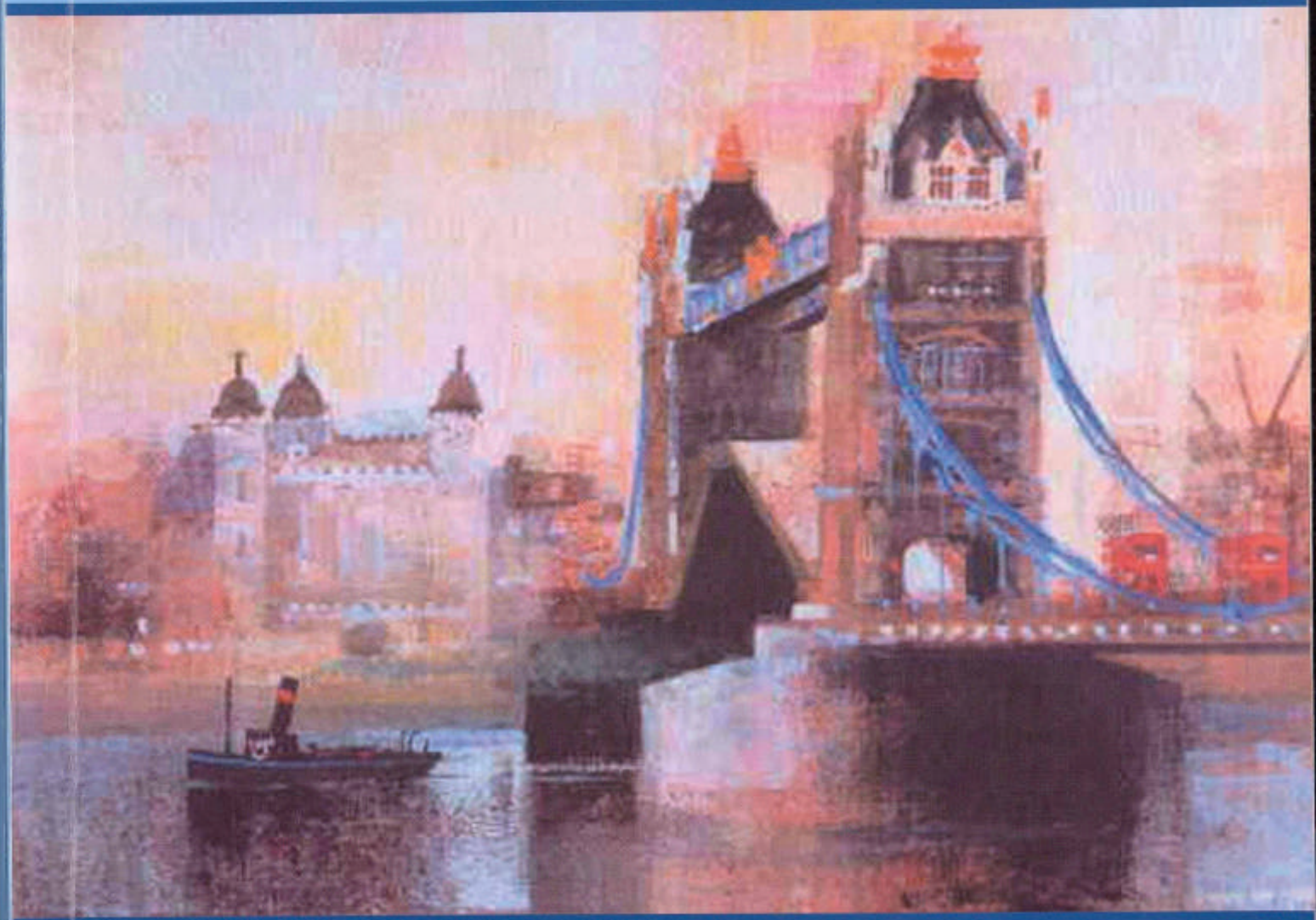
British Transplantation Society

6th Annual Congress, London



8-10th April 2003

Commonwealth Institute,
Kensington

O1

Renal warm ischaemia and cyclosporine exert a synergistic effect on injury and fibrosis in the kidney

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Interaction between cyclosporine (CsA) and renal warm ischaemia (RWI) in generating renal injury is a controversial area. Previous animal studies demonstrated variable results. The aim of this study was to investigate the effect of treatment with CsA on RWI injury in the rat. Male Sprague-Dawley rats (6 per group) were anaesthetised with halothane and the left renal pedicle clamped for 30 minutes through a flank incision. Animals were injected daily intraperitoneally either with CsA 15 mg/kg or the drug vehicle for 30 days. Kidney sections were stained with Masson's trichrome for fibrosis scoring. Immunohistochemical staining was performed for tubulointerstitial (TI) collagen III, collagen IV, TGF-β_1,and ED_1(macrophage marker). mRNA expression was measured for collagen III, collagen IV and TGF-β_1 by Northern blot analysis using GADPH as the housekeeping gene. Gelatin was used as a substrate to assess overall homogenate matrix metalloproteinases activity.

Either CsA or RWI alone had no significant effect on any measured variable of renal injury. However, in rats treated with CsA, RWI for 30 min induced a significant increase in fibrosis score (0.1 ± 0.05 vs. 1.96 ± 0.49, P<0.05). Immunochemical analysis also showed an increase in TI collagen III (8.5 ±± 5.0% vs. 37.9 ± 12.1%, P<0.05), TI collagen IV (39 ± 4.9% vs. 58.6 ± 3.1%, P<0.01), TI TGF-β_1(3.7 ± 1.4% vs. 39.5 ± 7.3% P<0.01) and TI macrophage influx (0.9 ± 0.1 vs.64.9 ± 15.4 cells/field, P<0.01) compared to drug vehicle .

There was significant upregulation of collagen III mRNA (3.3 ± 0.5 vs. 12.3 ± 3.8, P<0.01), collagen IV mRNA (4.7 ± 3.9 vs. 14.9 ± 1.72, P<0.05) and TGF-β_1 mRNA (8.5 ± 0.6 vs. 18.1 ± 2.7, P <0.01) compared to the drug vehicle. The MMP activity demonstrated significant reduction following CsA treatment of RWI (1.3 ± 0.2 vs. 0.9 ± 0.01, P<0.05) compared to CsA alone.

We conclude that treatment with CsA potentiates the renal response to warm ischaemia through increasing the synthesis of matrix proteins and the major fibrogenic growth factor TGF-β_1. RWI also potentiates the nephrotoxic effect of CsA through inhibition of MMP system.

O2

Normothermic preservation of Non-heart-beating donor porcine livers- Impact of prior cold preservation

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Background: Normothermic perfusion has been shown to resuscitate non-heart beating donor (NHBD) livers, which have undergone significant warm ischaemic injury. However, the logistics of clinical organ retrieval are complex and a period of cold storage prior to warm preservation would be of great benefit in simplifying the process. We investigate the effects of a short period of cold preservation before normothermic preservation.

Methods: Porcine livers were subjected to 60 minutes of warm ischaemia and then assigned to following groups.
Group I (n=5): Normothermic preservation for 24 hours.
Group II (n=4): Cold preservation in University of Wisconsin solution for 4 hours followed by normothermic preservation for 20 hours (total preservation time 24 hours).
Bile production, hepatocellular damage and metabolic function were compared on the circuit during preservation.
Mann-Whitney U test was used for statistical analysis using spss software.

Results: Group I livers had greater bile production (Consistently greater than 9 ml/hour) and metabolic activity (Lower base deficit and greater glucose utilisation). In Group II livers, by the end of preservation the bile production was less than 1.5 ml/hour, the perfusate became progressively more acidic and livers showed no evidence of glucose utilisation suggesting that these livers were non viable. Group II livers also had greater hepatocellular damage and sinusoidal endothelial cell dysfunction as measured by hyaluronic acid. On histology, group II livers had > 33% necrosis. In contrast group I livers had preserved tissue architecture with minimal necrosis.
See table 1 below.

Conclusion: Normothermic perfusion failed to resuscitate porcine livers after 60 minutes of warm ischaemia and 4 hours of cold preservation. Whether an even briefer period of cold preservation would be compatible with liver viability remains to be established.

Table 1.

At 20 hours of perfusion	Group I (n=5)	Group II (n=4)	P Value
	Mean± SEM	Mean± SEM	
Bile Production ml/hour	9.1±1	1.5±0.4	0.014
Base excess mEq/litre	-5.8±2.8	-17.5±2.2	0.027
Glucose mmol/litre	22.5 ± 2.9	35 ± 0	0.011
ALT U/litre	68.5 ± 19.3	237 ± 55	0.057
AST U/litre	671±408	4711±1300	0.021

O3

The predictive nature of intra-renal resistance during machine perfusion after warm and cold ischaemic damage

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Background

Non-heart beating donor (NHBD) kidneys have been shown to increase transplant activity, but their use is associated with a high rate of non-function due to warm ischaemic damage. Viability assessment would allow accurate estimation of warm ischaemic time (WIT) and prediction of future function. One method is the measurement of intra-renal resistance (IRR), which is thought to increase with WIT.
We aimed to measure IRR on machine perfused (MP) porcine kidneys subjected to different WITs, with additional, varying, cold ischaemic times (CIT), in an attempt to mimic the injury suffered by NHBD kidneys.

Methods

Landrace pigs were killed by lethal injection, and the kidneys were subjected to varying in-situ WITs of 10-90 minutes prior to explantation. Kidneys were subsequently stored for varying cold times of 2 to 48 hours (n=6 for each group). Perfusion pressure and perfusate flow were measured during 6 hours cold pulsatile machine (RM3 Renal Preservation System with Marshall's hyperosmolar citrate), and expressed as IRR (pressure/flow).

Results

For all WITs, IRR was higher at the start than the end of machine perfusion ($P<0.001$). There was a strong correlation between IRR on MP, and WIT ($r = 0.962$, $r2 = 0.9278$, $P<0.002$), but no correlation after 6 hours MP.
Intra-renal resistance increased as kidneys were exposed to longer CITs; this effect was most marked for the longer WITs ($P<0.004$). The slope gradient ($y=mx+c$) was similar for the two WITs.

Conclusions

Early IRR accurately reflects kidney in-situ WIT. Machine perfusion reduced IRR, with the greatest reduction for the longest WITs. Cold ischaemia imposed on periods of warm ischaemia increases IRR and attenuates the beneficial effect of MP.
Machine perfusion may partially ameliorate the effects of WIT in terms of IRR, and may prove useful in pre-transplant viability assessment of NHBD kidneys.

O4

Portal venous resistance for evaluation of ischemia/reperfusion injury (IRI) after orthotopic liver transplantation (OLT) in pig

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Introduction: The IRI following organ transplantation is one of the major causes for primary non function (PNF) of donated organs. Until today there is no reliable examination which might detect IRI directly after successful transplantation.The IRI mainly causes morpho-logical alternations at the site of the sinusoids, which lead to changes in haemodynamics. Aim of this study was to investigate haemodynamic alternation after OLT in pigs.
Material and methods: After premedication (Stresnil 3mg/kg, Dormicum 0.3 mg/kg and Ketamine 2mg/kg i.m,) narcosis was inducted (Hypnomidate 1.2mg/kg) and was 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. Pressure and flow measurements were performed before donor and recipient hepatectomy in a total of 58 pigs (German Landrasse, 25-30 kg) for obtaining physiological data. Pressure of liver veins (PVH) and portal vein pressure (PVP) were detected directly with inserted katheters, mid arterial pressure was taken for pressure in the A. hepatica (PAH). Flow measurements in A. hepatica (FAH) and in the portal vein (FPV) were obtained by run-time ultrasound detection. Resistances in A. hepatica (RAH) and portal vein (RVP) were calculated. After a mean cold ischemic time of 297 ± 47 min OLT was performed in 24 animals. Haemodynamics were measured at times 30, 60, 120, 180 and 240min after reperfusion. P- values < 0.05 (Mann-Whitney Test) were regarded to be significant. All animals were sacrificed in narcosis by overdose.
Results: The mean weight of the transplanted organs was 633.8±123.6g. Physiological arterial blood flow resulted in 37.3ml/100g, portal flow 129.3ml/100g liver tissue. The RAH was 0.266mmHg*min*ml-1, the RVP was negative with -.00545mmHg*min*ml-1. The FAH decreased significantly at all times after reperfusion, with the lowest flow after 120min. The portal venous flow was only reduced significantly 30 min after reperfusion, with a second reduction after 180min. There was no significant change in RAH, but for RVP there were significant increases at 30, 120 and 240min after reperfusion. The highest increase in RVP was noticed parallel to the lowest arterial flow at 120 min. These changes were mirrored by laboratory and histological investigations.
Conclusions: We report for the first time a physiological negative RVP, which correlates with the FAH. Liver haemodynamics present a potential examination method for direct evaluation of IRI. Explanations for negative RVP might either be a Bernoulli-effect of the A. hepatica and/or conduction of the negative intrathoracal pressure.

	physiogical	30 min	60 min	120 min	180 min	240 min
FAH in ml*100 37.3		21.4*	19.3*	17.7*	19.6*	21.2*
FVP in ml/100 129.3		106.1*	118.5	125.5	113.9	115.8
PAH	.2680	.3229	.2911	.3222	.3156	.3246
RVP (minus)	.00545	.00150*	.00393	.00225	.00343	.00360

O5

Modular Extracorporeal Liver Support (MELS) based on primary human hepatocytes originating from discarded donor organs

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Introduction

Cell-based extracorporeal liver support is an option to assist the failing organ until regeneration or until transplantation can be performed. The use of porcine cells or tumo cell lines is controversial. Primary human liver cells, obtained from explanted organs, found to be unsuitable for transplantation, are a desirable cell source as they perform human metabolism and regulation. Aim of our pilot study is to demonstrate the feasibili of an extracorporeal liver support system based primary human hepatocytes originating from discarded donor organs.

Methods

The Modular Extracorporeal Liver Support (MELS) concept combines different extracorporeal therapy units. A multi-compartment bioreactor is loaded with human liver cells, obtained by 5-step collagenase liver perfusion (cell mass: 400g - 600g). A detoxification module enables continuous single pass albumin-dialysis and veno-venuous hemodiafiltration via a standard high-flux dialysis filter.

Results

To date, cells from 93 human livers were isolated (donor age: 56 ± 14 years, liver weight: 1841 ± 527 g). These grafts were not suitable for liver transplantation (LTx), due to steatosis (53%), cirrhosis (11%), fibrosis (14%), arteriosclerosis (8%), and other reasons (14%). Cell recovery and adaptation to the system was observed after three days. Enzyme release and electrolytes were balanced within the first three days of culture period. A slowly declining metabolism was observed up to day 21. Out of 59 prepared bioreactors (viability 60 ± 12%), 11 bioreactors were clinically used in a pilot study in nine patients (one patient was connected to two bioreactors – one prior to LTx one post transplantation). Two patients with acute liver failure and two patients with acute-on-chronic liver failure (A-o-C LF) were successfully bridged to LTx. Two patients with A-o-C LF were not eligible for LTx due to continuing alcohol consumption: They were supported during a period of acute deterioration. Three patients were successfully bridged to re-transplantation after primary non-function of their liver graft. The overall treatment time was 7 - 144 hours. No adverse events were observed.

Conclusion

Isolation and culture of living primary human liver cells from discarded organs in bioreactors is feasible. Therapy with MELS bioreactor based on human hepatocytes is safe and well tolerated. The combination of cell-based liver support systems with detoxification and dialysis in a modular approach is promising.

O6

De-repression of heat shock transcription factor-1 in Interleukin-6 treated hepatocytes is mediated by down-regulation of glycogen synthase kinase 3B and MAPK/ERK-1.

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Aims: Heat shock proteins (HSP) are cytoprotective and upregulation of these chaperones may reduce ischemia-reperfusion injury in transplanted organs. HSP induction requires activation of heat shock transcription factor-1 (HSF-1) and we hypothesized that IL-6 would prime the HSP response by causing de-repression of HSF-1 resulting in augmented expression of HSP in stressed cells.

Methods: Huh-7 human hepatocytes were exposed to IL-6 1ng/ml at 37°C and or treated with HS at 43°C for 45 minutes with recovery at normothermia. Following SDS-PAGE, Western blots were performed for HSF-1, active (pAbTEpY) and total MAP kinase pERK, glycogen synthase kinase 3B (GSK3B) and HSP70. GSK3B activity was measured by kinase assay. DNA binding was studied by EMSA and transcriptional activity by transient transfection of a plasmid containing the inducible HSP70 promoter coupled to b-galactosidase.

Results: IL-6 treatment decreased active MAPK/pERK nd GSK3B expression and GSK3B kinase activity. In IL-6-treated cells, monomeric HSF-1 accumulated in the cytoplasm and nucleus, bound DNA but was transcriptionally inactive. On exposure to heat shock, this modified monomer asumed the transcriptionally active phenotype with trimerization and hyperphosphorylation evident. HSP70 transcription ($p<0.05$) and expression ($p<0.01$) was significantly increased in cells treated with IL-6 and subsequently exposed to heat shock but not in cells exposed to IL-6 at 37°C.

Conclusions: IL-6, via inhibition of the repressive kinases MAPK/pERK and GSK3B, converts inactive HSF-1 to an intermediate DNA-binding form augmenting transcriptional activation in the presence of a second stressor. This priming mechansism may provide a way of facillitating pre-conditioning strategies for use in transplantation.

O7

Preservation modality can effect graft function and survival

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Purpose: This retrospective review of national and local outcomes was to validate the renewed practice of using machine preservation.

Methods: Outcome data from a multicentered study group and US national data of single transplant recipients were sorted by organ characteristics (expanded criteria (ECK) and traditional criteria (TCK)) stratified by graft function and preservation modality (machine preservation (MP) vs. non-machine preservation (NMP)). The graft function and survival outcomes were compared and analyzed using the Chi-square and Fisher's exact test and Cox proportional hazards regression. Outcomes for all groups were adjusted for donor and recipient risk factors. The study group was comprised of two transplant hospitals and used only ECK while controlling for variation by utilizing a standardized MP protocol; recipient care and patient selection criterion remained unique. The study group used a more relaxed criterion to define ECK than the published national criteria. The analysis was therefore performed using both defined characteristics.

Results 40,472 transplants were reviewed. Approximately 11% were ECK and 12% were treated with MP. In both national groups (traditional and expanded) MP kidneys have a significantly reduced risk ($p<.0001$) of DGF (odds ratio (OR)=0.63 and 0.57 respectively. Significant differences ($p<.0001$) were also observed in those grafts with immediate function with an odds ratio of 1.54 in the traditional criteria kidney and 1.68 for the expanded group. Both definitions show that the odds of DGF (or PNF) are significantly lower ($p<.0001$) for kidneys that are MP, with OR of 0.53 and .054 for adjusted models.

Conclusion: Machine preservation reduces DGF in the traditional and expanded criteria kidneys. This effect is magnified when recipient risk factors were adjusted . There was no outcome differences observed between the national and study group defined expanded criteria kidneys.

Key Words
Machine preservation
Expanded Criteria Kidneys
Traditional Criteria Kidneys
Delayed Graft Function

Parallel Session IV(a)

Immunosuppression

Tuesday 8 April 2003

16:00 – 17:00

O8

Influence of sirolimus on human mesangial cell (HMC) cholesterol homeostasis: evidence for its anti-atherosclerotic effect

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The high prevalence of lipoprotein abnormalities in transplant patients and the generally accepted involvement of dyslipidaemia in atherosclerosis suggest that lipid abnormalities may play a significant role in the development of chronic graft dysfunction. Ross established the concept that atherosclerosis is an inflammatory disease (1). We have previously reported that inflammatory cytokines TNFα and IL-1β increase lipid accumulation in HMCs by increasing lipid uptake through LDL receptors (2), scavenger receptors (3), and by reducing cholesterol efflux pathways via ATP binding cassette transporter A1(ABCA1).

The antiproliferative properties of sirolimus have recently found clinical application in the sirolimus-eluting coronary stent (4) and a more specific antiatherosclerotic effect of sirolimus is suggested by results from the ApoE-knockout mouse model of hyperlipidaemia (5). We investigated the effect of sirolimus on cholesterol homeostasis in human mesangial cells in culture to determine possible mechanisms for an antiatherosclerotic effect.

Our study demonstrated that sirolimus reduced lipid accumulation in HMCs in culture in the presence of inflammatory cytokine IL-1β; shown as a reduction in Oil Red O lipid droplet staining. Since intracellular lipid content is governed by influx and efflux mechanisms, the balance between lipid uptake through lipoprotein receptors and cholesterol efflux mechanisms is important. Using real-time PCR, we screened the mRNA expression of lipoprotein receptors, which mediate lipid uptake. Sirolimus significantly suppressed LDL receptor, VLDL receptor, and CD36 gene expression. Furthermore, sirolimus also increased cholesterol efflux from HMCs by increasing PPARs, LXRα and ABCA1 gene expression. More interestingly, sirolimus overrode the suppression of cholesterol efflux and ABCA1 gene expression induced by inflammatory cytokine IL-1β .

These results provide a possible basis for the quantitative reduction in atherosclerotic plaque formation that has been observed in sirolimus fed ApoE knock out mice. Sirolimus may have a significant effect in preventing cholesterol accumulation, even in the presence of hyperlipidaemia and inflammation, by regulating both cholesterol influx and efflux pathways.

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O9

A randomised controlled trial of immunosuppression conversion for patients with chronic allograft nephropathy

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Objective To assess the effect of changes in immunosuppression on progression of chronic allograft nephropathy (CAN).

