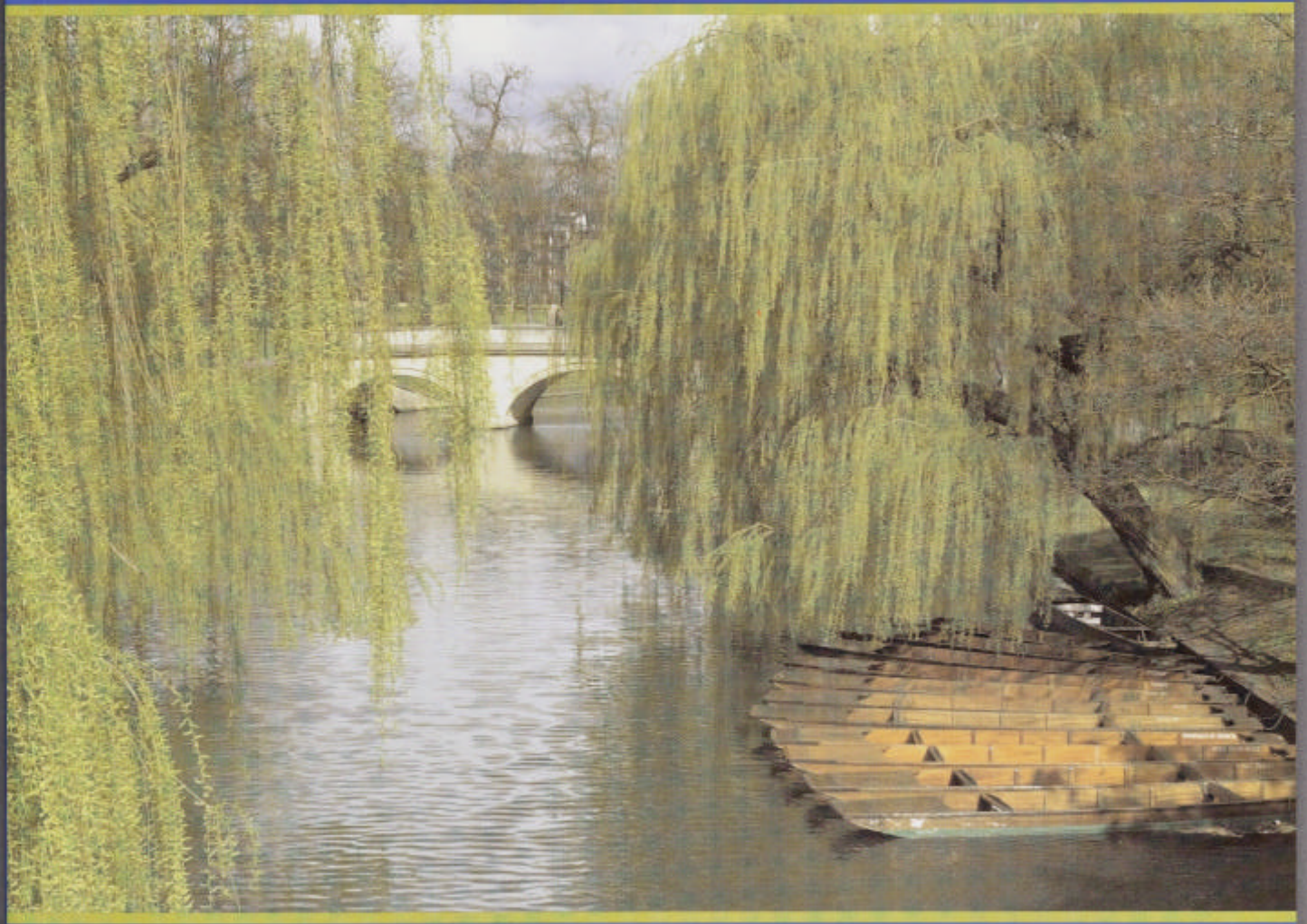
British Transplantation Society

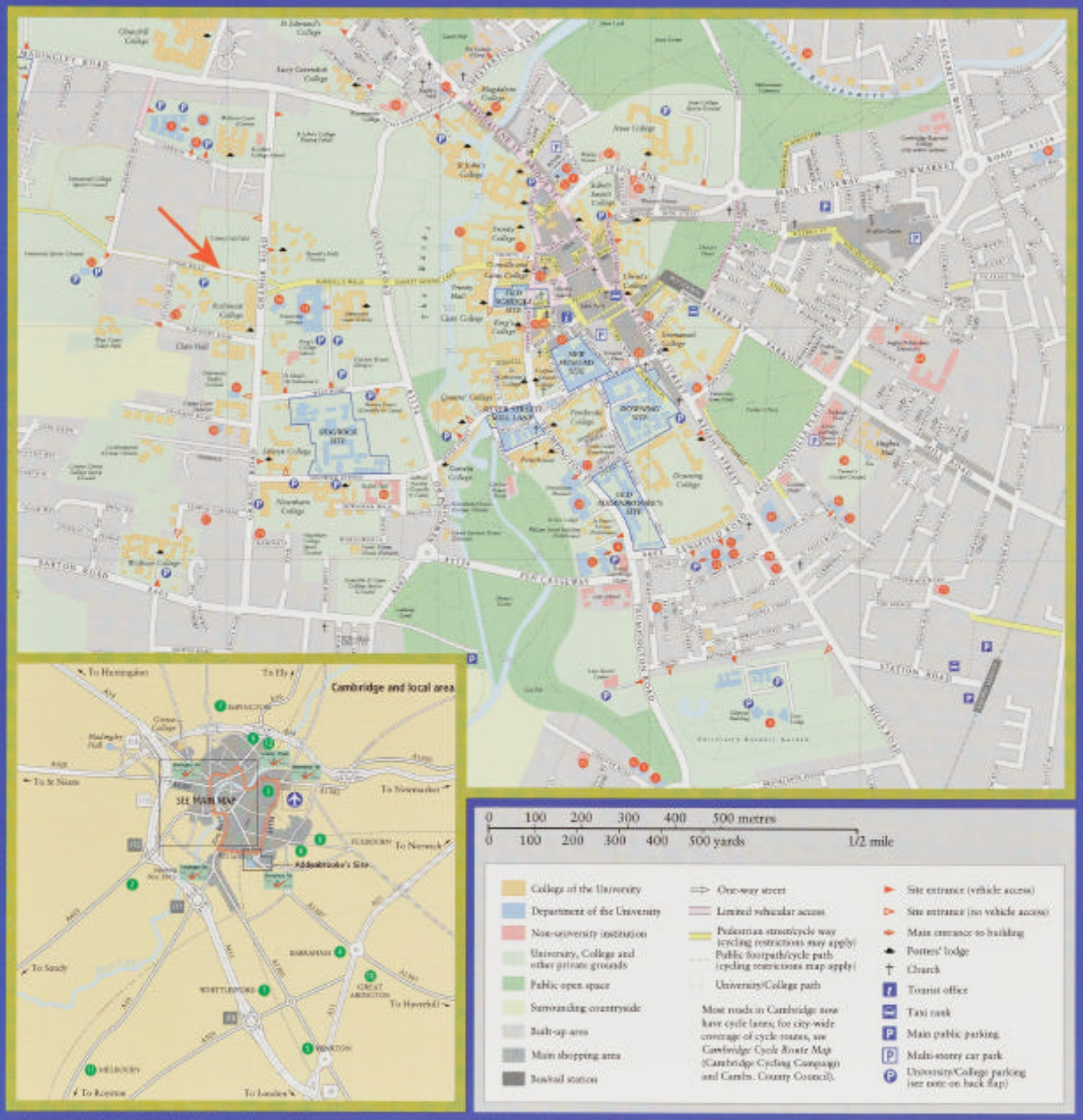


5th Annual Congress 2002

Robinson College, Cambridge

17-19th April 2002

CAMBRIDGE CITY MAP



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O1

PORTAL VENOUS PRESSURE CHANGES FOLLOWING SEQUENTIAL CLINICAL ISLET TRANSPLANTATION

John J Casey[1], Jonathan RT Lakey[2], Edmond A Ryan[2], Breay Paty[2], Richard Owen[3], Kevin O'Kelly[3], Sulaiman Nanji[2], Ray V Rajotte[2], Gregory S Korbutt[2], David Bigam[2], Norman N Kneteman[2], and AM James Shapiro.[2]
[1]Scottish Liver Transplant Unit, Royal Infirmary, Edinburgh, [2]Clinical Islet transplant Unit and [3]Department of Radiology, University of Alberta Hospital, Edmonton, Canada

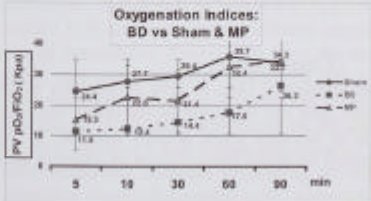
Background:
Sequential pancreatic islet transplantation via the portal vein has led to insulin independence in patients with type I diabetes. Complications associated with the injection of islets into the portal vein have been reported previously, and so in this study we sought to further characterise changes in portal venous pressure associated with islet infusion.
Methods:
