF (n = 15)	DGF (n = 10)	p value (MWU)
Donor age(yr)	35.9	50.4	< 0.05
Recipient age(yr)	48.3	47.7	NS
CIT(hrs)	19.3	20.2	NS
Preparation time(mins)	37.9	41.7	NS
2[nd] WIT(mins)	39.2	42.3	NS
Mean temperature during preparation (˚C)	5.4	5.3	NS
Mean temperature during implantation (˚C)	19.4	21.7	<0.05
AUC – preparation(minute.celsius)	225.9	427.1	< 0.01
AUC - implantation(minute.celsius)	847.4	1101.2	< 0.01
AUC – preparation+ implantation(minute.celsius)	1071.7	1526.2	< 0.01

P91

HTK Ameliorates Warm Ischaemic Endothelial Damage.

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Introduction

Endothelial preservation is a key component of long term successful transplantation. In recipients of non-heartbeating renal transplants we have noticed a high incidence of vascular endothelial lesions on protocol biopsies. We hypothesise that warm ischaemia and *in situ* cooling prior to organ retrieval leads to endothelial damage.

Methods

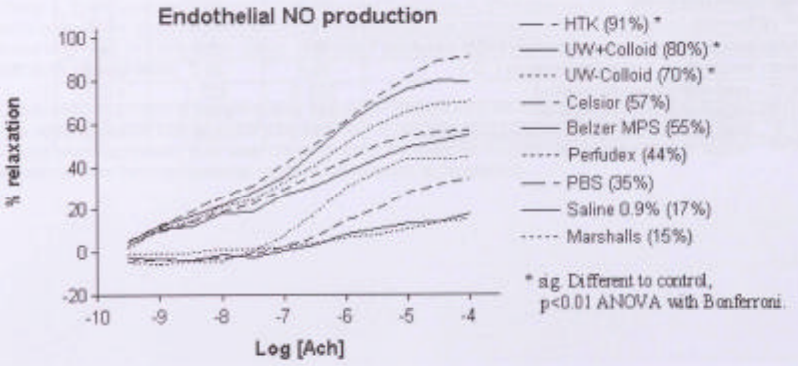
We compared the production of endothelial nitric oxide (NO) in vascular tissues treated with 8 different preservations solutions. After 1 hour of post-mortem warm ischaemia the abdomens of adult male Lewis rats were opened and the aorta cannulated. 55mls of chilled heparinised preservation solution were then pumped through the aorta and viscera over a further 60 minutes. After careful retrieval the thoracic aorta was stored for 24 hours in chilled preservation solution (4°C). Each aorta was sliced into rings and placed on a force transducer in warmed (37°C) oxygenated Krebs-Hensleit. Cumulative doses of acetyl-choline were then added to each bath to stimulate viable endothelium to relax the pre-contracted ring.

Results

Dose-response curves are presented in the attachment (n=32 per group). Figures in brackets represent maximum relaxation. The control group was treated with normal saline.

Significance

HTK, which is cheaper and less viscous than UW, may represent the optimal flush solution for human non-heartbeating endothelial preservation.



P92

Nitrone Based Compounds As Superior Anti-Oxidants To Combat Ischaemia Reperfusion Injury

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Aim:

To develop novel anti-oxidant compounds to mitigate acute the effects of oxidant stress.

Introduction

Oxidant stress is a major contributory factor in ischaemia reperfusion injury. It has also bee implicated in late graft dysfunction, cardiovascular disease , neurodegeneration, stroke and the early death of cloned animals. Reactive oxygen species, such as hydroxyl radicals, peroxyl radicals and superoxide radical anions cause significant damage to cellular macromolecules leading to a decline in cellular integrity and potentiating growth arrest. This impacts both on the immediate function of an organ, and subsequent ability to deal with further stress (e.g immune injury). Evidence in animal models and in transplant patients supports the hypothesis of increased oxidative stress within a graft post transplant. Conventional anti-oxidants, such as SOD, show limited efficacy in ameliorating such stresses. We, however, have developed a series of superior, novel nitrone based, anti-oxidant compounds to combat oxidant stress associated with ischaemia/reperfusion injury in transplantation. These have been tested for their ability to mitigate oxidant challenge in a human cell culture model.

Methods.

Cultures of primary human foreskin fibroblasts were treated with 150uM H2O2 in the presence and absence of nitrone compounds or conventional anti-oxidants. Cultures were investigated for markers of oxidative stress and related senescence associated (p16 and p21, Sirtuins1,2,3,7) gene expression by Real Time quantitative RT-PCR (TaqMan). And by staining for senescence associated beta galactosidase(SA b Gal).

Results.