Patients Adult cyclosporin (CsA)-treated renal transplant recipients (RTRs) in 2 regional centres. *Inclusion criteria*: negatively sloping reciprocal of creatinine versus time (ROCT) plot over ≥ 6/12; normal transplant ultrasound/ Doppler; biopsy-proven CAN. *Exclusion criteria*: previous tacrolimus (FK506)/ mycophenolate mofetil (MMF); serum creatinine > 400 umol/l.

Intervention A. *MMF / reduced dose CsA*: MMF 500mg bd for azathioprine, increasing to 1g bd, trough blood level (C_0) of CsA 75-100 ng/ml B. *FK506 for CsA*: target C_0 5-10 ng/ml C. *Continuation of a CsA-based regimen.*

Methods and main results A computer-generated randomisation sequence was used to allocate treatment. The study involved 42 patients over 36/12 (7/99-6/02). Study groups had similar characteristics. GFR (99mTc-DTPA clearance) was measured at baseline and after 6/12. 2 patients started dialysis within 6/12 of study entry (1 A, 1 B). 1 patient in A was intolerant of MMF and withdrew from the study. 6 patients in A reported gastro-intestinal symptoms, and 3 developed progressive anaemia necessitating MMF dose reduction. No study patients developed AR or *de novo* diabetes mellitus. CsA dose was reduced by 24% (interquartile range (IQR) 14%-27%) in A, giving an end-of-study C_0 of 99 (IQR 90-113) ng/ml. The maintenance dose of MMF in A was 1.5 (IQR 1.5-2) g/day. End-of-study FK506 C_0 in B was 7 (IQR 5-9) ng/ml. End-of-study CsA C_0 in C was 163 (IQR 145-215) ng/ml.

Comparison of ROCT slopes before (-12/12 - 0) and after (0 - 6/12) intervention revealed a treatment advantage for A ($p < 0.05$). Comparison of the pre-study and 3/12 - 12/12 ROCT slopes (to isolate the early effect on transplant function of CsA dose reduction) showed a non-significant difference between groups (A vs C, p=0.08). The GFR analysis also suggested a treatment advantage for A (p=0.05). Exclusion of patients with GFR < 20 ml/min/1.73m^2 gave a more significant result ($p < 0.05$).

Conclusions This study provides evidence of a treatment advantage for MMF/ reduced dose CsA over FK506 and standard dose CsA regimens in patients with CAN, at least in the short term. The CsA dose reduction component of the regimen is likely to be of particular importance. Other findings suggest that early intervention is beneficial.

O10

Sub-clinical rejection and borderline changes in early renal allograft protocol biopsies: significance for long term graft function

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Sub-clinical rejection, as detected by protocol biopsies in patients with stable graft function, varies greatly in incidence between units and its impact on long-term graft function is uncertain. In our unit protocol biopsies are performed on days 7 and 28 pos transplantation in all patients. In order to determine the frequency and long term significance of early sub-clinical rejection, we have reviewed day 7 and 28 biopsies performed during a 4-year period (1992-5).

The study was confined to patients with a serum creatinine (sCr) <200μmol/l and stable graft function when the protocol biopsies were performed, in order to exclude patients clinical rejection at the time of biopsy; number of patients 134, 92 biopsies at day 7 and at day 28. After 6 years, 22 grafts (16%) failed and 17 patients (13%) died with functio grafts. Immunosuppression was triple therapy with cyclosporine, azathioprine or mycophenolate mofetil and prednisolone in all patients, with methylprednisolone (MP) induction in 27 and anti-thymocyte globulin (ATG) induction in 9. All biopsies were revie by a single pathologist and classified using Banff 97.

Of the 92 day 7 biopsies, 75 were adequate, of which10 (13%) showed acute rejection and 9 (12%) borderline changes. Of the 10 patients with AR, 8 were treated immediate with pulsed MP and one untreated patient developed clinical rejection within three days the 9 patients whose biopsies showed borderline changes, 4 were treated with MP and untreated patients developed clinical rejection within three days.

Of the 108 day 28 biopsies, 79 were adequate, of which 6 (8%) showed AR and 13 (16 borderline changes. Of the 6 patients with AR, 3 were treated immediately with pulsed M and one untreated patient developed clinical rejection within six days. Ten of 13 patient with borderline changes had been treated for AR in the previous 3 weeks. Two of the 1 were treated for rejection following the day 28 biopsy. One untreated patient developed clinical rejection within one week.

Fourteen patients with sub-clinical rejection or borderline changes at days 7 or 28 were never subsequently treated for rejection. Two died with functioning grafts at 3 months an years, one suffered graft failure at 6 years and 11 had good graft function at the end of t years follow-up, median sCr 119μmol/l (range 90-185).

Compared to some other units, we have found a low incidence of sub-clinical rejection (at day 7, 8% at day 28) in early protocol biopsies. Furthermore, our findings suggest that majority of these "sub-clinical" rejections and borderline changes in protocol biopsies performed in the first month are either early clinical rejections or resolving rejections following treatment. The group of patients who were not treated for rejection following a diagnosis of sub-clinical rejection or borderline changes had a generally good long-term outcome.

O11

Phase III prospective, randomised study to evaluate the safety and efficacy of concentration controlled rapamune (sirolimus) with ciclosporin dose minimisation or elimination in *de novo* renal allograft recipients at 12 months

AG Jardine and On behalf of the European and Sou

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Objective: To assess the safety and efficacy of Rapamune plus low dose steroids as maintenance regimen with or without low dose ciclosporin (CsA) adjunctive therapy in recipients of kidney transplants.

Methods: We present a twelve-month interim analysis of data from this international study. A total of 280 recipients of kidney allografts from cadaveric or living donors have been enrolled; 172 patients are presented in this analysis. All patients received CsA (125-250 ng/mL) plus Rapamune (4-12 ng/mL) and low dose steroids daily after transplantation. At three months, eligible patients were randomized 1:1 to CsA elimination (eCsA) or CsA dose minimization (mCsA). In the mCsA group, drug levels were maintained between 50-100 ng/mL. In both arms, Rapamune trough levels were increased to achieve maintenance levels of 8-16 ng/mL. Antibody induction was prohibited while steroids were generally tapered to reach 5 mg at 10 weeks.

Results: One hundred and seventeen patients were randomised to eCsA (n = 59) or mCsA (n = 58) of which 102 patients completed 12 months of study therapy. There were no significant differences in demographic and donor variables between the groups. At twelve months, creatinine clearance was significantly higher in the eCsA group vs mCsA group respectively (70.9 mL/min vs., 54.6 mL/min, p=0.0001). The mean serum creatinine values were also significantly lower in the eCsA vs mCsA group respectively (124.6 μmol/L vs., 153.4μmol/L, p=0.0032). There were no significant differences in serum cholesterol, triglycerides, LDL, or HDL between the groups. No malignancies have been reported to date. Discontinuation of the Rapamune/CsA combination prior to randomization occurred in 55 patients for the following reasons; adverse events (18), acute rejection (8), graft loss (6), protocol violation (15), death (1), patient request (1), not known (6). The overall first biopsy proven rejection rate at 12 months was 25% (43 episodes in 172 patients) of which 34 episodes (19.8%) occured during the first 3 months of the study. In the post-randomization period 4 patients in the mCsA group and 8 patients in the eCsA group experienced acute rejection, all of which were mild or moderate in severity. 3 of the 8 patients experiencing acute rejection in the eCsA group had experienced acute rejection prior to randomisation, while on combination therapy. Overall, patient and graft survival at 12 months were 98.3% and 95.9% respectively (n = 172). For the randomised patients (n = 117), patient and graft survival at 12 months were 100% vs 98.3% and 98.3% vs 100% for eCsA and mCsA respectively.

Conclusions: These data suggest that the addition of low dose CsA to a Rapamune + steroids maintenance regimen does not increase immunosuppressive efficacy and has a detrimental effect on renal function. This interim analysis supports the hypothesis that Rapamune and low dose steroids is effective maintenance immunosuppressive therapy in recipients of kidney transplants.

Disclosure Study sponsored by Wyeth

O12

The anti-fibrotic agent pirfenidone reduces cyclosporine and tacrolimus induced nephrotoxicity in a salt-depleted model of renal fibrosis.

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Background

Chronic renal allograft dysfunction (CRAD), with its histological counterpart chronic allograft nephropathy (CAN), is the major cause of allograft loss after the first post-transplant year. CAN is in part due to the nephrotoxic effects of calcineurin inhibitor (CNI) immunosuppression. Renal interstitial fibrosis, part of the clinico-pathological entity, is mimicked in the rat salt-depleted model, which reflects some of the morphological and physiological changes seen in humans. Thus, the model allows examination of the effects of CNIs. The piridone pirfenidone has been shown to attenuate fibrosis in a number of non-renal animal models and is presently undergoing clinical trials for treatment of idiopathic pulmonary fibrosis. We employed the salt-depleted model to examine the potential antifibrotic effects of this novel agent on CNI-induced nephrotoxicity.

Methods

Male Sprague-Dawley rats (350-500g) were fed a low salt diet for seven days prior to the introduction of a regimen of CyA (15mg/kg/day) or FK (6mg/kg/day) treatment, with or without addition of pirfenidone at doses of 250, 500 and 750 mg/kg/day for 28 days. Serum creatinine and urinary protein/creatinine ratios were measured at 7, 14, 21 and 28 days, together with daily weight measurement. After sacrifice at 28 days, collagen deposition was calculated by pico-sirius red staining. mRNA expression of the fibrosis-associated genes TGF-beta, collagen III, MMP-2, MMP-9 and TIMP-1 was assessed by reverse transcriptase polymerase chain reaction.

Results

An increase in serum creatinine compared to positive controls was seen for both CyA (94 vs 61 µmol/l, P=0.002) and FK (76 vs 61 µmol/l, P=0.01) treatment at 28 days. This effect was more marked for CyA (P=0.009). Pirfenidone significantly attenuated serum creatinine for both drug treatments at 28 days, in a non-dose-dependent manner. For all drug dose combinations, 24 hour urinary protein excretion was non-significant compared to controls. CyA reduced MMP-2 mRNA expression, an effect abrogated by addition of pirfenidone (P=0.015). The reverse was found for the profibrotic TIMP-1 (P= 0.09). Collagen III (P=0.026) and TIMP1 (P=0.04) mRNA expression was reduced below FK-alone and control levels when pirfenidone was added to FK. TGF-beta and MMP-9 were unchanged compared to controls. Collagen III deposition, detected by pico-sirius red staining, was slight (1-4%) and not significantly different between groups.

Conclusions

Pirfenidone attenuates expression of profibrotic gene mRNA, and reduces serum creatinine in salt-depleted rats treated with cyclosporine and tacrolimus in this model of CNI-induced nephrotoxicity. This suggests the agent has an antifibrotic action in renal fibrosis. The lack of collagen III staining suggests that other components of the extracellular matrix should be examined to provide a histological explanation for this effect.

O13

Tacrolimus pharmacogenetics: a single nucleotide polymorphism associated with cytochrome P4503A5 expression identifies patients who fail to achieve target blood concentrations during the first week after transplantation.

I.A.M. MacPhee[1], S. Fredericks[2], T. Tai[2], P. Syrris[3], N.D. Carter[3], A. Johnston[4], L. Goldberg[5] and D. Holt[2]

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Previously, we have shown that the dose-normalised blood concentrations of tacrolimus at 3 months after renal transplantation were related to a single nucleotide polymorphism (SNP) in the CYP3AP1 pseudogene (A/G_{-44})that is commoner in Black subjects and strongly associated with hepatic CYP3A5 activity. Patients with a G-allele require two-fold higher doses of tacrolimus to achieve target concentrations. This study addresses the question as to whether concentration-controlled dosing with tacrolimus during the early period after transplantation can overcome this problem.

Results for 178 renal transplant recipients transplanted between 1995 and 2001 were examined (CYP3AP1 –44 genotype: AA, n=125, AG/GG n=53). Target blood tacrolimus trough concentrations, measured 3 times weekly for 2 weeks, were 15-20 µg/L during the first week, then 10-15 µg/L for the next 3 months.

The mean blood tacrolimus concentration during the first week was significantly lower for patients with the G-allele (Median 13.5 vs 18.5 µg/L, p<0.0001). More importantly, there was a significant delay in achieving target blood concentrations for patients with a G-allele (Table).

A significantly higher proportion of AA patients had at least one blood tacrolimus concentration above target during the first week (73.6% vs 35.8%, p=0.003). There was no significant difference in the rate of biopsy-confirmed acute rejection over the first three months post-transplant but the episodes of rejection occurred earlier in the AG/GG group (median 7 days vs 12 days, p=0.006).

In conclusion, initial dosing with tacrolimus, based on a knowledge of the CYP3AP1 genotype and, subsequently, guided by concentration measurements, has the potential to increase the proportion of patients achieving target blood concentrations early after transplantation.

Parallel Session IV(b)

Donation I

Tuesday 8 April 2003

16:00 – 17:00

O14

The total impact of streptokinase preflush to a NHBD programme

MA Gok[1], BK Shenton[1], M Pelser[2], JFC Glatz[2], D Mantle[3], C Cornell[4], R Peaston[4], BC Jaques[1], N Soomro[1], DM Manas[1] and D Talbot[1]

[1]Renal / Liver Transplant unit, Level 5, The Freeman Hospital, Newcastle Upon Tyne, NE7 7DN, [2]Dept of Physiology, Maastricht University, Maastricht, Netherlands, [3]Dept Of Agriculture, University Of Newcastle, Newcastle Upon Tyne, NE2 2HH and [4]Dept of Biochemistry, The Freeman Hospital, Newcastle Upon Tyne, NE7 7DN, United Kingdom

Introduction

Predicted estimates suggest that the donor pool (especially kidneys) could be substantially expanded by 20 – 40 % if widely used. Viability assessment is crucial to select out damaged NHBD kidneys. Therefore the potential yield from NHBD is hampered by the high discard rate especially from uncontrolled donors. Disseminated intravascular coagulation after cardio-respiratory arrest is believed to be the precedent for poor perfusion and thereby failure of viability assessment. Streptokinase has been shown to be of benefit in animal and human studies for perfusion characteristics though the discard rate was not significantly different. This study was conducted retrospectively to determine the impact of thrombolysis on the whole NHBD programme.

Materials and methods

Streptokinase preflush (1.5 MIU) was introduced into the Newcastle NHBD programme in August 2000 (n = 14 donors). Earlier NHBD procurements incorporated the use of heparinised in-situ preservation flush (n = 46 donors). All NHBD kidneys were machine perfused prior to transplantation with assessment of perfusion characteristics (pressure, flow, temperature, resistance) and perfusate enzymes (glutathione S transferase GST, alanine aminopeptidase Ala-AP and fatty acid binding protein FABP).

Results

See Table

Conclusion

The use of streptokinase preflush in the NHBD was associated with improved initial procurement appearance, with reduced perfusate biomarkers (GST, Ala-AP and FABP). There was a 15 % improvement in category III NHBDs, though the discard rate is low. For category II NHBDs there was a 15 % improvement in the discard rate.

	SK (n = 28 kidneys)	Non SK (n = 92 kidneys)	Mann Whitney U p value
Good Procurement (pale, clear flush)	80.0 %	35.2 %	< 0.05
Maximum perfusate biomarker			
GST	144.1 ± 16.6	163.2 ± 15.3	NS
Ala-AP	118.5 ± 14.6	191.9 ± 23.8	< 0.05
FABP	125.0 ±14.7	390.9 ± 38.9	< 0.05
% of kidneys transplanted	65.9	56.5	Chi sq p = NS
1st year graft survival (%)	92.9	90.9	Logrank p = NS
Discards			Chi squared
Category II donors (% of II)	43.8	58.5	p < 0.05
Category III donors (% of III)	11.1	25.0	p < 0.05
Severe necrosis on histology (%)	46.7	68.8	Chi sq p < 0.05

O15

Do donor factors influence organ quality and renal transplant outcome?

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Oxford Transplant Centre, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LJ, United Kingdom

There is mounting evidence from experimental and clinical studies that the quality of organs from cadaver donors may be influenced by events that occur in the Intensive Care Unit and around the time of brain death, and that these may affect subsequent transplant outcome. The aim of this study was to investigate the influence of donor factors on renal allograft outcome in a homogeneous cohort of 518 patients transplanted in a single centre over a nine-year period (1991-1999). During this time a standard cyclosporine based triple therapy immunosuppression regimen was used, with no change in the protocol for the presence of delayed graft function (DGF).

In this cohort of transplants, the level of HLA DR matching was: 0DR mismatches (MM)-269/518 (51.9%), 1DR MM-219/518 (42.3%), 2DR MM- 30/518 (5.8%). Delayed graft function (requirement for dialysis within the first week post transplant) occurred in 30% (150/518) of patient and 54% of patients had at least one episode of acute rejection (AR). One-year and five-year graft survival was 85.9% (445/518) and 68.2% (217/318), respectively.

The end points of the study were DGF, AR, 1-year graft survival and long-term survival of those grafts that reached 1 year. Multivariate analysis was performed to determine factors that may influence the graft outcome indicators.

DGF was found to be significantly influenced by cold ischaemia time (CIT) ($p<0.0005$), donor age ($p<0.0005$) and donor creatinine ($p<0.005$). Other donor factors, such as cause of death, ventilation time, requirement for inotropes and other factors associated with intensive care management were not risk factors for DGF, nor for rejection episodes or graft survival.

The risk factors for the number of acute rejection episodes were HLA-DR MM ($p<0.005$) and DGF ($p=0.009$), although this was influenced mainly through CIT. 1-year graft survival, after censuring for death, was only influenced by DGF ($p<0.0005$), no other factors were significant. When grafts surviving for one year were considered, only CIT ($p<0.009$), recipient age ($p<0.001$) and creatinine at one-year ($p<0.001$) were found to affect graft survival significantly. The results of this analysis of our series of well-matched transplant recipients show that CIT is the most important predictor of poor short and long-term graft survival. Donor factors and ITU parameters did not affect the outcome. Therefore in order to improve the long-term survival of renal allografts efforts should focus on limiting CIT.

O16

Ratio of monocyte chemotactic protein-1 (mcp-1) to mcp-2 expression in the donor kidney predicts delayed graft function in the transplant recipient

S Narayanan[1], KM Rigg[2] and M Shehata[2]

[1]Dept.of Surgery, Glan Cwyd Hospital, Rhyl, LL18 5UJ, United Kingdom and [2]Nottingham Transplant Unit, City Hospital, Nottingham, NG5 1PB, United Kingdom

Delayed graft function (DGF) following renal transplantation has been shown in many studies to be associated with poor long-term allograft survival and may be caused by a variety of ischaemic and immunological factors. The evaluation of DGF requires the integration of clinical, laboratory, imaging and possibly histological data, followed by changes in immunosuppression and a period of dialysis. The inability to predict with a degree of confidence those patients at highest risk of DGF results in these interventions occurring only after DGF has already occurred, with the result that the mechanisms that affect long-term survival are already set in motion. We report chemokine changes in the donor kidney that have the potential to be used as a predictive test for DGF.
METHODS. Pre-transplant biopsies from 50 (35 cadaveric and 15 living donor) renal transplants performed at Nottingham were snap frozen and analysed by the multiplex PCR technique for expression of a range of cytokines and chemokines – MCP-1, MCP-2, RANTES, IL-1beta, IL-6, IL-8, IP-10, TNF-alpha, TGF-beta and GM-CSF. Raw expression values were then standardised against GAPDH expression in each sample. Pre-transplant and post transplant (if taken) histology was recorded. All recipients received standard calcineurin-inhibitor based triple therapy as immunosuppression and were classified as having DGF if they required dialysis in the first week post transplantation. Standardised expression values were used in the analysis and the unpaired t-test used to compare groups with a p-value of <0.05 taken as significant.
RESULTS. 5 patients (4 cadaveric and 1 living donor) developed DGF following transplantation. Transplant biopsies taken from them in the first week showed that 2 had acute tubular necrosis whilst 3 had ATN with acute rejection (Banff 1A, 1B, 2A). An exploratory viewing of the expression data revealed that patients with DGF had higher levels of MCP-1 expression relative to MCP-2. Analysis of ratios of MCP-1 to MCP-2 showed a mean ratio of 4.9 (+/- 1.12) in those with DGF and 1.2 (+/- 0.84) in those without DGF (p <0.001). All patients with DGF had MCP-1/MCP-2 ratios greater than 3.5. Only 2 patients without DGF had ratios higher than 3.5, and both had initial non-function with their transplants functioning on days 3 and 5 respectively. Taken as a test with a cut off at 3.5, the MCP-l/MCP-2 ratio has a positive predictive value of 71.4%, sensitivity of 100% and a specificity of 95.6% in our experiments. No such significant relationships could be established between the expression values of the other cytokines studied and DGF.
CONCLUSIONS. These results suggest that measuring ratios of MCP-1 to MCP-2 in human renal allografts before transplantation can help identify patients at a high risk of developing DGF after renal transplantation. This raises the possibility of measures such as calcineurin inhibitor sparing regimens being introduced prophylactically in these patients. Standardising the technique of PCR for measuring these ratios, which can be done as an overnight test, could mean routinely using the MCP-1 to MCP-2 ratio as a test to predict the development of DGF in clinical practice.

O17

Non-heart beating versus cadaveric and living donor livers: Differences in inflammatory markers prior to transplantation

W Jassem[1], DDH Koo[2], P Muiesan[1], L Cerundolo[2], M Rela[1], SV Fuggle[2] and ND Heaton[1]

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Background: Liver transplantation from non-heart beating donors (NHBD) has been recently introduced into clinical practice to increase the donor pool, however little is known about the immune status of NHBD livers. There are clear differences between NHBD and conventional cadaver donors including the influence of brain death and exposure to longer warm ischemia times. The aim of this study was to assess intragraft cell populations and inflammatory markers in biopsies taken from NHBD livers prior to transplantation and to compare the findings with those from cadaveric and living related (LRD) donor livers.