Eight novel nitrone were synthesised and the compounds tested in the human cell culture model and two were selected for further evaluation on the basis of efficacy and cell growth in the absence of oxidant challenge. Both provided significant and enhanced protection from acute oxidant stress resulting from exposure to150uM H2O2. P16 and p21, expression were significantly repressed versus controls indicating that DNA damage responses were limited in the presence of the nitrones. SA b Gal staining, a classical cytological marker of senescence was also diminished versus controls, indicative of the efficacy of these compounds. Both compounds provided better or equivalent protection to PBN and a commercial anti-ischaemia/ reperfusion agent.

Conclusions.

We have successfully developed and shown efficacy for the anti-oxidant capabilities of two novel nitrone compounds. These compounds appear to ameliorate DNA damage responses and reduce macromolecular damage in the cell. These abilities make these compounds promising candidates for combating transplant related I/R injury.

P93

Orthotropic Heart Transplantation For High-Risk Patients: A 15 Years Experience

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Introduction:
Orthotropic heart transplantation (OHTx) is an established treatment for end-stage heart diseases and the outcome has improved in the last two decades with the advances in the organ preservation and immunosuppression. Pre-operative optimisation of the recipient is vital and more so in unstable patients. This high-risk group of patients remain as inpatients requiring pharmacological and mechanical cardiac support. It can potentially lead to infections and also multi-organ failure, which can influence their survival. We studied a group of high-risk patients and their post-operative morbidity and survival following OHTx.

Method:
A total of 341 patients underwent OHTx between 1987-2002 for ischemic heart disease (IHD) and dilated cardiomypathy (CM), of which 22% of them were unstable and inpatients. Donor demographics, body mass index (BMI), cause of death, the need for inotropic / hormonal support were collected. Recipient demographics, BMI, preoperative haemodynamics, perioperative / postoperative characteristics, endomyocardial biopsy score, recent left ventricular ejection fraction, a median follow-up of 10 years, and cause of death were collected. Patients who underwent OHTx for cardiac pathologies other than IHD and CM were excluded in order to obtain an unbiased representation. The collected data was analysed against the duration of the recipient's preoperative stay, requirement & nature of cardiac support, and a risk of acquiring infection. The data was analysed using SPSS.

Results:
There was no significant difference (p-value: 0.429) was observed in the cumulative survival between inpatient and standard patient groups. But there was significant difference in the survival in patients underwent OHTx for ischemic heart disease was observed:

	One year	5 years	10 years
Inpatient	79%	65%	43%
Standard	83%	72%	61%

(cox regression analysis : significance - < 0.005)

Conclusion:
The study clearly demonstrates the significantly reduced survival in recipients undergoing OHTx as an inpatient for IHD. The inherent and global ischemic process could be accountable for these results.

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Does Donor Catecholamine Administration Affect Early Lung Function Post-transplantation?

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Background

Exogenous donor catecholamine administration (EDCA) appears to improve outcome in liver and renal transplantation but worsens prognosis in heart transplantation. EDCA can also increase alveolar fluid clearance following brainstem death. Our aim was to assess the effect of EDCA on early lung fuction following lung transplantation (LTx).

Methods

A retrospective analysis of donor and 6 hour recepient gas exchange was performed in a series of 60 LTx (27 single: 33 bilateral). The reduction in PaO_2/FiO_2 ratio ($\Delta PaO_2/FiO_2$) from pre-harvest to 6 hours post-implantation was compared according to donor catecholamine (but not vasopressin) treatment at the time of organ retrieval.

Results

Catecholamines were used in 29/60 donors. There was no significant difference in initial mean PaO_2/FiO_2 ratio between catecholamine treated and untreated donors [504 (SD 74) & 486 (SD 86)] respectively. Although a significant fall in PaO_2/FiO_2 ratio was seen in both groups {$\Delta PaO_2/FiO_2$ 200 (SD137, $p<0.001$) & 272 (SD111,$p<0.001$) for catecholamine un-treated and treated respectively, this fall was significantly greater in the catecholamine treated group ($p=0.05$). $\Delta PaO_2/FiO_2$ did not correlate with the ischemic time, preservation technique, operation type or reperfusion strategy.

Conclusion
Impairment in early gas exchange is a uniform observation post-lung procurement, preservation and implantation. This impairment is increased when the donors receive exogenous catecholamines. Possible explanations include a direct effect on donor left atrial pressure or other haemodynamic parameters. Alternatively a requirement for EDCA may identify a sub-group of donors in whom peri-brainstem phenomena, previously shown to affect function, are more severe.