Methods: True-cut biopsies were obtained from controlled NHBD (n=9), cadaveric (n=22) and living donor (n=10) livers at the end of the period of cold storage, before implantation. Cryostat tissue sections were stained with antibodies for CD68 (macrophages/monocytes), CD3 (T lymphocytes) and ICAM-1. The percentage area of leukocyte staining was quantitated by morphometric point counting. ICAM-1 staining was assessed semi-quantitatively; Grade 1 - <70% sinusoidal endothelium positive, hepatocytes negative; Grade 2 - <90% positive sinusoidal endothelium with occasional positive hepatocytes; Grade 3 - >90% sinusoids positive with multiple foci of positive hepatocytes.

Results: The levels of leukocyte infiltration in NHBD reflected those found in conventional cadaver donors and were significantly higher than in LRD livers. Similar levels of CD68+ monocyte/macrophages were detected in cadaver (4.0 + 1.2%) and NHBD livers (4.6 + 1.2%) that were significantly greater than in the LRD livers (2.6 + 0.5%; p<0.01). Furthermore, the level of T lymphocytes in NHBD (1.1 + 0.6%) and cadaver donors (1.5 + 0.8%) were similar, and higher than in LRD (vs. 0.47 + 0.3%; p<0.05). There were significant differences in the expression of ICAM-1 between conventional cadaver and LRD livers. 12/22 (60%) of cadaver livers had high levels of expression (Grade 3), whereas high levels were found in only 1/10 (10 %) of LRD livers (p=0.02). The expression n of ICAM-1 in NHBD (Grade 3 – 4/9 (44%)) was intermediate between conventional cadaver donors and LRD.

Conclusions: The results from this study demonstrate that livers obtained from NHBD prior to transplantation are similar to conventional cadaver donors in the level of leukocyte infiltration. Nevertheless, lower levels of ICAM-1 were detected in NHBD suggesting less exposure to inflammatory mediators than conventional cadaver donor livers.

O18

The Effect of Cold Ischaemia in an Experimental Model of Kidney Transplantation in the Presence of Tacrolimus, Mycophenolate and Rapamycin

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The Transplant Unit, City Hospital, Nottingham, NG5 1PB, United Kingdom

There is no doubt that changes in organ sharing in the UK have resulted in better matching of kidney allografts. However, this has been at the expense of prolonged periods of cold ischaemia (CI). Increasing CI is associated with increased rates of delayed graft function and acute rejection and therefore it has a negative effect on long-term graft survival.

In these experimental studies we have investigated the effects of different periods of CI on kidneys treated with Tacrolimus (Tac) in combination with either Mycophenolate Mofetil (MMF) or Rapamycin (Rapa) in an animal model of kidney transplantation. Left kidneys were retrieved from Fisher (F344) rats and transplanted orthotopically into Lewis recipients immediately after retrieval or after 6 or 24 hours of cold storage in Marshalls solution. Animals (n=6) were treated with Tac and either MMF or Rapa. Animals were sacrificed 1 or 2 months post transplantation (n=3). Kidneys were perfused with phosphate buffered saline and processed for light microscopy. Morphometric analysis was carried out to study the effects of CI on the development of interstitial fibrosis (IF), glomerulosclerosis (GS) and arteriolar hyalinosis (AH). Controls included isografts (F344 to F344) and untreated allografts.

Morphometry Results From 1 Month

	Untreated Allograft			Tac + MMF treated Allograft			Tac + Rapa treated Allograft			Untreated Isograft
CI (hrs)	0	6	24	0	6	24	0	6	24	24
IF	74	38	55	19	36	37	41	65	73	51
GS	65	45	44	14	24	31	40	30	55	27
AH	27	12	19	8	10	18	21	6	19	17

Data represents % of cortical area affected or % of normal structures affected

Twenty-four hours of CI resulted in a moderate degree of IF and mild AH and GS in untreated isografts (non-immunological model). These changes were no different from those seen in untreated allografts (immunological model). Interestingly, the changes associated with chronic allograft nephropathy decreased with increasing periods of CI in untreated allografts, possibly due to under scoring caused by heavy interstitial infiltrates in kidneys exposed to a cold CI insult. Its significance is unclear.

After 1 month, a progressive increase in IF and GS in rats treated with Tac and MMF or Tac and Rapa was seen (immunological model with drug manipulation). However, the latter group showed a 2-fold increase in IF and GS compared to those treated with Tac and MMF. At 2 months, there was no apparent difference in these parameters compared to 1 month (results not shown). Although the combination of Tac and MMF prevented graft rejection, increasing CI reduced this effect and the chronic changes seen approached those of untreated grafts. Tac and Rapa had a more potent immunosuppressive effect but caused severe morphological damage, in particular IF, regardless of the CI time.

These studies demonstrate that CI has a major detrimental effect on the kidney. Immunological injury does not appear to have an additive effect on this injury after prolonged CI. Immunosuppression is of benefit if the CI times are short.

O19

Plasma Atrial and Brain Natriuretic Peptides in the Assessment of Donor Heart Function

B Nyawo[1], M Buckley[2], M Koban[2], NR Banner[2] and M Yacoub[2]

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Early failure of the donor heart is a serious complication of heart transplantation. Current methods of assessment of the donor heart prior to retrieval (echocardiography[TTE/TEE] are complex and cannot always be used. In addition the interpretation of TTE and TEE is subjective. Therefore, a simple rapid blood test that can assess donor heart function would be highly desirable. The natriuretic peptides ANP and BNP are increased in acute and chronic heart failure and their level is related to both prognosis and to the response to therapy.

Methods: We studied 32 consecutive potential cardiac donors between 11 and 56 years(Table1). Blood was drawn at the time of organ retrieval. In all patients transoesophageal echocardiography and pressure volume loops were used to document global ventricular function. Twenty of these hearts were subsequently transplanted while 12 were judged to be unsuitable if ejection fraction was less than 25%.

Results Clinically measured variables were similar in the 2 groups except EF which was lower in the unused group(Table 2).Plasma ANP (mean ±SEM) was 42.0±13.6pcg/ml in the 'unused' group compared to 23.9 ± 8.4 in the 'used' donors (p=0.01). Mean BNP was 70.7 ± 14.5 in the 'unused' compared with 32.8 ± 9.6 in the 'used' (p<0.05).RNA abundance (delta ct)for ANP was 17.6±1.8and 11.81±3.9 in used and unused donors respectively.For BNP the values were 17.0±3.0 and 10.7±3.1 respectively.

Conclusion Measurement of natriuretic peptides may be a rapid, simple and objective method to assess donor heart function and can be used as a bedside assay at the donor hospital.Further studies are indicated to determine its role in donor assessment and the response to treatment.

Table 2 (Clinical variables)

	Used Donors	Unused Donors
Ejection fraction(%)	52 ± 6	22.5 ± 3 (p<0.01)
CVP	7.1± 3	11.3 ± 4
LA pressure	8 ± 2	13.8 ±2 (p<0.05
Mean BP	73 ± 10.2	65.3 ± 8.1

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Mean BP	73 ± 10.2	65.3 ± 8.1

Parallel Session IV(c)

Liver/Thoracic

Tuesday 8 April 2003

16:00 – 17:00

O20

Long Term Outcomes in Heart Transplant Recipients Using Older Donor Hearts

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Objective: Heart transplantation, as a treatment modality, is limited by donor availability and strict selection criteria. The long term outcomes of transplant recipients of hearts from donors 50 years of age and older were compared to those of recipients of young donor hearts, to evaluate the potential to expand donor selection criteria.
Methods: Between 1989 and 1999, 27 patients recieved hearts from donors 50 years of age and older (mean 52 +/- 1.7, range 50-55 years) and 383 patients recieved hearts from young donors (mean 28 +/- 9.8, range 8-49 years). The results and outcomes were reviewed by retrospective analysis of the Stanford Transplant database.
Results: Patients and donor selection criteria were otherwise similar in the two groups. The average wait for recipients of older hearts was 166 days compared to 176 days for recipients of young hearts (p=ns). Recipient age, graft ischemic time and hospital stay were similar for both groups. Actuarial survival rates at 1, 3 and 5 years were 85%, 79% and 73%, respectively, for recipients of older hearts versus 85%, 79% and 72%, respectively for recipients of young hearts (p=ns). The rejection rates at 1, 3 and 5 years were 63%, 69% and 69%, respectively for recipients of older hearts versus 67%, 71% and 73%, respectively, for recipients of young hearts (p=ns). The mean time to first rejection episode was 56 days for recipients of older hearts versus 105 days for recipients of young hearts ($p<0.05$). CMV infection rates at 1, 3 and 5 years were 21%, 26% and 26%, respectively, for recipients of older hearts compared to 42%, 51% and 54%, respectively for recipients of younger hearts ($p<0.05$). The incidence of allograft coronary artery disease at 1, 3 and 5 years was 1%, 10% and 31%, respectively, for recipients of older donor hearts versus 7%, 14% and 20%, respectively, for recipients of young hearts (p=ns). No patient in either group required revascularization; 1 patient in the older donor group and 5 patients in the young donor group underwent retransplantation.
Conclusions: The recipients of hearts from donors over 50 years of age have similar survival rates compared to recipients of young donor hearts. The recipients of young donor hearts have a higher incidence of CMV infection but a longer delay to first rejection episode. Therefore, the use of carefully selected donors 50 years and over is possible without compromising transplant recipient outcomes and thereby ultimately expanding the limited donor pool.

O21

Duct-To-Duct Anastomosis Is An Acceptable Option During Liver Transplantation In Patients With Primary Sclerosing Cholangitis.

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Introduction: Traditionally, all patients with Primary Sclerosing Cholangitis (PSC) need a Roux-en-Y reconstruction at transplant because of risk of stricturing in the recipient duct and possibly of subsequent malignancy. However, a roux loop reconstruction adds to the operative time and complexity, has been shown to be associated with more infective complications and also denies subsequent access to the biliary tree at ERCP. We retrospectively reviewed prospectively collected data on a cohort of PSC patients managed with attempted duct-to-duct reconstruction, as against patients with Primary Biliary Cirrhosis (PBC) as a classical biliary disorder with normal ducts and a good prognosis.
Materials and Methods: During the period of March 1994 to August 2002, a total of 540 patients received a liver transplant at our centre. The attempt in all patients with PSC was to perform a duct-to-duct reconstruction unless preoperative imaging had shown disease in the distal recipient duct or the duct was found to be diseased and strictured at surgery and would not allow easy passage of a probe.
Results: 37 patients with PSC received a primary Orthotopic Liver Transplant and 58 patients with a diagnosis of PBC received a primary liver transplant. 25 of the 37 patients with PSC had a duct-to-duct anastomosis while 12 patients needed a roux loop. During this same period, 3 out of 58 patients with PBC and 47 out of 503 patients overall received a roux loop reconstruction. Patient demography, operative and other parameters (such as operative blood loss, CIT) were similar in the two groups.
The duration of follow up was similar in the two groups of patients with PSC (average follow up 57.9 months, range 3.7 – 105.7 months) and PBC (average follow up 57.4 months, range 7 – 104.7 months).
Six patients were excluded from subsequent analysis as they died within the first month (one in the PSC group and five in the PBC group). Table 1 outlines the occurrence of biliary complications in the remaining patients on follow up.

Table 1. Biliary Complications

	PSC			PBC		
	D-D N=24 (%)	Roux N=12 (%)	Total N=36 (%)	D-D N=50(%)	Roux N=3 (%)	Total N=53 (%)
Bile leak	2 (8.3)	3 (25)	5 (13.9)	3 (6)	1 (33.3)	4 (7.5)
Stricture	3 (12.5)	1 (6)	5 (13.9)	3 (6)	0	3 (5.7)
Overall Complns	5 (20.8)	4 (33.3)	10 (27.8)	6 (12)	1 (33.3)	7 (13.2)

There is thus no significant difference in the incidence of biliary complications between PSC patients with either duct-to-duct or roux loop even when compared to the PBC group ($p > 0.05$, chi square test).
Conclusion: It is therefore clearly possible to perform a duct-to-duct biliary reconstruction in patients with PSC with comparable results. Given the disadvantages of a roux loop discussed earlier and the fact that no case of a de novo cholangiocarcinoma has been reported in the recipient duct remnant, the data supports that an attempt should be made to perform a duct-to-duct anastomosis even in patients with PSC.

O22

The Utility of Intra-operative Blood Salvage During Liver Transplantation

SD Phillips, RR Deshpande, D Maguire, S Cottam, MJ Bowles, P Muiesan, M Rela and N Heaton

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Introduction: Adult liver transplantation (OLT) is associated with significant use of allogeneic blood products, which may place considerable demands on finite resources. Theoretically, this could be reduced by the use of autologous red cell salvage, but previous reports have suggested that this was not cost-effective. We therefore evaluated this in a prospective study.

Patients: Autologous red cell salvage was used in 660 adult patients undergoing OLT between January 1997 and July 2002. Of these, 134 (20.3%) had acute liver failure and 62 (9.4%) were re-transplants. Of the remainder, 90 had alcohol-related liver disease (ALD), 183 viral, 98 cholestatic and 93 had other aetiology (including autoimmune, cryptogenic and metabolic).

Results: The total volume of red blood cells transfused in these groups was 3641±315ml, 2805±234ml, 2603±443ml, 2785±337ml for ALD, viral, cholestatic and others respectively (p=0.19 ANOVA). Multivariate analysis revealed no correlation between transfused volumes and pre-operative haematological or biochemical parameters. Of note, blood volumes transfused at re-transplantation were significantly higher (7076±1110ml Vs 2864±138ml; p<0.001). Autologous blood volumes transfused were similar in all diagnostic groups (1677±154ml ALD, 1469±133ml viral, 1418±211ml cholestatic and 1750±221ml others; p=0.14 ANOVA), but were significantly greater in re-transplantation (2132±363ml Vs 970±57ml; p=0.02). Total savings per case were similar for all diagnostic groups (£279±49, £240±35, £300±86, £250±66 for ALD, viral, cholestatic disease and others respectively) but were greater in cases of re-transplantation (£864±222 Vs £238±24; p<0.001). Without use of autologous transfusion over the five and a half-year study period, the overall cost of allogeneic red blood cells would have amounted to £503,443. With the use of autologous transfusion this was reduced to £371,542, saving £131,901 (26%).

Conclusions: Intra-operative red blood cell salvage and autologous transfusion is cost-effective in adult liver transplantation. In the current era, where optimum resource utilisation and fiscal constraint are of paramount importance in the delivery of healthcare, autologous transfusion is an important adjunct in liver transplantation.

O23

Combined Heart and Kidney Transplantation: UK Practice and Outcomes

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Objective

Whilst the indications for isolated heart transplantation are well established, there is little agreement regarding the management of patients with heart failure who have major impairment of other organ systems. There is a select group of patients for whom combined heart and kidney transplantation is an appropriate option, with published single centre 5 year survivals as high as 80%. No data on UK-wide practices or outcomes has been published: we aimed to establish the current status of combined heart and kidney transplantation in the UK.

Method

We obtained outcome data from United Kingdom Transplant on the 26 combined heart and kidney transplants carried out in the UK between January 1986 and January 2002. The two heart-lung and kidney transplants were excluded from the analysis. In order to establish current practice in the UK we carried out a telephone survey of the eight units currently responsible for heart transplantation.

Results

The 30 day mortality was 11.5% (3/26) and actuarial survival at 1 and 5 years was 69.0% (95% confidence interval 55.6 – 78.5) and 45.0% (34.5 – 55.4) respectively, with wide variation between centres. Where used, the level of creatinine clearance considered a contraindication to isolated heart transplantation ranged from <50 to <30 mls/min. There was a similar variation in the level of creatinine clearance considered a contraindication to isolated heart transplantation. There was no formal agreement on the criteria for combined heart and kidney transplantation.

Conclusion

UK-wide survival for combined heart and kidney transplantation is significantly lower than published single centre and overseas survivals, and lower than isolated heart transplantation survivals. There is a strong case for establishing UK-wide guidelines for eligibility for combined heart and kidney transplants, and limiting procedures to selected centres with proven expertise.

O24

CUSUM and VLAD: Effective monitoring tools in Intrathoracic Transplantation?

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On behalf of the Steering Group, UK Cardiothoracic Transplant Audit, Clinical Effectiveness Unit, The Royal College of Surgeons

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Background:
The CUSUM (cumulative sum test) procedure was developed as a monitoring tool for quality in industrial production lines and laboratories. It has been used to monitor individual surgical performance and in learning skills. Its strength is the avoidance repeated statistical testing and its weakness an inability to account for patient risk. The variable life adjusted display (VLAD) allows for risk adjustment and so provides a means of standardisation for comparing individuals or centres carrying out similar procedures.
Methods:
30-day mortality after first time heart, lung and heart-lung transplantation in 9 centres was subjected to CUSUM monitoring. Risk-adjusted VLAD plots were constructed for the adult heart and lung programmes. 30-day mortality rates for the UK as a whole were taken as the reference values for the CUSUM and the upper limit of the 95% confidence interval for the overall estimate provided the basis for the construction of the upper alert and alarm lines.
Results:
2426 transplants were reported in the UK between July 1995 and December 2001. The unadjusted CUSUM after adult heart transplant remained within the alarm line boundaries for five centres. One crossed the lower boundary, indicating a significantly lower 30-day mortality than seen nationally, and two 'hit' the upper alarm line at the end of the study period. Risk-adjusted VLADs suggested that for one of these two centres the increased mortality could be attributed, at least in part, to patient risk. For the adult lung programme the CUSUM for one centre 'hit' the upper alarm but then returned to within limits, one crossed the lower boundary towards the end of the study period and the remainder remained within the alarm-line limits throughout. Risk-adjustment impacted on one centre, whose unadjusted results were within limits, and were improved further after adjustment for risk.
Discussion:
CUSUM and VLAD plots provide a means of the on-going monitoring results and provide a basis for visually comparing results between different centres or individuals performing the same procedures, especially in high-risk surgery like heart and lung transplantation. This study, although retrospective, provides a useful quantification of centre-specific 30-day post-transplant mortality and serves to identify particular periods of 'bad runs'. As a next stage in the process, we are evaluating the feasibility of using sequential probability ratio test, which allow alert and alarm lines to be added to risk-adjusted estimates.

O25

The Role of Reduced Size Liver Transplantation in an Adult Transplant Programme

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Aim – To review the practice of reduced size liver transplantation (RSLT) in an adult liver transplant programme.

Patients & Methods – Reduced size liver transplantation is widely practiced in paediatric liver transplant units but seldom in an entirely adult transplant unit. Since 1997, we have performed 20 cut down/split procedures resulting in 25 orthotopic liver transplants – 19 adults and 6 children. During this period 232 whole liver transplants were performed in the unit.

Data were collected prospectively on the unit database and outcome of 'exported' split livers (n=6) was established by direct contact with recipient units. Data collected included donor and recipient details, outcome and complications, operative time and blood loss. Data were analysed using Microsoft Word Excel 97.

Results – All reduction procedures were performed in our unit. The splits were performed by paediatric transplant teams prior to exporting the adult portion . There were 19 adult recipients (3 men and 16 women) and 6 children. The median body weight of adult recipients was 48kg and the median waiting time for these adult patients on the routine list was 126 days (range 4-432) compared to 35 days in the patients on the list who received whole livers during this period. 7 patients with fulminant hepatic failure (FHF) were transplanted with 8 RSLs. There were 4 post op deaths (4/19= 21%), all in fulminant patients. Of these 4, 3 succumbed to sepsis and 1 to iron toxicity.

The only patient who had primary non-function (PNF) was retransplanted, with a whole liver and is alive and well.

Summary – 25 patients benefited from RSLT in an adult transplantation programme. Mortality was related to patients' preoperative diagnosis (fulminant hepatic failure as opposed to chronic liver failure) and both mortality and major morbidity in the remaining patients was similar to 'whole organ' liver transplantation.
Conclusion – the technique of RSLT should be considered in specific groups of patients who are at a disadvantage on the National Waiting List. If used in FHF, the results are inferior but this may reflect the overall increased risk in these patients.

Plenary Session II

Medawar Medal

Wednesday 9 April 2003

10:30 – 12:30

O26

$CD25^+CD4^+$ regulatory T cells in transplantation: Evidence for bystander suppression after antigen-specific reactivation *in vivo*

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$CD25^+CD4^+$ regulatory T cells (Treg) have been shown to contribute to the induction and maintenance of tolerance to donor alloantigens. A complete understanding of their functional characteristics and potential in the setting of transplantation is critical if Treg are to be exploited in the future.

In mice, alloantigen-specific Treg can be induced by exposure to alloantigens *in vivo*. We wished to establish whether the generation of Treg capable of controlling the rejection of fully allogeneic grafts could be achieved by pre-treatment with cells expressing only a single donor MHC class I alloantigen ($H2K^b$) given under the cover of anti-CD4 therapy.

T cell-deficient recipients (CBA-$Rag^{-/-}$; $H2^k$) reconstituted with syngeneic $CD45RB^{high}CD4^+$ effector cells alone rejected C57BL/10 ($H2^b$) skin grafts acutely (MST 17 days, range 15-20 days; n=4). In clear contrast, co-transfer of $CD25^+CD4^+$ cells from mice exposed to a single donor alloantigen, $H2K^b$, in the presence of anti-CD4 was able to prevent rejection of fully allogeneic C57BL/10 skin grafts expressing $H2K^b$ plus the additional major and minor histocompatiblity antigens of the $H2^b$ haplotype (all grafts survived >100 days; n=5). These data demonstrate that $CD25^+CD4^+$ Treg can contribute to linked unresponsiveness / suppression *in vivo*.

Studies of naturally-occurring Treg have demonstrated that these cells must be activated by signals delivered via the T cell receptors in order to exert their regulatory effect but that, once activated, such Treg are able to regulate in an antigen non-specific manner. To determine if this phenomenon could be exploited in the setting of transplantation, we next pre-treated mice with blood from a third party strain (BALB/c; $H2^d$) under the cover of anti-CD4 antibody. An additional blood transfusion from the same third party donor was administered immediately before isolation of $CD25^+CD4^+$ cells with the objective of activating any putative Treg cells present in the pre-treated mice. $CD25^+CD4^+$ cells isolated from mice after reactivation with the same third party BALB/c alloantigens were able to prevent rejection of C57BL/10 skin grafts initiated by $CD45RB^{high}CD4^+$ cells (all grafts survived >100 days; n=4), whereas $CD25^+CD4^+$ cells from pre-treated mice that did not receive the repeat transfusion did not exhibit regulatory activity (all grafts rejected at day 25; n=4).

These results demonstrate that $CD25^+CD4^+$ Treg are able to regulate the rejection of grafts expressing alloantigens that they have not previously experienced only when they are reactivated in an antigen-specific manner immediately before their functional activity is tested. The ability to harness potential of Treg in this way could be of value in clinical transplantation where pre-treatment of recipients with donor-specific antigen is not usually possible, except in the case of live donor transplantation.

O27

Renal transplantation from non-heart-beating donors: Leicester supports government proposals for the expansion of further non-heart-beating centres

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Introduction: The success of renal transplantation continues to be restricted by a lack of available organs. The use of non heart-beating donors (NHBDs) has been advocated as an alternative to conventional cadaveric organs. Nonetheless, widespread acceptance of their use is limited by concerns regarding the effects of prolonged warm ischaemia on long-term renal function. Recommendations from the Quinquennial Review of UKT (2001) aim to increase the number of transplants performed from NHBDs from 42 to 210 per annum by 2005. In the present paper we report the outcomes of renal allografts retrieved from NHBDs in Leicester from1988-2002.

Methods: Outcome data from renal allografts performed in Leicester between 1988-2002 was prospectively collected. The series included both controlled and uncontrolled donors. Recipient and donor factors were analysed and the following outcome measures assessed: Primary non-function rate (PNF: transplants that never worked), incidence and duration of delayed graft function (DGF: requirement for dialysis in the first post-transplant week), initial graft function rate (IF), acute rejection episodes, renal function and actual allograft survival rate.

Results: 103 NHBD kidney transplants were performed in the study period. 15% of donors were controlled and 85% uncontrolled. The mean (range) donor age was 46 (18-63) yrs. The transplanted kidneys suffered a mean (range) first warm ischaemic time of 24 (10-60) minutes. The mean HLA A, B and DR mis-matches were 1.0, 1.2, and 0.6. The rates of PNF, DGF and IF were 12, 83 and 5% respectively. Acute rejection occurred in 41% of patients in the first post-transplant year. Allograft function and survival rates was 88%, 79% and 77% at 1, 3 and 5 years respectively. Mean duration of delayed graft function was 20 days.

Conclusions: In 1998, the British Transplant Society recommended cadaveric renal allograft survival should exceed 80 and 60% at 1 and 5 years respectively. The Leicester experience clearly refutes misconceptions that NHB kidneys are marginal organs and supports the further expansion of NHBD programmes.

Time post-transplant (mth)	Serum creatinine µmol/l (std)	Allograft survival (%)
3	189 (84)	88
12	179 (74)	85
36	182 (85)	79
60	200 (101)	77

O28

Presentation of conformational donor MHC class I by recipient dendritic cells - direct recognition by an indirect route

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BACKGROUND

T cells that recognise MHC alloantigen indirectly, as processed peptides, play a critical role in graft rejection. However, the nature of co-operation between T cells that recognise alloantigen indirectly and effector mechanisms that are directed against intact alloantigen requires clarification. Complex four cell cluster models (comprising donor cell, recipient effector cell, recipient antigen presenting cell and recipient CD4 T cell) have been proposed. The recent observation that dendritic cells can internalise and re-present antigen, intact and non-processed, suggests that the recipient dendritic cell is uniquely capable of presenting MHC alloantigen both as an intact protein and as peptide fragments, thus providing an important link between the direct and indirect allorecognition pathways. We provide evidence that the presentation of intact alloantigen by recipient dendritic cells results in an immune response directed against conformational MHC alloantigen.

METHODS

Dendritic cells (DC) were prepared from PVG.RT1u rat bone marrow by culturing with IL-4 and GM-CSF. Upon maturation, dendritic cells were pulsed in vitro with 10-100 micrograms of recombinant, conformationally-folded, MHC class I alloantigen (RT1.Aa) for 90 minutes. DC were then washed repeatedly before infusion of 1 million cells into naïve syngeneic PVG.RT1u recipients. Serial blood samples were analysed for the presence of antibody against conformational RT1.Aa protein in groups of rats treated with Aa-pulsed DC, or with DC alone, or with Aa-pulsed DC that had been lysed. Alloantibody was typed and quantified by ELISA using conformational class I bound to 96-well plates, and anti-rat immunoglobulin as detection antibodies.

RESULTS

Cultured bone marrow cells displayed typical DC morphology from day 5 onwards and were negative, by flow cytometry, for T cell, B cell and macrophage markers but positive for rat DC markers. By day 10 of culture dendritic cells had acquired a mature phenotype with increased levels of costimulatory ligand expression.
Following infusion of DC, high levels of anti-Aa antibodies of IgG class were detectable by day 10 in rats treated with Aa-pulsed DC but not in rats treated with DC alone or with lysed, Aa-pulsed DC. Antibody levels rose by day 14 and decreased by day 21, and were comparable in magnitude and kinetics to anti-Aa antibody levels in PVG.RT1u rats immunised with an infusion of blood from a PVG.R8 donor (RT1.Aa).

CONCLUSIONS

Our results provide the first in vivo evidence that recipient dendritic cells can present intact alloantigen for the generation of alloimmunity. Recipient DC may play a fundamental role in co-ordinating the recognition and effector arms of the alloimmune response, both in mediating graft rejection and in the induction of transplant tolerance.

O29

Mycophenolate Mofetil for Patients with Deteriorating Renal Transplant Function: Long Term Results of a Clinical Trial

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The evidence for a role for calcineurin inhibitors in the pathogenesis of chronic allograft nephropathy (CAN) is expanding. We first reported our early results of Cyclosporin (CyA) withdrawal and its substitution with either Tacrolimus or Mycophenolate Mofetil (MMF) in patients with CAN and deteriorating renal function in early 2000. We demonstrated that both drugs improved renal function initially. On follow up, however, it became apparent that only MMF sustained this improvement. As a consequence all patients with CAN, worsening renal transplant function and CyA based immunosuppression are switched to MMF based therapy. We report long term follow up of the first cohort and patients recruited subsequently as part of our clinical practise.
54 patients (15 from the original cohort) had CyA withdrawn and replaced with MMF. This involved starting MMF and building up to a maximum tolerable dose over 2 weeks followed by complete CyA withdrawal. In dose increments of 1/3rd over 3 months. Transplant biopsy was performed routinely in the first 15 patients only. Primary renal diseases were 8 reflux nephropathy, 8 glomerulonephritis, 5 adult polycystic kidney disease, 4 reno-vascular/hypertension, 3 IgA nephropathy, 2 diabetic nephropathy, 7 other and 15 unknown. Data is expressed as mean±standard deviation. See table 1 for demographic data. Mean follow up was 21.2±12.8 months.

Male: Female ratio	35:19
Age at Transplantation	39.7±11.9
Time Post Transplantation to Conversion (years)	6.8±4.3
Serum Creatinine at Conversion (μmol/L)	224±73
Cockroft Gault GFR at Conversion (ml/min)	40.8±15.8
Rate of Deterioration of serum creatinine in 6 months prior to conversion (μmol/L/year) (determined by linear regression analysis)	72±89

No episodes of acute rejection were seen in the conversion period. One graft was lost to progression of CAN and 3 patients died with a functioning graft. Two patients failed conversion to MMF and were converted back to CyA because of diarrhoea. Side effects noted included diarrhoea, infections (pneumonia, cellulitis, VZV, CMV, oral candida, salmonella and giardia), anaemia and a perforated oesophagus.
Significant improvements in serum creatinine (see table 2) and Cockroft Gault GFR were seen at all time points after the time of conversion with the greatest improvement in the first 3 months during the CyA withdrawal phase. Significant improvements were also seen in systolic and diastolic blood pressures and in lipid profiles.

	At Conversion	3 months	6 months	1 year	2 years	3 years
Serum Creatinine (μmol/L)	224±73	183±53	165±44	184±51	171±76	140±27
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Our data continues to support our previous findings that the use of MMF in these patients is safe and prolongs renal allograft survival in those patients with deteriorating transplant function.

O30

Direct alloantigen recognition by $CD4^+$ T cells is critically dependent on CD40-CD154 interactions in response to a donor-specific transfusion but not to a cardiac allograft.

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Introduction Blockade of CD40-CD154 costimulatory pathway is an effective strategy to inhibit $CD4^+$ T cell-mediated allo-immune responses. However, the exact role of the CD40-CD154 costimulatory pathway in $CD4^+$ T cell responses to alloantigen has yet to be fully elucidated. The aim of this study was to establish an *in vivo* model in which we could follow the fate of $CD4^+$ T cells that recognise MHC class II alloantigen by the direct pathway. We used this novel experimental approach to directly examine the response of alloantigen-reactive $CD4^+$ T cells when stimulated by a donor-specific transfusion or a cardiac allograft under conditions of CD40-CD154 costimulation blockade.

Methods $6x10^6$ CFSE labelled alloreactive TCR-transgenic $CD4^+$ T cells (-D T cells; recognition of MHC class II $H2A^b$ is confined to the direct pathway) were adoptively transferred into T cell-depleted syngeneic B10.BR mice. These mice were either pre-treated with a $H2A^{b+}$ DST i.v. and 3x 500µg of an anti-CD154 mAb, MR1, or received an $H2A^{b+}$ cardiac allograft and MR1. The proliferation (as measured by loss of CFSE) and activation of alloreactive $CD4^+$ T cells was assessed in the spleen at several time-points after alloantigen challenge.

Results In the absence of CD40-CD154 costimulation (DST+MR1), i.v. alloantigen challenge in the form of a DST initially (day +1) activated alloreactive $CD4^+$ T cells, as demonstrated by the increase in cell size and upregulation of various cell-surface activation markers such as CD25, CD69 and CD44, in a manner similar to that observed in mice that received DST + hamster control antibody (Hlg). However, by 7 days after i.v. alloantigen challenge most $H2A^b$-reactive $CD4^+$ T cells had been deleted by blocking CD40-CD154 costimulation ($4.4x10^4 \pm 1.0x10^4$ –D $CD4^+$ T cells in spleen) but not following DST+Hlg treatment ($22.0x10^4 \pm 6.0x10^4$ –D $CD4^+$ T cells in spleen). Kinetic analysis revealed that the deletion of alloreactive $CD4^+$ T cells was evident as early as 2 days post DST challenge.

Next we examined the response of adoptively transferred $H2A^b$-reactive $CD4^+$ T cells to a $H2A^{b+}$ cardiac allograft under conditions of CD40-CD154 blockade. We found that 7 days after cardiac allograft transplantation splenic alloreactive $CD4^+$ T cells had proliferated ($19.1 \pm 3.6\%$ divided). In clear contrast to the results from the DST experiments, alloreactive $CD4^+$ T cells were not deleted following CD154-blockade. Indeed, the proliferative response of alloreactive $CD4^+$ T cells was not significantly different to that in control mice that had received an $H2A^{b+}$ cardiac allograft and Hlg ($22.0 \pm 8.4\%$ divided).

Summary & Conclusion These data demonstrate that $CD4^+$ T cells that are reactive to alloantigen via direct recognition can have distinct costimulation requirements depending on the form and microenvironment of primary alloantigen contact.

In contrast to a DST, following transplantation, blocking CD40-CD154 interactions does not abrogate direct $CD4^+$ T cell responses. Therefore these data suggest that long-term allograft acceptance induced by anti-CD154 mAb treatment may be confined to $CD4^+$ T cells responding via the indirect pathway and not the direct pathway of allo-recognition.

O31

Nitric Oxide Production Is Associated With Acute Rejection In Kidney Transplantation. Have we finally found a marker for rejection?

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Background and Aim: Diagnosis of acute rejection relies on biopsy (Bx), with all non-invasive tests failing to show satisfactory predictive value. Inducible Nitric Oxide Synthase (iNOS) has been shown to play a role in acute rejection (AR) and once stimulated generates a large amount of nitric oxide which is mostly oxidized to its products nitrite (NO2) and nitrate (NO3). Aim of this study is to analyse the relationship between NO production (as measured by the level of NO2 and NO3) and biopsy proven allograft rejection. Furthermore to explore the use of measuring NO3 as marker of rejection during periods of allograft dysfunction.

Patients and Methods: Fourteen consecutive renal allograft recipients aged 23-72 years, that were transplanted in a single centre were prospectively recruited (recruitment is ongoing with a target of 50 patients). Blood and urine samples were collected before the transplant, at 15min, 75 min post-perfusion and thereafter at regular intervals (every two days initially and then at every visit for the first 8 weeks following their transplant). Extra samples were collected when an event requiring admission or causing renal dysfunction took place. The samples were centrifuged and stored to -80°C. To determine endogenous serum and urinary nitrite levels Griess reagent is added to the samples and nitrite measured spectrophotometrically at 540nm. Nitrate levels are measured after reduction of NO2 to NO3 with a nitrate reductase. Biopsies were performed as clinically indicated.

Results: 14 patients, who had complete samples for 8 weeks are included in this report (mean number of serum samples per patient was 22). Nitrite levels were constantly very low and not found useful. Pretransplant Serum Nitrate levels (SNO3) were between 20 and 481mmol/l (median 59.5 and mean 105mmol/l). These levels of SNO3 at 15 and 75 min post perfusion were reduced by a mean value of 15 and 18mmol/l respectively. There was a strong association of preoperative with the immediate postoperfusion values of SNO3 in each patient (t-test correlation coefficient 0.986 and 0.988).
There were 14 Bx performed for clinical reasons. **7 Bx did not show AR and in all of those the concurrent SNO3 levels were< 50mmol/l**. In 5 out 7 Bx that showed AR the levels of SNO3 were > 130mmol/l. Two of those patients required OKT3 and had levels over 250 and 550mmol/l respectively. The levels of SNO3 returned to base value with successful treatment. Two patients with AR did not increase their SNO3 above 80mmol/l. The 10 patients that never had a rejection episode had nitrate levels of less than 80mmol/l in all their samples.
In 4 patients that experienced DGF the SNO3 was less than 55mmol/l during the duration of delayed function. There were 11 UTI's in 8 patients. There was no instance that SNO3 was above 40mmol/l during an episode of UTI.
There was a total of 33 serious events including Bx, DGF (without Bx), UTI's, Creatinine increase>10% (without Bx). **During those events a level of SNO3 > 130mmol/l was 100% specific and 78% sensitive for AR.**

Conclusion: Serum Nitrate measured spectro-photometrically,post kidney transplant,increases with episodes of acute rejection but not with other causes of renal dysfunction. Therefore it can serve as a marker of acute rejection with specificity of 100% and good sensitivity.

O32

Human innate regulatory nkt and allopeptide-specific CD4+CD25+ cells control both direct and indirect alloresponses ex-vivo

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Innate NKT and CD4+CD25+ T cells have been identified recently as spontaneously occurring regulatory cells in the control of autoimmunity. Although these regulatory cells appear to mediate transplantation tolerance, little is known concerning the regulatory role of human NKT cells in alloresponses and the antigen-specificity of CD4+CD25+ cells. Here we describe the characterisation of naturally occurring NKT cells from human peripheral blood and the induction of allopeptide (HLA-A2: 138-170) specific human CD4+CD25+ regulatory T cell lines by priming purified CD4+CD25+ cells ex-vivo. Methods: T cell receptor (TCR) Vα24+Vβ11+ cells were purified by FACS sorting from peripheral blood lymphocytes. In order to establish the A2 peptide-specific CD4+CD25+ regulatory T cell lines purified CD4+CD25+ cell populations from HLA-A2 negative individuals were primed with immature DCs pulsed with the A2 peptide. Results: Vα24+Vβ11+ cells represented a very tiny population of T cells ranging from 0.01% to 0.1%. They suppressed IL-2 production by CD4+ T cells stimulated by allogeneic DCs in the presence of α-galactosylceramide. The suppression was dose-dependent. More than 95% inhibition was seen at a ratio of Vα24+Vβ11+ cells to responder CD4+ T cells of 1:50. The suppression was partly cell contact-dependent. When the two cell populations were stimulated separated by a semi-permeable membrane, minimal suppression was observed. Addition of anti-GITR antibody could partially reverse the suppression effected by Vα24+Vβ11+ cells. These results indicate that innate NKT cells are the most potent regulatory cells described so far and they may serve to the early phase of controlling alloresponses. We next compared NKT cells to the in vitro-generated allopeptide-specific CD4+CD25+ T cell lines. The CD4+CD25+ cells were anergic, but could be expanded by weekly re-stimulation with the peptide-pulsed immature DCs in the presence of exogenous IL-2. The CD4+CD25+ cells showed sustained high CD25 expression, and retained their ability to suppress antigen-driven responses of CD4+CD25- cells. They inhibited not only IL-2 secretion by CD4+CD25- T cells specific for the same allopeptide (suppression of indirect alloresponse), but also the direct alloresponse of naïve CD4+CD25- T cells stimulated by semi-allogeneic DCs in the presence of the peptide ("linked suppression"). Interestingly, anti-GITR antibody had no effect on the suppression effected by the CD4+CD25+ cells. These findings suggest that peripheral CD4+CD25+ regulatory cells are a precommitted cell lineage from which cells with specificity for non-self-peptides can be selected. Taken together, these data may pave the way for using innate NKT cells and "customised" allopeptide-specific CD4+CD25+ regulatory T cells as potential therapeutic tools in manipulating both direct and indirect alloresponses in vivo.

O33

Marginal donors – the importance of cold ischaemia time for successful liver transplantation

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Introduction:
Increasingly the liver transplant (LT) community is willing to accept more marginal donors because of the discrepancy between patients waiting and available organs. The optimal use of this group of organs is essential to minimise early allograft dysfunction which has been shown to have a profound influence on recipient outcome. This study aims to propose a method whereby donor characteristics can be determined and used to predict an acceptable cold ischaemia time to attenuate the risk of early allograft dysfunction.

Method:
We performed an analysis on data collected prospectively of 758 first time cadaveric liver transplants between January 1994 and January 2001 in non-fulminant recipients at our institution. Both univariate and multivariate analysis were performed on donor (plasmaNa, bilirubin, AST, inotrope use, hypotensive episodes, ITU stay, age, BMI, steatosis) and recipient factors (plasma bil, PT, Cr , ITU stay, blood requirements) with relation to early allograft dysfunction (primary non function + delayed graft function). This data was then used to classify donors into marginal and non-marginal populations, and the influence of cold ischaemia determined for each group.

Results:
Out of a cohort of 758 recipients 21 (2.8%) developed PNF (death or urgent re-transplantation days 2-7), and 85 (11.2%) developed PDF (PT>20 + AST>2500 in the first week). Multivariate analysis determined donor age (p=0.008) and steatosis (moderate/severe) (p=0.013) were independent predictors of deranged function. This enabled us to produce a scoring system to differentiate marginal donors with respect to risk of early allograft dysfunction as follows:

Score = [0.35x donor age] +[11x(steatosis: 0 if no steatosis/mild, 1 moderate/severe steatosis)]

ROC curve analysis determined an ideal cut-off value of 17.5, below which (non-marginal donors) there was no correlation with cold ischaemia time and early allograft dysfunction and above which (marginal donors)there was a strong correlation (p=0.002). In the marginal group the cut off value of cold ischaemia time was 12.6 hours.

Conclusion
From this cohort of liver transplant donors and recipients we developed a scoring system that classified an organ as either marginal or non-marginal depending on the donor age and degree of steatosis. Marginal livers have a strong risk of developing early allograft dysfunction with increasing cold ischaemia times and should be transplanted within 12 hours. Cold ischaemia time was not found to be an important factor in the development of early allograft dysfunction in non-marginal donors.