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The Rights Of Donors, Recipients And Patients Awaiting Transplantation

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Although renal transplantation has become routine, it still carries a significant mortality and morbidity. Furthermore, transplanted organs vary significantly in their quality, with clear implications for the recipient. The rights of the recipient to be informed about the risks of transplantation with a particular organ may need, according to some, to be balanced against the responsibility to those on the transplant waiting list not to waste valuable organs. In living donation, issues of ownership of the transplanted organ may conflict with traditional concepts of patient autonomy.
To explore these issues a questionnaire (and 2 reminders) was sent to transplant surgeons at all renal units in the UK. 37 replies were received.
Surgeons were asked whether they would inform a potential recipient about a below average outcome from a barely transplantable kidney. 27 (73%) said they would always do so, 6 (16%) said only if asked and 4 (10%) said they would not use the organ at all.
In response to a question about a CMV positive donor and CMV negative recipient, 19 (51%) said they would inform the patient of the risk of CMV infection, 11(30%) said only if asked, 6 (16%) said they would never do so and 1 (3%) thought this not applicable.
A third question asked about a biopsy from a living donor kidney after nephrectomy but before transplantation. 4 (10%) would seek consent only from the recipient, 2 (5%) only from the donor, 24 (65%) from both donor and recipient and 3 (8%) from neither (in 4 this was not applicable).
27 (73%) respondents thought recipients should have more information about donor organs, 7 (19%) thought they should not, and 3 (8%) did not respond. 3 (8%) also commented that more information might lead to wasted organs.
In summary, there is significant variation amongst the surgical transplant community regarding the rights of donors and recipients. Although the majority would always inform the recipient of risks related to organ quality, fewer would do so for outcomes with a lower but still significant risk. A significant minority did not believe that ownership of a living donor organ was related to both donor and recipient. A clear majority felt that recipients need more information than they are currently given. Further work is needed to define and resolve the ethical conflicts raised, and more debate is needed to increase recipient awareness of risk.

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An Attitudinal Survey On Facial Transplantation Of Healthcare Professionals And Patients

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Introduction
Extensive loss of facial tissue remains a serious reconstructive challenge. Facial transplantation is a potential solution. It, however, poses serious medical, surgical, ethical and psychological consequences that have to be addressed. The use of immunosuppressant therapy with its resultant metabolic, infective and malignancy complicatons on an otherwise healthy individual needs to be addressed. The psychological impact of the procedure on the patient and on his family as well as the surgical risks involved have to be explored. We have carried out a study to explore the acceptability of facial transplantation.

Aims and objectives
To explore the acceptability of facial transplantation. The objectives were to identify the concerns of health professionals and patients as well as to identify factors vital to consent.

Materials and methods
A questionnaire based study was used which enabled us to collect both qualitative and quantitative data. There were 200 participants made up of a sample of health professionals, members of the public, renal transplantation patients and patients with facial deformity. Emerging issues were identified between the different groups and statistical relationships were established using SPSS, a statistics software.

Results
Male gender and familiarity with a transplantation programme significantly increased the likelihood of donating for facial transplantation. The risks of surgery and the side effects of the immunosuppressive regimen reduced the tendency to participate in a facial transplantation programme. Identity has emerged as one of the most important issues with 81.5% positive about receiving a facial transplant provided there was no resemblance to the donor.

Conclusion
Facial transplantation continues to stimulate a lot of debate. This study identified the major issues around the subject of facial transplantation. It provides the impetus for further study to address the major concerns before facial transplantation could become a clinical reality.

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Does Donor Cardiopulmonary Resuscitation Time Affect Outcome In Uncontrolled NHBD Renal Transplants?

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Introduction In Maastricht category II uncontrolled NHBD transplants there is a period of cardiopulmonary resuscitation (CPR) of variable duration. Even during optimal CPR, cardiac output by precordial compression is reduced to around 30-40% of normal(1), and we were therefore concerned that kidney viability would be reduced by prolonged CPR.

Methods We reviewed our series of 46 category II non-heart beating donors from 1998 to 2003, recording duration of CPR (excluding bystander CPR) and outcome. Primary endpoints were discard rate, primary non-function rate and duration of delayed graft function.

Results The mean and standard deviation of the CPR duration for all donors was 60±20 minutes; of those where the kidneys were transplanted it was 58±17 minutes (range 38-99 minutes), and of those where both kidneys were discarded it was 62±23 minutes (range 34-124 minutes) (unpaired t test, p=0.523). There was no statistically significant difference in discard rates using 60, 70, 80 or 90 minutes of CPR duration as cut-off points.
We took 60 minutes as our hypothetical cut-off point. The two groups of donors were well matched in terms of sex, total warm ischaemic time and total ischaemic time, but the group with CPR duration <60 minutes had a significantly longer primary warm ischaemic time (27.3±7.9 vs. 20.2±7.5 minutes, p=0.009) and were almost significantly older (51.4±5.6 vs. 46.6±10.4 years, p=0.105).
There was no statistically significant difference between the groups in terms of discard rate (58% vs. 57.3%, p=1.000), primary non-function rate (8.5% vs. 10%, p=1.000), or duration of delayed graft function (15.3±14.4 vs. 12.6±6.6 days, p=0.548).