Parallel Session III(a)

Regulatory Cells

Wednesday 9 April 2003

14:20 – 15:30

O34

Gene expression associated with tolerance and rejection – kinetic expression studies in different transplantation models

B Sawitzki[1], A Bushell[1], N Jones[1], U Steger[1], K Risch[3], M Lehmann[3], M Fisser[2], N Profanter[2], H Volk[2] and K Wood[1]

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INTRODUCTION: Due to the development of new improved strategies the induction of transplantation tolerance in patients might be possible within a few years. But at present there are no accurate methods available to measure such tolerance, to enable induction, development and maintenance to be monitored. In order to identify "tolerance" or "rejection" associated markers we have previously compared the gene expression of allo-specific graft infiltrating cells (GIC) from rejecting and during the development of tolerance kidney allograft recipient rats treated with anti-CD4 antibody. Using this approach, we were able to define 11 gene fragments that were differentially expressed in GIC during tolerance induction: 7 were up-regulated and 4 were down-regulated.
AIM: Identification of reliable markers whose mRNA expression is consistently associated with either rejection or long term acceptance of allografts following transplantation of different organs and in different species with the aim of assessing the success of tolerance induction therapy or of predicting immune mediated rejection episodes.
METHODS: The gene expression of the 11 fragments identified was analysed using Real Time RT-PCR in 3 different transplantation models of long term graft acceptance in 2 species: 1) The above mentioned rat kidney transplantation model, 2) a mouse heart transplantation model, where the unresponsiveness is induced by pretreating the recipient mice with an anti-CD4 antibody and a donor specific blood transfusion 4 weeks before transplantation and 3) a mouse liver transplantation model of spontaneous graft acceptance. Gene expression was analysed in the graft of the recipient as well as in peripheral lymphoid tissues including peripheral blood.
RESULTS: Among the 7 genes upregulated in GIC during tolerance induction, T8 (no homology to any known gene) and 1A50 (alpha-1,2-mannosidase) showed a consistently high expression in grafts from long term graft survivors in all 3 models examined whereas, expression of these two genes was down-regulated in rejecting grafts. Interestingly, we also observed a down-regulation of their expression in peripheral blood cells from rejecting recipients and that down-regulation was already detectable 3 days before the rejection occurred. Among the 4 down-regulated genes in GIC from tolerance developing recipients 1A6 (no homology to any known gene), 2A5 (RHAMM) and 2A15 (RhoGAP) were highly expressed during kidney and heart rejection whereas there was only a minor and transient increase in their expression detectable during tolerance induction.
CONCLUSIONS: From the profile of differentially expressed genes identified we hope to have obtained some reliable tolerance and rejection associated markers, that can be used to monitor the induction, development and maintenance of tolerance. As the expression of some of these genes was also regulated in peripheral blood cells of rejecting recipients we believe that we may also be able to use these markers to recognise clinical rejection episodes during or after treatment before impaired graft function is detectable. This may be applicable to tolerance induction strategies as well as conventional immunsuppressive therapies.

O35

Random blood transfusion in the absence of any additional therapy induces CD25+ CD4+ regulatory T cells: A probable explanation of the blood transfusion effect

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Background The blood transfusion effect has had a significant influence on the success of clinical cadaveric kidney transplantation and several potential explanations have been proposed including (1) the selection of low responder recipients, (2) the production of anti-idiotypic or blocking anti-donor antibodies, (3) a shift in the relative Th1/Th2 T cell balance, (4) the generation of suppressor cells.
Context and Aims Renewed interest in suppression or regulation in transplantation has been stimulated recently by observations suggesting that T cell regulation is an important peripheral mechanism for preventing autoimmune disease. In the transplantation setting we have previously shown that pretreatment of CBA (H-2^k) mice with a single DST under the cover of anti-CD4 antibody generates $CD4^+$ $CD25^+$ T cells that regulate responses to skin allografts using mechanisms shared with naturally occurring $CD25^+$ regulatory T cells. The aim of the present study was to use the same sensitive in vivo model to determine whether blood transfusion alone without additional immunotherapy can generate $CD25^+$ $CD4^+$ regulatory T cells that contribute to prolonged allograft survival.
Results $CD25^+$ $CD4^+$ T cells were isolated from CBA (H-2^k) mice given a single DST of B.10 (H-2^b) blood at day -7. These cells were co-transferred with naive $CD4^+CD45RB^{hi}$ effector cells into syngeneic CBA.Rag deficient mice which were then transplanted with full thickness donor-specific (B.10) skin grafts. Graft survival in this single-DST group was unimpressive with 3/5 grafts rejected acutely and 2/5 showing significant necrosis at day 100. Aware of clinical data indicating that the blood transfusion effect increased with the number of transfusions given, a second group of mice received multiple B.10 DST's at intervals and 7 days after the last transfusion, $CD25^+$ cells were isolated and their ability to regulate rejection determined as above. In contrast to the results seen with a single transfusion, mice that received $CD25^+$ $CD4^+$ cells from donors given the multi-DST protocol (n=7) all accepted their grafts for greater than 150 days with no evidence of rejection, no graft shrinkage, luxuriant hair growth and normal histology. In an attempt to model the clinical situation where random rather than donor-specific transfusions were used, an additional group of CBA mice received multiple transfusions of whole blood pooled from three donors MHC-unrelated to the skin graft donor. Seven days after the last transfusion $CD25^+$ cells were isolated from these cell donors and their regulatory capacity determined. $CD25^+$ cells from these random-blood mice were fully able to prevent rejection mediated by purified RB^{hi} effector cells (n=4, MST >150 days) and like their multi-DST counterparts showed full hair growth and normal histology.
Conclusion The results of this study provide a basis for understanding the blood transfusion effect seen and exploited in clinical transplantation. $CD25^+$ regulatory T cells probably arise in this situation because the blood transfusion mimics the expression of peripheral self antigen which is known to drive the generation of $CD25^+$ T cells with regulatory capacity.

O36

Inhibition of alpha-1,2-mannosidase in regulatory t cells reduces their potential to inhibite activation of naïve CD4+ T cells

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Introduction: CD4+CD25+ T cells have potent immunoregulatory activity (Tregs). Several groups have reported a lack of IL-2 production by regulatory T cells after antigen stimulation in vitro. Recently we demonstrated, that long term surviving heart grafts express high levels of alpha-1,2-mannosidase. It has been shown that inhibition of alpha-1,2-mannosidase in anti-CD3 antibody stimulated T cells enhances their IL-2 production.

Aim: The aim of this study was to analyse alpha-1,2-mannosidase expression in alloantigen specifc Tregs after alloantigen stimulation. Furthermore, we tried to determine the importance of alpha-1,2-mannosidase for the function of regulatory T cells in regulating responsiveness to alloantigens in vitro and in vivo.

Methods: CBA (H-2k) mice were pretreated with an anti-CD4 antibody on two consecutive days and donor specific B10 (H-2b) blood transfusion (DST) at day -27. At day -3 or -1 the mice received a second DST in order to mimic exposure to alloantigen normally provided by the allograft. 28 days after initiation of treatment, spleens were harvested and leukocytes sorted into CD4+CD25+ or CD4+CD25- populations. Total RNA was isolated and cDNA reverse transcribed. The mRNA expression of alpha-1,2-mannosidase was analysed applying Real Time TaqMan PCR using expression of CD3 to normalise the cDNA concentration in each sample. Additionally, alpha-1,2-mannosidase activity was inhibited by administration of 2.5-5 mM 1-deoxymannojirimycin during in vitro and in vivo regulation assays (Kingsley et al.).

RESULTS: Surprisingly in pretreated animals additional exposure to alloantigen resulted in a 4 fold increase of alpha-1,2-mannosidase mRNA expression in CD25+ cells 1 day after restimulation. By 72 hours after alloantigen challenge alpha-1,2-mannosidase in the CD4+CD25+ cells from pretreated mice had fallen below that of naïve CD25+ cells. This expression kinetic was highly antigen specific. Interestingly, inhibition of alpha-1,2-mannosidase during regulation assays resulted in abolished suppression of activation and proliferation of naïve CD4+ T cells by Tregs.

CONCLUSIONS: Our data show, that CD4+CD25+ regulatory T cells express alpha-1,2-mannosidase at higher levels than CD25- cells. Furthermore, Tregs are dependent on alpha-1,2-mannosidase expression in order to regulate the activation and proliferation of naïve CD4+ T cells. These data support a unique role for alpha-1,2-mannosidase in the development and function of regulatory T cells after alloantigen pre-treatment.

References:

Kingsley CI, Karim M, Bushell AR, Wood KJ. CD4+CD25+ regulatory T cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses. J Immunol. 2002 Feb 1; 168(3):1080-6.

O37

Generation of CD25$^+$CD4$^+$ regulatory T cells in a transplantation model: development is thymus-independent and does not require CD25$^+$ precursors

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CD25$^+$CD4$^+$ regulatory T cells (Treg) play an important role in the control of immune responses *in vivo*. It has been demonstrated that naturally-occurring autoreactive CD25$^+$CD4$^+$ Treg undergo positive selection within the thymus and leave as committed CD25$^+$ Treg. We have previously shown that CD25$^+$CD4$^+$ Treg with the capacity to prevent the rejection of skin allografts in mice can be generated by pre-treatment with donor-specific blood transfusion (DST) given under the cover of anti-CD4 therapy. We wished to establish whether these Treg develop via a similar thymus-dependent pathway to naturally-occurring Treg.

Adult CBA ($H2^k$) mice that had previously undergone thymectomy were pre-treated with blood transfusion from C57BL/10 ($H2^b$) donors under the cover of anti-CD4 antibody, and CD25$^+$CD4$^+$ cells were then isolated for adoptive transfer. T cell-deficient (CBA-Rag$^{-/-}$) recipients were reconstituted with CD45RB^{high}CD4$^+$ effector cells from naïve mice with or without CD25$^+$CD4$^+$ cells from the pre-treated animals, and then received a C57BL/10 skin allograft. Adoptive transfer of effector cells alone resulted in acute rejection of the skin allografts whereas, in clear contrast, co-transfer of the CD25$^+$CD4$^+$ cells overcame this rejection and allowed long term skin graft survival, demonstrating that these Treg were generated in a thymus-independent process.

We next wished to establish whether these CD25$^+$CD4$^+$ Treg arise as a result of the peripheral expansion of naturally-occurring CD25$^+$CD4$^+$ Treg that cross-react with alloantigen, or by the conversion of mature peripheral T cells (which may be CD25 positive or negative) to a Treg phenotype. We therefore reconstituted CBA-Rag$^{-/-}$ mice with CD25 *negative* CD4$^+$ cells, treated these animals with C57BL/10 blood and anti-CD4 antibody, and administered CD45RB^{high}CD4$^+$ effector cells prior to performing a C57BL/10 skin allograft. These mice all accepted their skin allografts long term, whereas animals reconstituted in the same way but without the tolerising anti-CD4 / DST protocol all rejected their grafts acutely.

These results demonstrate that, in contrast to the development of naturally-occurring autoreactive CD25$^+$CD4$^+$ Treg, the anti-CD4 / DST pre-treatment in this model does not generate alloreactive Treg through a thymus-dependent process or by the expansion of pre-existing CD25$^+$CD4$^+$ Treg populations, but rather by the conversion of mature peripheral T cells to a regulatory phenotype. These observations may have important implications for the design of clinical protocols to induce allograft tolerance in adult recipients.

O38

Changes in MICB expression are associated with cellular stress following renal transplantation

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MICA and MICB (MHC class I-related chain A and B) are highly polymorphic genes that encode molecules closely related to MHC class I and are induced on epithelial cells in response to stress. Their precise function is not fully understood, but they are recognised by γδ, CD8+ αβ T cells and NK cells via NKG2D and may play a role in autoimmunity, tumour and virus recognition. Importantly, incompatible donor MIC genes have recently been shown to initiate the formation of antibodies in recipients of organ transplants. The aim of this study was to determine whether there are changes in expression of MICB following renal transplantation and whether changes are associated with graft rejection and function.

Paired renal biopsies obtained from living donor (n=10) and cadaveric renal allografts (n=50) before and 7 days post-transplant were stained with a rabbit polyclonal antibody specific for MICB. The level of staining was compared to that of HLA class II, molecules known to be up-regulated on renal tubules during inflammatory responses.

Variable levels of tubular expression of MICB were evident in pre-transplant biopsies [high 6/60 (10%), low/negative 13/60 (22%), intermediate 35/60 (58%)]. High levels of expression of MICB were significantly associated with induction of MHC class II on renal tubules in pre-transplant donor biopsies. Following transplantation, MICB was up-regulated on the renal tubules of 17/60 (28%) biopsies. This was significantly associated with the induction of MHC class II antigens ($p = 0.04$). Acute rejection (AR, diagnosed using the Banff '97 classification) and delayed graft function (DGF, requirement for dialysis in the first week after transplantation) can both be considered objective markers of cellular stress within the transplanted kidney. At day 7, 37/60 transplants were found to have AR and/or DGF. There was a strong association between upregulation of MICB and cellular stress, 15/17 biopsies with up-regulated MICB expression had AR and/or DGF ($p=0.003$).
In summary, this is the first study that demonstrates that MICB expression is induced following renal transplantation. MICB expression is associated with HLA class II induction and with cellular stress in the transplanted kidney.

O39

CD4+CD25+ T cells are dependent on interferon gamma expression in order to prevent skin graft rejection by naïve CD4+ T cells

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INTRODUCTION: It is well documented that CD4+ CD25+ T cells are potent immunoregulatory cells (Tregs) both in vivo and in vitro. However, there are conflicting data about cytokine production and dependence by Tregs following antigen exposure. We have previously shown that pre-treatment with donor alloantigen under the cover of anti-CD4 antibody generates donor specific CD4+ CD25+ T cells, that can regulate rejection of a donor specific skin graft mediated by naïve CD4+ T cells.
AIM: The aim of our study was to determine cytokine mRNA expression by donor alloantigen specific regulatory T cells in vivo after exposure to donor alloantigen.
METHODS: CBA (H-2k) mice were pretreated with an anti-CD4 antibody on two consecutive days. On the second day of pretreatment the mice received a transfusion of donor specific B10 (H-2) blood (DST). 25 and 27 days later they received a second DST in order to mimic exposure to alloantigen that would normally be provided by the allograft. 28 days after initiation of treatment spleens were harvested and leukocytes sorted into CD4 CD25+ or CD4+ CD25- populations. Total RNA was isolated and cDNA reverse transcribed. The mRNA expression of cytokines was analysed applying Real Time TaqMan PCR using expression of CD3 to normalise the cDNA concentration in each sample.
RESULTS: Surprisingly in pretreated animals additional exposure to alloantigen resulted in a 5 fold increase of Interferon gamma mRNA expression in CD25+ cells 1 day after restimulation with donr alloantigen whereas CD25- cells showed no such increase in Interferon gamma mRNA expression. By 72 hours after alloantigen challenge, Interferon gamma in the CD4+ CD25+ cells of pretreated mice had fallen below that of naïve CD25+ cells. This expression kinetic was highly antigen specific, exclusive to Interferon gamma and not observed for any other cytokine analysed. Neutralisation of Interferon gamma after co-transfer of CD4+CD25+ regulatory T cells together with CD4+CD45RBhigh effector T cells into RAG(-/-) skin graft recipients resulted in dramatically reduced regulation and subsequent skin graft rejection.
CONCLUSIONS: Our data show, that CD4+CD25+ regulatory T cells do express Interferon gamma. Furthermore, Tregs are dependent on early Interferon gamma expression in order to prevent rejection of skin grafts by naïve effector T cells. These data support a unique role for Interferon gamma in the development of tolerance after alloantigen pretreatment and are consistent with observations showing that tolerance cannot be induced in the absence of Interferon gamma (Interferon gamma k.o. mice).

O40

Signals through CTLA-4 are not required for the generation of CD25+CD4+ regulatory T cells that prevent graft rejection

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Background and Aims We have recently shown that pretreatment of recipient mice with donor-specific transfusion (DST) under the cover of anti-CD4 antibody generates a population of $CD25^+CD4^+$ T cells that prevent allograft rejection. These regulatory T cells arise as a consequence of the pretreatment alone, are present prior to transplant and like their naturally occurring counterparts, regulate in a manner that is dependent on CTLA-4. Given that blockade of the B7 family of surface molecules is likely to enter clinical trial in the near future, the aim of the present study was to determine whether signals delivered through CTLA-4 are required for the *generation* of these powerful $CD25^+$ regulatory T cells in vivo.

Results CBA mice ($H\text{-}2^k$) were tolerized with anti-CD4 antibody (either YTS 177.9, IgG2a or KT6, IgG2a) on days –28 and –27 plus a B.10 (H-2b) DST on day –27. During this tolerance induction phase the mice also received anti-CTLA4 antibody (UC10-4F10-11) at 250µg per dose on days –28, -27, -26 and –25. We have previously shown that this antibody completely blocks the regulatory function of $CD25^+$ T cells on adoptive transfer and when given at the time of transplant this dose of 4F10 prevents the engraftment of primary cardiac allografts in otherwise tolerant mice. At day 0, $CD25^+CD4^+$ cells from these mice were transferred together with naive $CD4^+CD45RBhi$ effector cells into syngeneic CBA.Rag deficient recipients which were transplanted 24 hours later with full thickness donor-specific (B.10) skin grafts. $CD25^+CD4^+$ T cells isolated from either cohort of anti-CD4/DST + anti-CTLA4 cell donors behaved exactly like their counterparts isolated from tolerized mice without antibody blockade and prevented graft rejection mediated by the RBhi effector cells (177/DST n = 4, MST >100 days; KT6/DST n = 4, >100 days) indicating that signals through CTLA-4 are not required for the generation of allo-specific $CD25^+$ regulatory T cells.

Conclusions and significance Although signals through CTLA-4 are required for their regulatory function in vivo, the results of the present study obtained from two different anti-CD4/DST tolerance models clearly indicate that the generation/expansion of allo-specific $CD25^+$ regulatory T cells is not dependent on CTLA-4. Co-stimulation blockade with CTLA4-Ig and its derivatives is entering clinical trial and although the principle aim of these reagents is to block T cell activation via CD28 such fusion proteins will also prevent signalling through CTLA-4 itself. The results of the present study give grounds for cautions optimism that this inevitable co-blockade of CTLA-4 should not preclude the development of regulatory T cells that may contribute eventually to operational tolerance in clinical transplantation.

Parallel Session IV(a)

Donation II

Wednesday 9 April 2003

16:30 – 17:30

O41

Variation in cadaveric organ donor rates in the United Kingdom

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Objectives - To determine whether or not observed variations in the number of cadaveric heart beating organ donors between Kidney Retrieval Areas (KRAs) within the United Kingdom (UK) are correlated with various possible explanatory factors.

Design - Geographical study involving Poisson regression analysis, with the number of donors as dependent variable, and the following as independent variables (adjusted for population size): deaths from road traffic accidents, intra-cerebral haemorrhage, and other trauma; the number of general and neurosurgical intensive care unit (ICU) beds; co-location of transplant and neurosurgical units in the same hospital; proportion of the population from minority ethnic groups; proportion of the population on the organ donor register; the number of transplant coordinators working in organ retrieval; the number of transplant units.

Setting - the 21 KRAs in England, Scotland, Wales and Northern Ireland in calendar years 1999 and 2000.

Main outcome measures - Donor rate in each KRA. Strength of any statistical association between the independent and dependent variables, and the magnitude of changes in the donor rate associated with changes in independent variables.

Results - The donor rate varied between 8 and 27.4 donors per million population per year across the KRAs, which is more than can be accounted for by chance. In multivariate analysis there was an association between the donor rate and the number of general ICU beds (i.e. more beds associated with a higher donor rate), but this was of borderline statistical significance ($p=0.065$). However the donor rate was negatively associated ($p<0.05$) with the number of neurosurgical ICU beds (more beds, fewer donors) and the proportion of the population from minority ethnic communities. There was no statistically significant association with the other independent variables.

Conclusions - There is significant variation in the organ donor rate between different parts of the United Kingdom. In those areas where there are more neurosurgical ICU beds, and a higher proportion of the population from ethnic minority origin, there are fewer donors. There is a weak link between the number of general ICU beds and the number of donors. The association between the number of neurosurgical ICU beds and donors is counter-intuitive, and merits more research both to confirm or deny it, as well as to understand the mechanisms for the link.

O42

Laparoscopic Live Donor Nephrectomy: A Single centre Experience of a New Surgical Procedure

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Increasing the rate of live donor renal transplants is one way of addressing the shortfall in the number of organs for transplantation. The donor operation has traditionally been performed through a loin incision. However, in 1995 laparoscopic live donor nephrectomy was introduced in the USA and this has now virtually replaced open surgery as the operation of choice there. There is still much debate in the UK as to which is the best approach. We present our units data and experience of laparoscopic live donor nephrectomy since it was introduced in March 1999.

We have currently performed 39 live donor nephrectomies (8 paediatric and 31 adult recipients of which 8 were unrelated). The recipient operation is done sequentially by a different surgeon. For demographic data see table 1. For results see table 2.

Donor Male:Female	22: 17
Recipient Male:Female	20: 19
Donor Age (years)	44.8±13.5
Recipient Age (years)	32.5±17.6
Right: Left kidney	2: 37
Haplotype Mismatch 0:1:2	3: 24: 12
Nos of arteries 1: 2: 3	31: 5: 3

Operative time (mins)	165±29
Anaesthetic time (mins)	209±36
1° warm ischaemic time (mins)	4.1±2.5
Cold ischaemic time (mins)	82.9±54.1
2nd warm ischaemic time (mins)	29.3±7.5
Time to full diet (days)	1.5±0.7*
Post operative stay (days)	3.0±1.0*

*1 patients data excluded

*1 patients data excluded

Two patients required conversion for soft tissue bleeding during the donor procedure. One patient had a small bowel injury that was recognised on the first post operative day. This resulted in a prolonged post-operative in patient stay and this data has been excluded from the above table. There have been 2 graft losses, one to non-compliance and one to arterial thrombosis. No delayed graft function has been seen. Other complications reported were nausea (n=5), constipation (n=2), stitch abscess (n=2), wound/retroperitoneal haematoma (n=2) and UTI, wound pain, hypertension, and wound emphysema (all n=1).

The 2 patients requiring conversion and the patient with a bowel perforation were operated on early in this series and it is felt that they had been part of the learning curve associated with a new procedure. Modifications have been made to our operative technique and post-operative management subsequently. We now feel that this is a safe procedure in our hands and offer it to all of our patients requesting living kidney donation.

O43

Perceptions of living kidney donation by patients on the cadaveric transplant waiting list

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Live donation is being promoted to increase the supply of donor organs for transplantation. Whilst physiological and psychological benefits to the donor are well documented, there is a dearth of evidence relating to the psychological and social acceptability, or otherwise, of this option for the potential recipient.
This study examines the attitudes of patients waiting for a kidney transplant, towards living donation. Preferred source of organ and willingness to accept a live donor from family and non-family members were investigated. The potential recipient's efforts to find a living donor were also documented. Ninety-five respondents with End Stage Renal Failure, currently on the waiting list for a cadaveric transplant, completed a postal questionnaire specifically designed for the study. Fourteen of the respondents were randomly selected for a follow-up interview. The interview data were analysed using Grounded Theory and the survey data were submitted to statistical analysis.
The majority of patients (44%), preferred to have a transplant from a cadaveric donor, 83% of them wishing to avoid harm to a relative. Of those preferring a living donor (17%), the main reason identified related to patient's perception of a better matched kidney (75%), better quality of kidney (69%) and greater chance of success (63%). The survey, showed that patients were statistically most willing to accept a 'stranger' as their living donor. Acceptability of donation from a sibling, parent or friend was relatively equal, but patients were least willing to accept a kidney from their offspring. The Grounded Theory analysis revealed a complexity of decision-making strategies, one of which involved crossing thresholds of acceptability of any donor depending on perceived donor insistence and recipients need. The survey results showed that most patients report not asking someone directly for a kidney (76%). Those who had asked someone had most frequently asked a blood relative, usually with some degree of difficulty (65%). Patients who asked were more likely to be younger (t=3.369 p=0.001). 20% of patients admitted to requesting by proxy. In most cases it was the patient's spouse who made the request for donation. Over half of those surveyed reported having someone volunteering to donate and of those, 10% were parents, 25% were siblings and 23% of patients had offers of donation from offspring, (identified as the least favoured donor). Interview data revealed that patients had experience of no one offering, conditional offers (depending on 'near death' of patient) and people refusing. This was observed to result in major personal and family conflicts, some of which may have left a permanent legacy of ill feeling. The subject of living donation was often avoided by the recipient within their families as evident from the interview data and the survey revealed low levels of information seeking regarding living donation.
The clinical implications of these findings indicate that when promoting this option as a solution to the organ donor shortage, the responsibility extends to the multidisciplinary team to ensure that the potential for psychological harm and family conflict is acknowledged and addressed. In our unit the live donor co-ordinator and the clinical psychologist are developing interventional strategies to address the complex and difficult issues described.

O44

Feasibility Study into the potential for a Controlled Non Heart Beating Organ Donation Programme in a Regional Neurosurgical Intensive Care Unit.

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There are currently several initiatives within the United Kingdom with the aim of increasing the rate of solid organ transplantation. One approach intends to increase the number of units retrieving organs from non-heart beating donors.

Before this was put in place we had appointed staff locally to fully explore the potential for non-heart beating organ retrieval. In this component of the study the potential for "controlled" (Mastrich category three) non heart beating donation was examined. In this abstract the summary results of a twelve-month audit of all the deaths within a single large regional neurosurgical intensive care unit that started in October 2001 are presented. See table below.

Of the remaining deaths further criteria was used to explore the potential for controlled non-heart beating organ donation. This included age between 16-65 years, the planned withdrawal of life supporting treatment, normal biochemistry on day of death. A case-by-case assessment of the suitability for organ donation was made by reviewing the medical records.

In the twelve-month period 39 patients who died were identified on the above criteria as potential non-heart beating donors. Of these 27 patients died within two hours of the withdrawal of life supporting treatment.

Although the frequency of refusal of consent for organ retrieval from relatives and the logistic barriers to organ retrieval from non-heart beating donors is not known in this setting, it appears likely that a significant number of solid organs could be retrieved from this and similar units.

Total number of deaths	98
Organ donors	19
Relative refusal following death diagnosed on brain stem criteria	4
Medical contraindication to organ donation	11
Remaining number of patients	64

O45

Potential donor audit

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As part of a series of measures to improve organ donation, UK Transplant has established a Potential Donor Audit (PDA). Several large scale donor audits were undertaken in the UK in the late 1980s/ early 1990s and UK Transplant's PDA aims to provide an up-to-date assessment of the potential for organ and tissue donation from Intensive Care Units (ICUs) in the UK. Although various local exercises are being undertaken to measure the number of brain stem dead patients, it was felt that a national approach was required.

A pilot of the PDA began in May 2002 using an audit form developed by UK Transplant in collaboration with members of the Transplant Co-ordinator's Advisory Group, other transplant co-ordinators and ICU link nurses. The 21 Donor Liaison Nurses (DLNs) already funded by UK Transplant complete one form for each death in an ICU that they cover. Several donor transplant co-ordinator teams also participate in the pilot study and to date, approximately 130 ICUs are involved.

An evaluation of the first three months of data collected from the pilot study (June-August) and recorded on the National Transplant Database as of 1 September 2002, took place during September. Reports of each unit's activity were sent to the ICU itself and to the relevant DLNs and transplant co-ordinators. A report detailing the overall national results of the three months pilot data was also produced and sent to appropriate personnel.

The results from the National report showed that at the time of the analysis, 50 hospitals (58 ICUs) had reported at least one patient death. A total of 617 patient deaths were therefore included in the analysis. Of these 617 patient deaths, 532 (86.2%) were on mechanical ventilation at sometime during their hospital stay; brain stem death was a possible diagnosis for 68 (11.0%); brain stem death tests were performed on 54 (8.8%) and brain stem death was confirmed for 53 (8.6%). In 46 cases the possibility of donation was suggested to relatives and consent for donation was given in 28 (61%) cases. 25 (54%) of these 46 cases became actual donors. In only 7 (13%) of 53 families was there no discussion of donation with relatives.

It is intended that a further report comprising data from six months of the pilot study (June-November) will be produced early 2003. This report will provide more up-to-date information to that presented in this abstract. The pilot study will continue until the end of December 2002. The National PDA will commence on 1 January 2003 and it is anticipated that every ICU in the UK will take part.

It is hoped that the implementation of the National PDA will continue to raise the profile of organ donation and heighten awareness of donation issues amongst all critical care staff. In addition it will allow a realistic estimate to be made of the true potential for organ donation in the UK, based on UK data, and will allow the identification of both local and national obstacles to reaching the potential.

O46

National kidney allocation scheme - Four year review

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

A revised Kidney Allocation Scheme was introduced in the UK in July 1998 based on HLA matching at three levels: 000 mismatches, favourable matches (ie 100, 010 and 110 HLA-A, B, DR mismatches) and non-favourable matches (all other HLA matches). Within these levels paediatric and local patients receive priority and since July 2000 priority has also been given to HLA-DR homozygous patients when the donor is HLA-DR homozygous. A second change to the Scheme in July 2000 gave priority to favourably matched paediatric patients anywhere in the country over local favourably matched adults for the second kidney from an adult donor. A further change to the Scheme was made at the end of its fourth year: blood group B patients were made eligible to receive blood group O donor kidneys, with overall priority retained by blood group O patients. Any ties are sorted on six points scoring factors. These are recipient age, donor-recipient age difference, matchability (a score based on HLA tissue type, unacceptable antigens and blood group), waiting time, sensitisation to HLA antigens and centre/alliance Balance of Exchange.

To assess the effectiveness of the revised scheme, results of the first four years have been compared with those of the last 18 months of the previous scheme. Through greater exchange of organs between centres and increased access to favourably matched adult kidneys for paediatric patients, there have been significant improvements in HLA matching for all adult and paediatric transplants ($p<0.0001$). The proportion of 000 mismatched grafts has increased from 7% to 14% for adults and from 5% to 11% for paediatric recipients and overall, HLA matchgrades for paediatric recipients are now comparable with those for adults. There has also been a significant improvement in HLA matchgrades for HLA-DR homozygous patients: 50% 000 mismatched or favourably matched compared with less than 30% prior to 1 July 2000 ($p<0.0001$). This is due to increased use of HLA-DR homozygous donor kidneys in these patients which has also resulted in a 14% increase in the number of transplants for HLA-DR homozygous patients.

Highly sensitised patients have received more transplants under the revised scheme and a decreasing number of positive crossmatch test results have been reported. For kidneys allocated to adults through the national scheme there have been some changes related to points scoring factors. Initially the revised scheme appeared to be favouring younger adults but this has not been borne out by subsequent experience. The mean donor-recipient age difference has decreased by two years. Also, patients who are moderately difficult to HLA match initially received proportionally more transplants at the expense of those who are easiest to match ($p=0.05$), although the trend has not been sustained in the latest two years.

In conclusion, the new UK Kidney Allocation Scheme has been associated with significantly improved HLA matching for all adult and paediatric patients. No change has occurred in age at transplant and there has been a decrease in donor-recipient age differences. HLA-DR homozygous patients are achieving significantly better HLA matched transplants than previously and early indications are that, as predicted, the most recent change to the scheme has benefited a small number of blood group B patients.

Parallel Session IV(b)

Chronic Rejection

Wednesday 9 April 2003

16:30 – 17:30

O47

Matrix Metalloproteinases & Their Inhibitors: Cadaveric Versus Live Related Allograft Recipients.

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Background: ECM degradation occurs predominantly through the matrix metalloproteinase (MMP) system, which is regulated by Tissue inhibitors of MMP (TIMP). Reduced ECM degradation can lead to ECM accumulation and scarring. We have previously reported reduced MMP and elevated TIMP levels in implantation, acute and chronic rejected biopsies of cadaveric allograft recipients suggesting these changes may have a pre implantation cause. Here we have compared MMP and TIMP levels between cadaveric and live related allograft recipients to determine if pre harvest events or organ preservation is effecting the MMP system.

Patients and Methods: Twenty six renal biopsies from cadaveric graft recipients and 17 renal biopsies from live related graft recipients were diagnosed and scored to acute and chronic allograft nephropathy. Fourteen biopsies of focal segmental glomerulosclerosis (FSGS) with a similar scarring index were included to control for the effects of cyclosporin on the MMP system. The expression of MMPs 1, 2, 3, & 9 and TIMPs 2 & 3 were localised using immunohistochemistry and evaluated by point counting.

Results: Point count analysis showed levels of all MMPs to be reduced and TIMPs elevated compared to normal sections. These changes were observed in acute and chronic rejection biopsies of both cadaveric and live related donors as well as in the FSGS biopsies. The reduction in MMPs was less in the live related group than the cadaveric groups, but this only reached significance for MMP1 (see table below).

Conclusions: The greater reduction in MMP 1 levels in allografts from a cadaveric donor than a live related donor suggesting that either pre harvest events or cold ischaemia influence MMP 1 expression. This may have implications for ECM processing and scarring in the allograft.

	Normal	Acute Cadaveric	Chronic Cadaveric	Acute Live related	Chronic Live related	FSGS
MMP1	44.22 ± 2.6%[C]	3.2 ± 2%	5±3.2%	50.6± 9.8%[C]	10.2±9.1%	25.1± 7.4%
MMP2	68.4 + 3.8%[B]	32.8+ 5.9%	28.2+ 6.9%	40.7 + 4.1%	35.7 + 8.4%	29.7 + 4.6%
MMP3	44.5 + 9.1%[B]	17.9+4.5%	32.1+ 6.5 %	40.2 + 12.1%	37.7 + 7.5%	27.6 + 6.6%
MMP9	46.7±12. 5%[A]	21.8±5.9%	26.7 ±7.8 %	35.7 ± 12.3%	24.5 ± 9.3%	20.3 ± 5.6%
TIMP2	14.5±4.3%[B]	29. ±4.6%	33.9±4.1%	38.4±11.4%	25.8±7.8%	34.3±4.5%
TIMP3	23.6±6.7%[A]	40.3±5.7%	48.2±6.2%	49.5±5.3%	40.9±4.5%	42.2±4.6%

A= P<0.05, B= P<0.01, C= P<0.001

O48

Interstitial fibrosis in chronic renal allograft dysfunction : A consequence of transdifferentiation of tubular epithelium to a myofibroblast phenotype?

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The fibro-proliferative process responsible for graft failure is initiated by acute alloimmune rejection during the first weeks after transplantation but the link between acute and chronic injury is not understood. We have reported development of a population of CD103+ T cells in the renal tubules during episodes of acute rejection that persists within the graft, maintained by locally produced IL-15. The primary lesion responsible for chronic graft dysfunction is tubulointerstital fibrosis associated with a loss of nephrons. Proliferating interstitial fibroblasts constitute the major effector cell type responsible for fibrosis. In the mouse the majority of these cells are generated from damaged and cytokine-stimulated tubular epithelial cells (TEC) by a process of epithelial to mesenchymal transdifferentiation (EMT). Demonstration of the induction of fibroblast specific protein 1 (FSP1) within damaged tubules in vivo allows direct visualisation of EMT. Importantly, expression of the human homologue of FSP1, S100A4, has not been investigated in the context of chronic renal allograft dysfunction. It is known that S100A4 expression increases the migratory potential of cancer cells, suggesting an association between this protein, change of shape and cell migration. TGFβ plays a crucial role in the induction of EMT within the kidney: its activity within the renal tubules is increased in both acute rejection and chronic graft dysfunction.
Methods: In this study a dual-colour immunolabelling procedure was used to visualise CD103 and TGFβ simultaneously in diagnostic tissue sections. An immunofluorescence method with scanning laser confocal microscopy was also used to show distribution of TGFβ. Biopsies were labelled using an immunoperoxidase technique to detect S100A4, αSMA and MIB-1 and sections were counterstained with periodic acid Schiff to outline basement membranes. Image analysis was performed using a Leica imaging system with QWin software. In addition, renal epithelial cells were co-cultured with CD103+ T cells derived from a mixed lymphocyte reaction in the presence of TGFβ.
Results: High levels of TGFβ were present in tubules and 'intraepithelial' cells during and after acute rejection. αSMA and MIB-1 defined proliferating myofibroblasts in the interstitium. S100A4 was differentially expressed in individual tubular epithelial cells in disrupted tubules during acute rejection. In chronic graft dysfunction CD103+ cells formed a characteristic 'chronic infiltrate' in the interstitium as well as within the few remaining tubules and TGFβ was also widespread. In vitro renal epithelial cells acquired a spindle shape and expressed S100A4.
Conclusions: Allospecific long-lived CD103+ T cells within the tubules generate a microenvironment which stimulates TEC to transdifferentiate to a proliferative, S100A4-expressing myofibroblast phenotype and to migrate into the interstitium where they become fully differentiated and express αSMA. CD103+ T cells migrate from the tubules alongside myofibroblasts to continue their association in the interstitial milieu which is rich in fibroblast-generated and chemotactic IL-15 and TGFβ. Clearance of intratubular T cells after any episode of acute rejection could prevent progression to chronic tubulointerstitial fibrosis.

O49

Bk virus nephropathy - Experience from transplant centres in the UK and Republic of Ireland

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BK virus nephropathy (BKVAN) is an important cause of renal allograft failure. Reports from single centres in Europe and the US suggest an increasing incidence of BKVAN in up to 5% of transplant recipients. This may reflect increasing use of newer immunosuppressive agents such as tacrolimus or mycophenolate mofetil (MMF). The UK incidence of BKVAN is unknown.

In a questionnaire survey of 30 transplant centres in the UK and Republic of Ireland (RoI), requesting the number of cases of BKVAN diagnosed at each centre, together with relevant clinical data, 24 centres (80%) responded, accounting for 1495 (80%) kidney or kidney/pancreas transplants in 2001.

34 cases of BKVAN were identified in 14 centres, the incidence as high as 3% in some centres. The mean time to diagnosis was 11.6 (2-48) months. Immunosuppressive regimens are tabulated below:

Immunosuppression at time of diagnosis	Number of patients	
Cyclosporine +/- prednisolone	2	CsA
Cyclosporine/Azathioprine/prednisolone	4	
Cyclosporine/MMF/prednisolone	2	9
Cyclosporine/rapamycin/prednisolone	1	
Tacrolimus/prednisolone	1	
Tacrolimus/Azathioprine/prednisolone	4	FK
Tacrolimus/MMF	6	
Tacrolimus/MMF/prednisolone	12	24
Tacrolimus/MMF/rapamycin	1	

Diagnosis was made by light microscopy in 6 cases, with electron microscopy in a further 11 and immunohistochemistry in a further 13 cases. Immunosuppression was reduced in 28 cases. 8 patients received intravenous cidofovir. In 13 cases outcome was monitored by serum creatinine alone. 12 patients were monitored with urine cytology and 7 by serial allograft biopsy. 15 patients were monitored using quantitative PCR in blood and/or urine. In 19 cases (56%) allograft function stabilised or improved after diagnosis. In 5 cases (15%) graft function continued to deteriorate and 7 (21%) grafts were lost. There was one death due to post-transplant lymphoproliferative disease and 2 patients had no outcome data.

BKVAN affects a smaller proportion of transplant recipients in the UK and RoI than reported elsewhere, though it is likely that the incidence will increase with increasing use of more potent immunosuppression. The outcome is poor with a high rate of allograft loss. BKVAN should be included in the differential diagnosis of renal allograft dysfunction. Appropriate management requires immunosuppressive dose reduction and close monitoring, including molecular diagnostic techniques.

O50

Serum Creatinine as a surrogate marker in the early phase of immunosuppression treatment, predicts long term patient and graft survival in Renal Transplant Patients better than acute rejection episodes.

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Using serum creatinine (SC) at 1 year rather than acute rejection episodes (AREs) makes sense as SC measures the damage to the kidney caused by AREs. We set out to prove that SC was a better surrogate marker than AREs through analysis of the relationships of both with long term outcomes. We compared the relationship between AREs within the first year post-transplant and SC levels recorded at one year as markers of patient and graft survival. Comprehensive data were available from 1978 to 2001 for 937 transplants in Cardiff. Survival estimates are reported using mean covariates using Kaplan-Meier and Cox proportional-hazards models. There were 352 (37.6%) females and 73 (7.8%) subjects had pre-existing diabetes (DM). Median age at first graft was 42 years (IQR 30-54) and median SC at one-year was 149 mmol.l-1 (IQR 119 to 200). In year-1, 283 (30.3%) subjects experienced one ARE; 149 (15.9%) two and 115 (12.3%) three or more. SC levels in those with no ARE versus those with at least one ARE were 134 mmol.l-1 and 166 mmol.l-1, respectively ($p<0.001$). Mean patient survival was 16.3 years for those with no ARE and 13.7 years in those with at least one ARE ($p=0.346$). Excluding patient deaths, mean graft survival was 15.3 years with no ARE versus 12.1 years with at least one ARE ($p=0.012$). After adjustment for age, sex, DM and SC levels, patient survival was not significantly associated with AREs. The relative hazard (RH) for graft survival (excluding deaths) with at least one ARE in year-1 was 1.36 (95%CI 0.997 to 1.843; $p=0.052$). The RH for graft survival including patient deaths was 1.45 (CI 1.14 to 1.84; $p=0.003$). After classifying AREs as 0, 1, 2, or 3 or more episodes, there was no association with patient survival. After excluding deaths, there was a significant association between graft survival and those patients experiencing three or more AREs (RH=1.63: CI 1.083 to 2.47; $p=0.019$). There was an association with graft survival with one or two AREs in the year-1. After adjusting for age, sex and DM, SC was associated with patient survival for a 10 mmol.l-1 increase (RH=1.001: CI=1.001 to 1.0142; $p<0.001$). After excluding patient deaths, SC levels were found to be significantly associated with graft survival for a 10 mmol.l-1 increase (RH=1.082: CI 1.053 to 1.131; $p<0.001$). SC levels recorded at one-year post transplant are a more reliable predictor of patient and graft survival than are acute rejection episodes in year-1. The predictive value of AREs is usually lost in multivariate analysis.

O51

Expression of human tissue factor pathway inhibitor and hirudin on vascular smooth muscle cells reveal role of clotting factors in neointimal formation during chronic vascular injury

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Neointimal formation and vascular remodelling resulting in luminal narrowing and ischaemia occurs in numerous conditions, including chronic allograft dysfunction, where it can have various aetiologies. Experimental models of chronic vascular injury have highlighted the potential role of activated clotting factors in pathophysiology, especially after acute vascular injury involving luminal thrombosis. This on-going study is designed to assess whether thrombin (IIa) or Xa have a role in chronic vasculopathy. We have generated two genetic constructs encoding novel chimeric proteins based on a human TFPI and the leech anticoagulant hirudin. The constructs have been incorporated into a transgene along with a modified alpha-actin promoter and mice have been generated by microinjection. They have a normal phenotype with no evidence of a bleeding diathesis. Northern analysis has shown expression of the transgene in all tissues examined and immunohistology has confirmed protein expression limited to vascular smooth muscle cells (VSMCs). VSMCs purified from the mice expressed surface human TFPI or hirudin or both at rest. To test the functional integrity of the fusion proteins, unilateral carotid artery injury was performed by wire insertion. Arteries in injured wild-type arteries showed immediate non-occlusive intravascular and extravascular deposition of fibrin, which disappeared after 3-5 days. Injured arteries from transgenic mice also showed acute fibrin deposition, but this was cleared within 24-48 hours. There was rapid upregulation of tissue factor expression in the VSMC of all injured vessels, but this had disappeared by 7 days in all mice. Neointimal formation was evident at 5 days in wild-type mice and was associated with near-total occlusion of the vascular lumen by day 28. In contrast, neointima formation was not seen until days 14-21 in any of the transgenic strains, and at day 28, luminal diameter was only slightly reduced and remained at near-normal patency up to day 56 (end of experiment). In vitro, proliferation of VSMCs from transgenic mice after exposure to either factor Xa or factor IIa was reduced by up to 50% compared to wild-type. These data suggest that neointima formation and VSMC proliferation in vivo is driven, at least in part, by activated clotting factors. Transplantation experiments are underway in murine models to determine the impact of these transgenic proteins on neointima formation in the vasculopathy associated with chronic rejection.