Conclusion Although the numbers in this study are limited, we found no significant difference in outcome depending on duration of CPR, and will continue to retrieve kidneys from donors irrespective of duration of CPR, with our usual viability testing after retrieval(2).

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From Presentation To Publication – How Does The British Transplant Society Compare To Other Meetings?

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Background
The annual meeting of the British Transplant Society (BTS) provides a forum for the discussion of work. For the dissemination of this information, peer reviewed publication is required. The aim of this study was to establish the amount of presentation which go on to publication and compare this with 3 other UK based surgical meetings.

Method
We obtained abstracts for all BTS presentations for 2000, 2001 and 2002. In October 2003 we determined whether a presentation had led to a successful publication using PubMed®. For 2001 we compared the BTS publication rate with the meetings of the Vascular Surgical Society (VSS; November 2001), the Association of Coloproctology of Great Britain and Ireland (ACGBI; July 2001) and the Association of Surgeons of Great Britain and Ireland (ASGBI; May 2001). We also compared the median impact factor (IF) of journals used for each BTS year and between meetings.

Results
The number of presentations successfully published for each BTS meeting (as of Oct '03) is shown in the table (below).

Publications were to be found in 39 different journals, with Transplantation Proceedings (41), Transplantation (40) the most frequently utilised. There was no difference between the median impact factor of journals utilised each year (Kruskal Wallis p=0.219). The 2001 meetings of the BTS, ASGBI and VSS had a significantly greater proportion of total publications within 2 yrs than the ACGBI (Chi sq. p<0.001). However there was no difference in the median impact factors of the journals used between the meetings (Kruskal Wallis p=0.883).

Conclusion
The BTS compares well with other meetings in the UK in terms of spreading of information from presentation to peer review publication.

Year	Total	Published		Total published	Median impact factor (IQR)
		<1yr	>1yr		
2000	102	22	34	**56** *within 42 months*	2.581 (0.478-3.265)
2001	132	23	31	**54** *within 30 months*	3.265 (1.497-3.355)
2002	141	31	5	**36** *within 18 months*	2.307 (0.478-3.399)

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The Role Of The Registered Nurse Surgical Practitioner In A Renal Transplant Unit (RTU)

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The aim of this presentation is to describe our initial experiences in establishing the role of a Nurse Surgical Practitioner (NSP) in a major transplant unit and discuss the ways in which this role may develop.

The Problem:

Most transplant surgical services have the all too familiar situation of:

· No SHO's to assist in theatre – therefore lack of consistency in juniors assisting at point of transplant.
· Excellent nursing care on the unit that was suffering from lack of continuity at point of transplant – when many patients are feeling scared and at their most vulnerable.
· Too many last minute cancellations of elective surgery for avoidable reasons.

The Answer?

To provide someone who could address these issues, give continuity of care below Consultant level, and who had undertaken nationally recognised academic and clinical training / assessment. The answer was:

· A non-medical practitioner who could work both in and out of the operating room and who could be taught to undertake specified surgical intervention under direct, indirect or proximal supervision.

The Aim of the Nurse Surgical Practitioner Role:

The primary aim of the role has been to improve and optimise patient care by:

· Providing continuity of individualised patient care from RTU to theatre and back.
· The introduction of a pre-operative visiting programme to help plan the patients peri-operative care and to allay any fears that they may have about coming to theatre.
· The introduction of a pre-admission clinic for patients undergoing major renal surgery aimed at reducing cancellations and so improve the service we offer our patients.
· Providing the operating surgeons with a level of consistent assistance at point of surgery…especially at SpR rotation time.

Future Role Development?

To provide the renal patient with the best service and patient care by:

· Faster access to stent and Tenchkoff removal lists by teaching the NSP to undertake these under proximal supervision.
· Faster access to Tenchkoff insertion by teaching the NSP to undertake these under proximal supervision.
· Ongoing audit of practise and outcomes – e.g. on-going audit of the efficacy of NSP-led pre - admission clinics in reducing cancellation rates.
· Instigating research to improve patient care e.g. an RCT into pre-operative skin preparation solutions on wound sepsis rates.

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For full details of the British Transplantation Society please refer to the Society website:

www.bts.org.uk

For more details of this meeting, please refer to the meeting website:

www.bts2004.org.uk