O52

The detection of cytomegalovirus (HCMV) replication in kidney transplant recipients using nucleic acid sequence based amplification (NASBA)

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The management of CMV mismatch in kidney transplant recipients remains debatable. Some centres advocate anti-viral prophylaxis, while others treats symptomatic patients. Recently, pre-emptive treatment based on detection of viral replication (VR) has been suggested. A number of diagnostic techniques have been advocated to detect HCMV replication including qualititative and quantitative PCR and NASBA. NASBA directly detects replicating late gene pp67 mRNA which may be more predictive in detecting VR than the early gene which is also detected in abortive infection.

We have investigated NASBA as an early detection marker for HCMV replication in 21 consequent kidney allograft recipients. Blood samples were collected pre-transplant, at weekly intervals for the first 3 months and then every 2 weeks up to 1 year post transplant. Prophylactic high dose acyclovir was administered to CMV -ve recipient receiving CMV +ve kidneys for 3 months. Patients suspected of developing CMV infection had their blood, throat and urine tested for CMV in the conventional way. CMV infection was treated by a course of intravenous Ganciclovir for 14 days. All patients were managed according to their clinical diagnosis and diagnostic tests, irrespective of the pp67 results.

12 patients were CMV –ve and 9 patients were CMV +ve. Of the CMV -ve recipients, 3 received CMV -ve kidneys and 9 received CMV +ve kidneys. Of the CMV +ve recipients, 4 received CMV –ve kidneys and 5 received CMV +ve kidney.
All patients had a –ve NASBA on the day of transplant. All –ve CMV recipients who received CMV +ve kidneys developed +ve NASBA post transplant (12-57 days) despite acyclovir therapy. Of these, 5 developed clinical CMV infection. In these patients VR was detected at a mean of 10 days before the onset of clinical symptoms. All received a course of i.v. ganciclovir. Post treatment, NASBA revealed VR in 3 patients, 1 required a further course of treatment (no VR was detected thereafter). The 5 CMV +ve patients who received CMV +ve kidneys developed +ve NASBA at varying time post transplant (mean 34 days). 3 of these patients developed mild symptoms and CMV was detected in urine and blood, however, they did not require ganciclovir. The NASBA also detected VR in the 4 CMV +ve recipients who received CMV –ve. None of these patients had clinical evidence of CMV. The positive NASBA coincided with acute rejection episodes in 2 and peritonitis in 1 patient. The remaining patient had CMV detected on biopsy and was treated with Ganciclovir.

This study demonstrates that acyclovir does not protect against CMV infection in CMV –ve recipients. In patients with clinical CMV, NASBA predicted VR on average of 1 week prior to presentation. The NASBA technique is a valid method for detecting VR. However, it appears that not all the detected VR is of clinical significance. Further studies are needed to evaluate the role of CMV screening using replicating gene detection in clinical transplantation.

Parallel Session IV(c)

Renal Outcomes

Wednesday 9 April 2003

16:30 – 17:30

O53

Renal transplantation in patients with diabetes. Clinical success or poor use of resources?

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AIM: The number of patients with diabetes referred for transplantation is increasing. This study set out to determine whether transplantation is worthwhile in this group of patients by comparing outcome in diabetic and non-diabetic patients.
METHODS: 1095 patients listed and transplanted between 01.01.1989 and 31.12.1999 in Scotland were included in the analysis. The outcome variables (patient survival, graft survival, acute rejection, delayed graft function, causes of death and graft failure) were compared between patients according to their diabetic status. A comparison of survival on transplantation versus dialysis in patients with diabetes was also performed.
RESULTS: 130 (12%) of the 1095 patients had diabetes. All patients, irrespective of their diabetic status, received comparable matched kidney grafts, from donors with a mean age of 40 years old and within an average cold ischaemic time of 20 hours. The incidence of acute and chronic rejection as well as delayed graft function was comparable between the two groups. Patient and graft survival are significantly lower in patients with diabetes (see table below). Twice as many patients with diabetes die with a functioning graft, but the incidence of the causes of death are comparable between the two groups of patients. When compared with the outcome on contiunous dialysis, transplantation leads to a comparable reduction in the risk of death in both groups of patients.
CONCLUSION: The results of transplantation in diabetics are not as good as those obtained in other renal diseases, but a kidney graft offers the best chance of survival and an enhanced life expectancy when compared with dialysis.

	Non-diabetics n=965	Diabetics n=130	p value
Patient half-life (years)	19.8	9.4	
Adjusted RR of death (95%CI)	1	2.65 (1.83 - 3.85)	<0.0001
Graft half-life (years)	11.8	7.8	
Adjusted RR of graft failure (95%CI)	1	1.68 (1.23 – 2.29)	0.001
Death with functioning graft (%)	29.1	50	0.004
Long term (>1yr) relative risk of death with a transplant compared with the risk of death on dialysis (RR=1)	0.33 (0.26-0.43)	0.30 (0.18-0.52)	<0.0001

O54

En bloc paediatric into adult recipients: The Newcastle experience

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Aim
To review and analyze the medium-term outcome of transplantation using paediatric en-bloc kidneys into adult recipients

Methods
15 en-bloc paediatric cadaveric renal transplants were performed between January 1996 to September 2000. The mean donor age was 26.9 ± 3.0 months (11 - 48 months) and the
mean weight was 13.2 ± 1.5 kg (10 - 25 kg). The mean recipient age was 35 ± 4.0 years (17 - 61 yrs) and the mean weight was 58.7 ± 3.5 kg (44 - 81 kg). The median HLA mis-match was 1:1:1 at the A:B:DR loci. Grafting of the en bloc kidneys was carried out by end-to-side anastomosis of the donor cava and aorta to the recipient¡¦s iliac vessels after a vascular switch (the Newcastle technique) 1 in 13 transplants, and grafting of en bloc using the conventional technique in 2. The mean cold ischaemic time was 1179.1 ± 131.7 minutes (7hrs 35mins - 30hrs 38mins) and the mean 2nd warm ischaemic time was 34.1 ± 1.6 minutes (27 ± 48 mins). Immunosuppression consisted of Tacrolimus based standard triple therapy in 14 transplants, and cyclosporine based triple therapy in one transplant. Rejection episodes were treated with steroids and ATG antibodies in non-responders.

Results
One graft failure occurred due to recurrence of the primary disease (FSGS) (6.7%), and the remaining 14 (93.7 %) functioned well with a mean serum creatinine of 167, 107, and 100 µmol/l at 1, 6 and 12 months respectively. Subsequently one patient died with gynaecological malignancy (6.7 %). One patient developed a lymphocoele requiring surgical drainage, and another one recipient contracted CMV infection, giving a complication rate of 13.3 %. Two patients required post-operative dialysis. The mean hospital stay was days 20.3 days (11 - 33 days).
Therefore the one year graft survival was 93.3 % and the one year patient survival was 100 %.

Conclusions
En-bloc paediatric kidneys can be transplanted successfully with excellent mid-term results.

1. Talbot D et al,"En bloc" paediatric renal donors into adult recipients -- the Newcastle technique. Transpl Int 1999;12(2):152-5.

O55

Acute rejection after renal transplantation is reduced if the recipient has received prior therapeutic blood transfusion, even in tacrolimus-treated patients.

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The deliberate administration, prior to renal transplantation, of three units of blood to previously untransfused recipients improved graft survival rates in the pre-cyclosporin era, and a controlled randomised trial (Opelz 1997) showed a significant benefit in cyclosporin-treated patients. In the modern era, the proportion of previously untransfused patients coming to transplantation is increasing, so it is important to determine whether a significant blood transfusion effect is still present.

Two hundred and sixty five consecutive recipients of renal transplants performed in a single centre were studied. Protocol induction immunosuppressive was with azathioprine, prednisolone, and either cyclosporin or tacrolimus. Twenty six patients received 'non-standard' immunosuppression (clinical trials, or individualised immunosuppression). Characteristics were:- median age 41 years; 85% first graft; 82% cadaveric grafts; 72% induction with cyclosporin-based therapy; 37% with biopsy-proven acute rejection in the first 6 months; 54% had 0 HLA-DR mm; 46% received 0-2 units of blood prior to the transplant.

Univariate analysis showed that a lower risk of rejection was associated with tacrolimus induction therapy (odds ratio (OR) 0.53 (95% confidence intervals (CI), 0.29-0.95), p=0.049); prior transfusion of 3 or more units of blood (OR 0.54 (95% CI, 0.33-0.90), p=0.024), older donor age (mean age rejection patients 38.96 (SD 12.37) years, non-rejecting patients 44.23 (SD 12.56) years, p=0.001). Rejection was not associated with year of transplantation; graft number; or cadaveric versus living donor. Although HLA-DR mismatching was associated with an increased risk of rejection, this was not statistically significant (versus 0 DR mismatch, 1 HLA DR mm OR 1.13 (95% CI 0.68-1.89), 2 HLA DR mm OR 3.16 (95% CI 0.72 – 13.77).

The rejection rates were 48% in cyclosporin-treated patients with 0-2 prior transfusions; 34% in cyclosporin treated patients with 3 or more prior transfusions; 35% in tacrolimus-treated patients with 0-2 prior transfusions; and 18% in tacrolimus-treated patients with 3 or more prior transfusions.

Multiple logistic regression modelling, after adjustment for all other variables, showed a final effect of 3 or more prior transfusions equivalent to OR 0.49 (95% CI 0.29-0.83), p=0.008. The independent effect of tacrolimus compared to cyclosporin was OR 0.36 (95% CI 0.18-0.70). Exclusion of patients who received more than 1 transplant, or who received 'non-standard' induction immunosuppression did not change the results significantly.

In conclusion, the prior administration of 3 or more units for therapeutic reasons was associated with a 50% reduction in the acute rejection rate, an effect independent of tacrolimus induction therapy.

O56

Outcome of renal transplantation in Indo-Asian recipients is similar to non-Asians.

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The United Kingdom has a large Indo-Asian (IA) population, who have a high rate of renal disease and form a significant percentage of the renal transplant waiting list. However, information about short and long-term transplant outcomes in this ethnic group is limited. It has been suggested that graft survival is poorer in this population compared with non-Asians (NA) [1].

We reviewed 245 renal transplants performed in 240 patients at St Mary's hospital from April 1995-April 2002, using tacrolimus-based immunosuppression. Fifty-three grafts were transplanted in IA patients, who had similar age, gender and donor characteristics to the NA group. The degree of HLA-matching, type and number of rejection episodes as well as incidence of delayed graft function was similar in both groups. Survival was analysed using the Kaplan Meier method and the log-rank test was used to detect statistical significance between survival curves. Graft survival was calculated with and without censoring for death with function (see table below).

		INDO-ASIANS	NON-ASIANS	P-VALUE
Graft survival CENS (patients at risk)	1-yr	94.3% (41)	93.6% (148)	0.88
	3-yr	91.1% (26)	91.8% (89)	0.96
Graft survival UNCENS (patients at risk)	1-yr	92.5% (41)	92.5% (148)	0.95
	3-yr	84.7% (26)	88.6% (89)	0.51
Patient survival (patients at risk)	1-yr	98.1% (44)	98.9% (159)	0.63
	3-yr	93.5% (29)	95.9% (100)	0.39

The main causes of death were cardiovascular events (IA=3, NA=4) and infection (IA=2, NA=3). The overall incidence of rejection during the follow-up period was 26.4% and 33.9% in the IA and NA groups respectively (p>0,05). The estimated glomerular filtration rates (eGFRs), calculated by the Cockroft-Gault equation were not significantly different in the two groups. At 2 years the mean eGFR for the IA group was 57.4mls/min and for the NA group 56.7mls/min (p=0.88). In a multivariate model including factors such as recipient age, sex, degree of HLA-mismatch and incidence of vascular rejection, the relative risk of graft failure (censored for death) in Asians was 1.21 (95 % CI, 0.39-3.70, p = 0.74).

We therefore conclude that in our patient cohort, IA ethnicity does not confer a survival disadvantage following renal transplantation.

References
1. Robin J et al. Indo-Asian experience of renal transplantation in Yorkshire: results of a 10-year survey. Transplantation 2002; 73(10): 1652-1657

O57

Changes in renal function and structure following elimination of cyclosporine in renal allograft recipients treated with rapamycin

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Previous studies have demonstrated progressive structural injury in renal transplant recipients treated with cyclosporine therapy. The introduction of rapamycin therapy has enabled clinicians to withdraw or eliminate cyclosporine. The aim of the present study was to examine the impact of cyclosporine minimisation or elimination on renal structural injury when compared to patients maintained on cyclosporine therapy. A subgroup of patients who entered the UK Rapamycin 02 study underwent protocol renal allograft biopsies at 3 and 12 months post transplantation. Renal structural injury in this group of patients was compared to a historical control group of patients treated with cyclosporine (Cya) azathioprine and prednisolone who also underwent protocol biopsies at 3 and 6 months. Patients entering the UK Rapamycin 02 study were treated with cyclosporine, rapamycin and prednisolone. At 3 months cyclosporine was either eliminated (Cya E) or minimised (Cya M) to target levels between 50-100ng/ml. ($p<0.05$ 3 months vs. 12 months; † $p<0.05$ Cya E vs. Cya).
Patients maintained on cyclosporine and azathioprine based immunosuppression developed progressive renal structural injury despite maintaining renal function. There was a significant increase in percentage glomerulosclerosis and interstitial fibrosis from 3-12 months in this group of patients. Patients treated with rapamycin in whom cyclosporine was eliminated (Cya E) demonstrated less progressive renal structural injury compared to the cyclosporine/azathioprine group (Cya). In particular there was less interstitial fibrosis and tubular atrophy in this group. In addition elimination of cyclosporine was associated with an improvement in renal function in this group of patients at 12 months. In summary this study has demonstrated an improvement in renal function following a withdrawal of cyclosporine in patients treated with rapamycin. We would hypothesise that this is as a consequence of an improvement in renal hemodynamics associated with cyclosporine elimination since structural injury progressed in this group. Elimination of cyclosporine is however associated with less structural injury when compared to patients in whom cyclosporine therapy was continued. We would suggest that this might result in maintenance of long term renal allograft function.

		Cya		Cya E		Cya M	
	n	15		12		14	
	month	3	12	3	12	3	12
GFR	ml/min	55±3	57±2	59±3	65±3*	55±3	57±4
CS	%	5±1	14±3*	6±3	11±4	5±2	13±3*
Vvi	%	17±2	39±5*	16±3	28±2†	18±4	37±5*
Vvt	%	56±2	42±4*	55±4	36±3†	55±5	41±3*

O58

Kidney/pancreas transplantation - UK summary

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Simultaneous pancreas and kidney transplantation (SPK) is a recognised treatment for diabetic patients requiring renal replacement therapy. It is a treatment that results in superior patient survival and quality of life and is becoming increasingly available in the UK. There are nine transplant units currently undertaking the procedure and the number of such grafts has more than doubled in the last five years. In 2002, the number of SPK is set to exceed 50 for the first time.

Potential recipients of an SPK are typically under 50 years of age with type 1 diabetes and are either on or are within 6 months of starting dialysis. Suitable pancreas donors would be 45 years or under with no history of diabetes mellitus.

At the end of August 2002, 82 patients were on the national waiting list for SPK. These patients were significantly younger (mean age 40, sd 8) than patients awaiting a kidney only graft at that time (mean age 47, sd 14), $p<0.0001$, and had been on the waiting list for a significantly shorter time: median 463 days (IQ range 189-724) compared with median 647 days (IQ range 264-1341), $p<0.0001$.

Comparison of kidney only and kidney/pancreas transplants for the 19 months ending 31 July 2002 also showed differences. Once again the SPK patients were significantly younger (mean age 40, sd 8) than kidney only patients (mean age 44, sd 15), $p<0.01$. The donors whose organs were used for SPK were also significantly younger (mean age 29, sd 9) than those used for kidney only grafts (mean age 43, sd 16), $p<0.0001$.

There is no proven benefit for HLA matching for SPK, and only the blood group should be matched. SPK operations can thus be initiated early with a view to minimising cold ischaemic times. Recipients are often called in and the operation set up before the HLA typing results for the donor are known and suitable kidney recipients have been identified on the national matching run. Patients awaiting SPK are given priority but pancreas transplant units have agreed that where possible they will stop the operation proceeding if a 000 HLA mismatched child is identified. A review of kidney only patients for whom kidneys would have been offered for transplant, had they not been used for SPK recipients, showed that virtually all kidneys could have been used in a 000 mismatched or favourably matched patient. In the 19 months to 31 July 2002, eight (11%) of 71 kidneys transplanted as part of a kidney/pancreas block could have been used for a 000 mismatched patient. Four of the eight patients identified as missing out on an offer of a 000 mismatched kidney were highly sensitised, including one child. Of these four highly sensitised patients, only the child has since received an alternative kidney transplant. It is not known whether or not any of the potential offers of kidneys for kidney only patients would have been accepted.

As the number of kidney/pancreas transplants continues to rise, so do the concerns about lack of equity of access between patients listed for SPK and those listed for kidney only transplant. There also appears to be geographical variability in the referral of patients for SPK.

Plenary Session I

Mechanisms

Thursday 10 April 2003

09:30 – 10:30

O59

Mice immunised with vimentin undergo acute rejection of syngeneic cardiac grafts.

S Nair[1], A McCormack[1], A Holder[1], D Simpson[2], M Burke[2] and M Rose[1]

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Introduction: Previous studies in humans have shown that antibodies to the protein intermediate filament vimentin are an independent predictor of transplant coronary artery disease after cardiac transplantation. Here we have used an experimental model of heterotopic cardiac transplantation to investigate whether the immune response to the autoantigen vimentin affects survival of syngeneic or allogeneic cardiac allografts.
Methods: C57BL/6 mice received subcutaneous injections of vimentin or saline in Complete Freunds Adjuvant and two weeks later they were examined for an antibody and cellular response to vimentin. Having established the presence of a strong immune response at two weeks, vimentin or saline immunised mice received heterotopic transplantation of syngeneic (C57BL/6) or allogeneic (from 129/Sv mice, representing minor differences only) hearts. Hearts were palpated every day and animals were sacrificed when heart contractility dropped to grade 1 or zero indicating rejection. Hearts were removed and examined histologically.

Results: Enzyme linked immunoassays demonstrated that anti-vimetin IgG titres were significantly higher in the vimentin immunised (mean titre 516+/-365) compared to saline immunised (mean titre 5.75+/-6.3), p=0.001. IFNg production from cultured splenocytes was 411+/-519pg/ml after vimentin immunisation compared to 5.25+/-10 pg/ml after saline immunisation (p=0.07). The survival of donor syngeneic hearts in saline immunised mice was 100% at 32 days whereas the mean survival of the syngeneic heart in vimentin immunised mice was only 14.1+/4.3 days (p=0.0007). The mean duration of survival of allogeneic transplants in both saline immunised and vimentin immunised mice was not significantly different at 7.38 and 7.0 days respectively (p=0.8). Histology demonstrated that rejection was associated with a diffuse infiltrate consisting of mainly mononuclear cells, myocyte necrosis and signs of vascular haemostasis. Histology of the animals' own hearts demonstrated no effects of vimentin immunisation. Vimentin immunised and saline immunised mice were treated with monoclonal antibodies to CD4 and CD8 T cells at days -6, -3 and the day of allogeneic transplantation to see the effect on graft survival. This treatment significantly prolonged graft survival in saline immunised (from 7.5+/-0.5 days to 16.7+/-0.76,p=0.0002) and vimentin immunised mice (from 7.4+/-0.5 to 12.71+/-0.52, p=0.0002). The fact that survival was not enhanced to at least 32 days (as obtained with syngeneic in saline immunised) suggest a role for antibody in vimentin mediated rejection.
Conclusion: The results suggest that the surgical process of transplantation is sufficient to sensitise syngeneic grafts for further damage by an autoimmune response. The nature of the damage and whether allogeneic hearts are affected is currently under investigation.

O60

Effect of ICAM-1 polymorphisms on efficiency of leukocyte adhesion to and migration through human vascular endothelial cells, *in vitro*.

C Lawson, P Pandya, AL Holder, A McCormack, RE Stanford, JD Smith, M Yacoub and ML Rose

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Intercellular adhesion molecule (ICAM)-1 is an adhesion molecule expressed on the surface of several cell types including leukocytes, endothelial cells and proliferating intimal smooth muscle cells. Its ligands are the membrane bound β2-integrin receptors LFA-1 (CD11a, CD18) and Mac-1 (CD11b, CD18) on leukocytes, although several other ligands have been described. It is critical in the firm arrest and trans-migration of leukocytes out of blood vessels and into tissues. Two biallelic polymorphisms have been described in the coding region of the human ICAM-1 gene, located in exons 4 and 6, modifying codons 241 (G/R; Mac-1 binding domain) and 469 (E/K; aggregation domain), respectively. E469 is linked with freedom from allograft rejection, however, the mechanisms for this protection have not been elucidated. In this *in vitro* study we have investigated interactions between genotyped human umbilical vein endothelial cells (HUVEC) and purified human peripheral blood mononuclear cells (PBMC), in two separate functional assays. The influence of ICAM-1 polymorphisms to ICAM-1 expression and ICAM-1-mediated adhesion and migration was analysed.
ICAM-1 cell surface expression was increased in HUVEC of GEGE genotype (MFI 3.06±0.46; GKGE 2.53±0.3; GKGK2.23±0.21; GKRE 2.20±0.31), however, the differences did not reach statistical significance. HUVEC of GEGE genotype supported significantly increased ICAM-1-mediated adhesion of PBMC (35.04±6.84%) compared to HUVEC of other genotypes (p=0.036 vs GKGE (17.41±2.56%); p=0.026 vs GKRE (14.99±4.01%)). There was no significant difference in ICAM-1-mediated migration of PBMC through HUVEC of each genotype. The frequencies of each ICAM-1 allele of the study population were similar to a control UK population.
In this study, HUVEC of GEGE genotype support increased ICAM-1-mediated adhesion of PBMC in a static *in vitro* assay. The G241 and E469 alleles have been linked with cardiac and renal transplant vasculopathy, as well as several chronic inflammatory diseases, including ulcerative colitis, Crohn's disease and multiple sclerosis. The findings presented here provide a functional basis for these previous observations.

O61

CD4+CD25+ regulatory T cells and the direct pathway alloresponse in human renal transplant recipients.

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CD4+CD25+ Regulatory T cells have been shown to regulate a variety of autoimmune and allogeneic responses in mice and humans. Mouse models have shown that adoptive transfer of this subset of T cells can prevent rejection of cardiac allografts in a donor-specific manner. In vitro studies in humans have shown that CD4+CD25+ cells are hyporesponsive to polyclonal stimuli and that they can suppress the responses of CD4+CD25- cells. To date there is no published data on the role of CD4+CD25+ cells in regulating alloresponses in human transplant recipients. The technique of limiting dilution analysis (LDA) allows derivation of the frequency of alloreactive T cells in peripheral blood of renal transplant patients; the influence of a regulatory population can also be assessed. Using this technique, it has previously been shown that there is a reduced frequency of direct pathway donor-specific T cells in renal transplant recipients when compared to the frequency of T cells reactive to an HLA mismatched third party. We hypothesised that if CD4+CD25+ cells are important in maintaining hyporesponsiveness in the direct pathway then depletion of these cells would influence the alloreactive cell frequency and/or other parameters of regulation obtained by LDA. We performed LDA for cell proliferation and IL2 secretion together with ELISPOT for IFNγ in 10 living-related renal transplant patients with stable renal function and one HLA-DR mismatch to donor. In no case did depletion of CD25+ cells significantly increase the frequency of donor-specific T cells nor indicate regulation against donor when compared to an equally HLA-DR mismatched third party. We conclude that the action of CD4+CD25+ regulatory cells is not the main mechanism of donor-specific hyporesponsiveness in the direct pathway of allorecogniton. Our interpretation is that T cell anergy is driven by overwhelming co-stimulation deficient direct pathway presentation and the additional contribution of the CD4+CD25+ cells is not significant/ detectable. We would predict that the low frequency indirect pathway would be more amenable to suppression by these cells.

O62

Inhibition of intravascular thrombosis in mice displaying regulated endothelial cell expression of human tissue factor pathway inhibitor

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Intravascular thrombosis occurs in disorders with diverse aetiology, including humoral rejection of transplanted organs. Formation of clot depends on the intraluminal expression of tissue factor (TF) and the cleavage or removal of regulatory proteins from the surface of activated endothelial cells (EC). These proteins include antithrombin III, thrombomodulin and tissue factor pathway inhibitor (TFPI). A rational strategy to prevent intravascular coagulation is to replace these EC-tethered anticoagulants. We have generated a genetic construct encoding a novel protein based on a human TFPI/CD4/P-selectin chimera to target expression to Weibel-Palade (WP) bodies in resting EC. The construct has been incorporated into a transgene along with a modified CD31 promoter and mice have been generated by microinjection. They have a normal phenotype with no evidence of a bleeding diathesis. Northern analysis has shown expression of the transgene in all tissues examined and immunohistology has confirmed protein expression limited to vascular EC. EC purified from the mice expressed no surface human TFPI at rest but displayed rapid expression after translocation of WP bodies by exposure to inflammatory cytokines. To test the strategy, mice were injected with lipopolysaccharide (LPS) and observed for up to six hours under terminal anaesthesia. Wild-type mice developed a severe thrombocytopenia (26 $x10^9$/l - < 10% of controls) and consumptive coagulopathy (fibrinogen levels undetectable and bleeding time >11 minutes). Histology revealed widespread deposition of intravascular fibrin in all tissues. In contrast, heterozygous transgenic mice developed mildly deranged clotting parameters (fibrinogen levels >30% normal and bleeding time <6 minutes) and retained physiological platelet counts (198 $x10^9$/l - approximately 50% of control). Little intravascular fibrin was visible on immunohistological analysis, despite intraluminal expression of TF. These data confirm the importance of TF in the pathophysiology of LPS-dependent DIC and demonstrate that a genetic strategy to replace EC-tethered anticoagulant TFPI is highly effective at limiting intravascular thrombosis. Organs from these mice are being transplanted to determine the impact of this approach on humoral rejection.

O63

Identification of Response to Minor Histocompatibility Antigen Mismatch in Clinical Renal Transplantation, using HLA-peptide Tetramer Staining.

NS Krishnan[1], RM Higgins[1], FT Lam[1], M Kashi[1] and A Morris[2]

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Donor-recipient mismatch for the minor histocompatibility antigen (mHag) HA-1 in bone marrow transplantation may result in both clinical graft vs host disease, and specific cytotoxic lymphocyte (CTL) responses, identified with peptide-tetramer reagents. The purpose of these experiments was to see if there was evidence of a cellular anti-HA-1 response in renal transplantation, although we have previously shown that HA-1 mismatching does not affect the clinical outcome.

We identified 5 recipients who were HA-1^R and had received kidneys from HA-1^H donors. These patients were all HLAA2 and DR matched with their donors. There was no significant tetramer binding to unstimulated PBML, measured by flow cytometry. PBML from recipients 1-4 were stimulated in MLC with the third party donor, who was matched for A_2 & DR and matched as closely as possible for other antigens but mismatched for HA-1. T cells were depleted from donor PBML so that in subsequent analysis only recipient T cells were studied. MLCs were incubated for 3 days. Cells were stained for CD25,CD8 and tetramers (Tet). The negative controls were (i) recipients' cells in culture on their own, (ii) two patients who were HA-1^R and was never exposed to HA-1^H, who were <0.12% positive in these experiments. Results are shown in Table 1.

To confirm that lymphocytes specific for an anti-HA-1 response were present, we stimulated recipient cells with HA-1^H peptide and found significant levels of binding with tetramer, shown in Table 2.

We conclude that anti-HA-1 specific CTL responses can be elicited in renal transplant recipients.

Table 1

Recipient	Control %CD25+ Tet+	Expt %CD25+Tet+	Control %CD8+Tet+	Expt %CD8+Tet+
1	0.12	0.79	nd	Nd
2	0.11	0.5	1.0	4.0
3	0.14	0.62	2.0	7.12
4	0.1	0.32	0.9	2.5

Table 2

Recipient	Control %CD25+ Tet+	Expt %CD25+Tet+	Control %CD8+Tet+	Expt %CD8+Tet+
5	0.18	2.5	1.4	14
2		1.75		7.4
HA-1 compatible transplant		0		1.9

O64

Immunosuppressive drugs inhibit the Sonic hedgehog-mediated induction of pro-inflammatory cytokines and vascular endothelial growth factor by human macrophages.

SJ Wakelin[1], SEM Howie[2], JR Lamb[3], OJ Garden[4] and JLR Forsythe[1]

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Introduction The Sonic hedgehog (Shh) signalling pathway has an important role in determining the proliferation and fate of cells during development. Hedgehog family members have been demonstrated to be important during renal and lung ontogeny. There is also evidence to suggest that the signalling pathway may be activated during repair and/or remodelling processes in the damaged lung. Chronic renal allograft nephropathy (CAN) is the major cause of long-term renal failure in kidney allograft recipients. Macrophages are consistently found in CAN with macrophage infiltration and activity correlating directly with graft survival. Components of the signalling pathway, including the receptor for Shh, Patched (Ptc) are expressed in macrophages thus allowing these cells to receive hedgehog-mediated signals. Furthermore, the products of activated macrophages, Transforming Growth Factor-beta (TGF-β) and Vascular Endothelial Growth Factor (VEGF), have both been implicated in the aetiology of CAN and both may be upregulated by Shh. Using immunohistochemistry, we have demonstrated the presence of Ptc on macrophages in culture and in the cellular infiltrate in the acutely rejecting and CAN kidney. Shh is expressed in the tubular epithelium of normal kidney. It is therefore possible that Shh may modulate the effector function of macrophages and thus play a role in mediating some of the changes seen in the failing allograft.

Methods Monocytes were isolated from peripheral blood mononuclear cells of healthy donors using positive selection, and matured into macrophages by plastic adherence for 7 days. The macrophages were stimulated with recombinant Shh and the supernatants harvested for cytokine and growth factor analysis using cytometric bead assays for pro-inflammatory cytokines and ELISAs for VEGF and TGF-β. These experiments were repeated in macrophages in the presence of the specific Shh signalling pathway inhibitor, cyclopamine and the immunosuppressants, dexamethasone, rapamycin and cyclosporin.

Results Shh mediated a marked dose-dependent upregulation of IL-6 and IL-8 secretion from macrophages. The effects of Shh on macrophage cytokine release were dose-dependently inhibited by cyclopamine and the immunosuppressants rapamycin, dexamethasone and cyclosporin. VEGF secretion was also upregulated by Shh. The effect of Shh on TGF-β secretion is currently under investigation.

Conclusions Here, we demonstrate a novel effect of Shh on macrophages. Shh differentially modulates the pro-inflammatory cytokine expression of macrophages *in vitro*. This effect is inhibited by cyclopamine, dexamethasone, rapamycin and cyclosporin. There is evidence that damaged epithelium can release Shh. This may provide a mechanism by which macrophages could initiate inflammation and/or repair at sites of epithelial damage. That immunosuppressants affect this response may have significant implications for a repair role of Shh in the rejecting kidney.

Plenary Session II

Liver

Thursday 10 April 2003

12:00 – 12:30

O65

Generation of $CD25^+CD4^+$ regulatory T cells is not a specific feature of spontaneous liver allograft acceptance

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Background- It is well documented that mouse livers transplanted across a major histocompatibility barrier can be accepted spontaneously without immunosuppression. Previously we have demonstrated that donor specific $CD25^+CD4^+$ regulatory T cells can be isolated during both the induction and maintenance phases of tolerance to alloantigens and that these cells can prevent skin graft rejection in a sensitive adoptive transfer model. We next sought to address a simple yet fundamental question. Is the spontaneous acceptance of liver allografts explained by a unique ability to drive the development of donor specific $CD25^+CD4^+$ regulatory T cells?

Methods- CBA ($H\text{-}2^k$) mice were transplanted with a B10 ($H\text{-}2^b$) liver or cardiac allograft, the latter as a rejection control. At day 10 spleens from these animals were harvested, and $CD25^+CD4^+$ T cells isolated and adoptively transferred into T cell deficient (CBA-$Rag^{-/-}$) mice together with naïve, syngeneic $CD45RB^{high}CD4^+$ effector T cells. One day later, these reconstituted animals were transplanted with a donor specific (B10) skin allograft.

Results- We have shown that B10 liver allografts are spontaneously accepted by naïve CBA recipients (MST >100 days), whereas B10 cardiac allografts are acutely rejected (MST = 7 days). However strikingly, despite these divergent outcomes, the $CD25^+CD4^+$ T cells isolated from both liver and cardiac allograft recipients at day 10 after transplantation were able to prevent B10 skin allograft rejection mediated by $CD45RB^{high}CD4^+$ effector T cells (MST >100 days and MST >100 days respectively).

Conclusion- Our data provide evidence that the generation of $CD25^+CD4^+$ regulatory T cells is not a specific feature of spontaneous liver allograft acceptance. We suggest that T cells with regulatory potential may be generated after transplantation of any solid vascularised graft regardless of its eventual fate as a consequence of the large alloantigen load provided by graft. The kinetics of induction and the number of regulatory cells that develop will all contribute to determining the outcome after transplantation.

O66

Patient survival after Liver re-transplantation according to indication for the primary transplant.

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Background

In view of the ongoing shortage of organs for liver transplantation retransplantation for certain patient groups has been questioned.

Identifying factors associated with poor post retransplantation outcome will assist patient selection.

Method

The UK & Ireland Liver transplant audit is a multi-centre prospective cohort study of all patients undergoing liver transplantation since 1 March 1994.

This study extended from 1 march 1994 to 28 February 2002, during which 4883 primary transplantations were recorded. Of these 423 patients had a second liver transplantation.

We studied patient and graft survival outcome upto 3-months after second liver transplantation, according to 6 indications for the primary transplantation.

Result

The overall 3-month post retransplantation mortality was 25.8% (95% confidence interval 21.7% to 30.2%). The confidence intervals of 3-month mortality, for all the primary indications, included the overall mortality. The 3-month post retransplantation graft survival was 69.7% (95% confidence interval 65.1% to 74.1%). For further detail of survival outcome across the groups please see table1.

Discussion

Indication for primary liver transplantation had no significant effect on the 3-month post retransplantation patient mortality, in this analysis.

We are undertaking a study looking at other potential pre retransplant risk factors that influence post transplant patient survival.

Table1. 3-month patient and graft survival by indication for first Liver transplantation in patients who received a second liver transplantation between 1 March 1994 and 28 February 2002 in the UK and Ireland.

Primary transplant Indication	Retransplant (%)	Deaths (n)	Percentage mortality (95%Confidence interval)	Graft survival (%) (95%Confidenceinterval)
"Acute Failure"	58(13.7)	10	17.2(8.6--29.4)	75.9(62.8--86.1)
Cancer	14(3.3)	3	21.4(4.7--50.8)	71.4(41.9--91.6)
Metabolic	22(5.2)	6	27.3(10.7--50.2)	68.2(45.1--86.1)
Cholestatic	74(17.5)	25	33.8(23.2--45.7)	64.9(52.9--75.6)
Cirrhosis	235(55.6)	59	25.1(19.7--31.2)	70.2(63.9--76.0)
Others	20(4.7)	6	30.0(11.9--54.3)	65.0(40.8--84.6)
Total	423	109	25.8(21.7--30.2)	69.7(65.1--74.1)

O67

Liver Transplantation from Controlled Non-Heart Beating Donors: the King's College Hospital Experience

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Background. Liver transplantation is an effective treatment for end-stage liver disease, however the current shortage of donor organs limits its clinical application. Split liver and living related donation have provided an alternative source of grafts. Despite these innovations, deaths occur in patients awaiting liver transplantation. To further increase the number of livers available for transplantation, a non-heart beating donor (NHBD) liver transplant programme was started in 2001 after obtaining hospital ethical committee approval.

Methods. Controlled donors with a warm ischaemia of less than 30 minutes were considered, preferably less than 55 years of age. A 5-minute period was observed from asystole to skin incision. A super-rapid technique with aortic and superior mesenteric vein perfusion was utilised for the retrieval. A number of methods were used to assess the suitability of these livers for transplantation and included serum liver function tests, morphological and histological assessment, isolation and estimation of hepatocyte viability.

Results. In the first 19 months of the programme 23 livers were retrieved from NHBD. Of these 10 were not used, because of steatosis in 5, prolonged hypoxia and hypotension post withdrawal of therapy in 4 and poor perfusion in one. Mean donor age was 40.6 years (range16-67), 14 were males. Eight donors suffered head trauma, 8 anoxic brain injury and 7 had an intracranial bleed. The average ITU stay was 3.5 days (1-9). The mean warm ischaemia time (systolic BP < 30 mmHg to aortic perfusion) was 13.8 minutes (9-30). Thirteen livers were used for 14 transplants and the indications included viral hepatitis in 6, primary sclerosing cholangitis in 2, acute liver failure in 2, and, in the remaining 4, alcoholic liver disease, autoimmune hepatitis, familial amyloid polyneuropathy and hepatoblastoma respectively. The mean cold ischaemia time was 9.7 hours (7-14.3). One patient receiving a right lobe split graft was retransplanted for primary non-function (PNF). All other grafts had good early function. There were three deaths. The patient transplanted for paracetamol overdose died 11 days post transplant from sepsis and severe multi-organ failure (MOF) with evidence of ischaemic brain injury. One patient died of chronic rejection 7 months post transplant. The patient retransplanted for PNF died 9 days after the 2nd transplant from sepsis and MOF. The overall patient and graft survival is 78.5%. The livers retrieved from NHBD but not transplanted were perfused using a two-stage collagenase perfusion technique followed by centrifugal purification of hepatocytes. Wedge biopsies from the transplanted grafts were mechanically digested with collagenase. Average hepatocyte viability rates were 33% for the livers not used for transplantation and 72% for grafts, which were transplanted ($p=0.02$).

Conclusion. Grafts from controlled NHBD should be considered for organ donation. Protocols have been established, but the procedure is demanding on staff, time and resources. Early results suggest that this is a significant source of grafts but careful selection and short cold ischaemia is mandatory. Further experience with hepatocyte viability testing is needed to evaluate its potential in assessing the suitability of livers from this source for transplantation.

Poster Session I

Tuesday 8 April 2003

P1

Effects of stem cell factor, FLT3-ligand and thrombopoietin on a population of human hepatic stem cells.

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Introduction: Oval cells are a well-known hepatic progenitor. In the rat, oval cells have been shown to express the surface antigen CD90 (Thy-1). CD34 is a surface antigen characteristic of haematopoietic stem cells (HSCs). We postulated that cells expressing the same antigens would represent a stem cell population in human fetal liver and that such cells give rise to hepatic cell precursors.
Methods: Fresh human fetal liver was digested with collagenase and maintained in culture for up to 7 days in the presence or absence of cytokines: stem cell factor [SCF], thrombopoietin [TPO] and Flt3-ligand [Flt3-l]. At days 3 and 7, cultured cells were fixed and immunostained by monoclonal antibodies for CD90 and CD34 followed by further intracellular staining with monoclonal antibodies for markers of biliary cells (cytokeratin 19 [CK19]) and hepatocytes (cytokeratin 18 [CK18] and fibrinogen [FIB]). In addition, flow cytometry for the detection of CD90 and CD34 was performed at the time of cell retrieval and also on flask-adherent cells 6 days after the addition of TPO and Flt3-l.
Results: Flow Cytometry: 2% of the fresh liver cell suspension was CD90+ve, 9% was CD34+ve/CD90-ve and 2% was CD34+ve/CD90+ve. Following culture in the presence of the TPO and Flt3-l, the proportion of CD90+ve cells was 30% (compared with 15% of cells in controls), CD34+ve cells were no longer detected and total cell number in the flasks had increased 8-fold. **Immunocytochemistry:** More cells were present on day 7 compared with day 3. At both time points, there were abundant cells staining for CK18, CK19 and FIB. Some cells were singly labelled and some cells were double-labelled for CK18 and FIB or CK19 and FIB. Nuclear staining showed that a proportion of cells did not stain for any of these cell markers. Culture with SCF, TPO and Flt3-l altered cell morphology. In their presence, more of the cells were small and round (possibly more primitive cells), some of which stained brightly for CK18, CK19 and FIB. Without cytokines, a greater proportion of the stained cells had a more mature morphology. CD34+ve cells were not seen at day 3 or day 7. CD90+ve cells were, however, seen at both time points, but none demonstrated dual-labelling with CK18, CK19 or FIB.
Conclusion: CD90+ve cells are known to be hepatic stem cells in the rat and we have demonstrated their presence in human fetal liver cell cultures for up to 9 days. The cells retrieved from the fetal liver were susceptible to manipulation by cytokines known to proliferate stem cells, demonstrating an increase in total cell number, an increase in proportion of CD90+ve cells and altered morphology. Finally, cultures of fetal liver revealed the presence of cells with bi-lineage potential (i.e. double-stained for both hepatocyte [FIB] and biliary tract cell [CK19] markers), thus demonstrating a population of human hepatic stem cells.

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Large scale human hepatocyte isolation - the work of the UK Human Tissue Bank

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Introduction
Human hepatocytes are the ideal tissue source for investigation of drug metabolism, bio-artificial livers and cell transplantation. However their acquisition is difficult and restricted by ethical and legal constraints. The UK Human Tissue Bank undertakes the acquisition of human livers, from fully consented patients, and isolates the hepatocytes for such use, on a cost recovery not for profit basis. We present a review of 1 year's output and illustrate that a significant number of good quality human hepatocytes can be obtained.

Results
Ninety-two patients were identified for liver resections and consented for tissue acquisition as well as 6 multi organ donors. Hepatocyte isolated occurred in 62 of these livers. Fifteen of the total were inoperable, and 13 not isolated due to lack of customers and 5 due to insufficient samples. Twenty-seven kilograms of tissue was donated and isolations from 7.7 kg were undertaken. A total of $3.1x10^{10}$ hepatocytes were isolated but only $1.3x10^{10}$ was sent to researchers.

Conclusion
An organisation like UKHTB can process and supply a large amount of tissue for research use, as well as potentially cells for transplantation or bioartificial liver construction. Flexibility to accept tissue outside of normal working hours is required to maximise this resource. An increase in the number of sources of resected liver tissue would significantly increase the potential, and this is currently being actively sort. Routine availability of high quality human hepatocytes in the UK is now a reality.