Pre and post transplant portal venous pressures were recorded in 50 consecutive transplant procedures in 26 patients receiving highly purified, heparinized allogeneic islet preparations between March 1999 and November 2001.Islets were delivered via a radiologically placed portal venous cannula. Doppler ultrasound scans of the portal vein were completed within 24 hours of transplantation.
Results:
Eight patients (31%) received one islet infusion, 12 patients (46%) received 2 islet infusions and 6 patients (23%) received 3 islet infusions. An average of 388,527 islet equivalents (IE) were infused during each procedure (5790 IE/kg) in a mean packed cell volume of 4.8cc (range 1.5-9cc). Post transplant portal vein pressures rose significantly with sequential transplantation (12.4mmHg vs 17.3mmHg, $p<0.05$). Portal pressure change correlated significantly with islet packed cell volume ($r=0.66$, $p<0.001$) and also with the number of islets transplanted ($r=0.49$, $p<0.001$). Segmental portal vein thrombosis was radiologically detected after 2 procedures (4%).
Conclusion:
Multiple sequential islet transplants can be safely carried out via the portal vein, provided care is taken with islet purification and attention paid to portal venous monitoring.

O2

REPERFUSION SYNDROME REDUCTION IN LUNG TRANSPLANTATION AFTER MODULATION OF INFLAMMATORY DONOR GRAFT DAMAGE

C. H. Wigfield, H. D. R. Gollege, B. K. Shenton, J.A. Kirby, J.H. Dark
Freeman Hospital and University of Newcastle upon Tyne, UK

Background: The incidence of reperfusion syndrome in lung transplantation has not decreased. Efforts to reduce the severity of early graft dysfunction (EGD) focused on interventions in the recipient. We hypothesized that modulation of the inflammatory graft insult associated with brain death prior to transplantation would result in improved early reperfusion. **Purpose:** We sought to modulate inflammatory donor graft damage with non-specific anti-inflammatory intervention administered after BD. We investigated whether reduction of graft injury prior to transplantation would result in ameliorated reperfusion syndrome after transplantation. **Methods:** Brain stem death was induced in Wistar rats by inflation of an epidural balloon in two experimental groups: **BD Group** (n=10) and **MP group** received 30mg/Kg methyl-prednisolone (n=8). Controls had balloons inserted only, not inflated (**Sham group**, n=9). Brain stem auditory evoked potentials (BAEPs) confirmed brain stem death or functional status. Neurological assessment, invasive cardiovascular monitoring of donors and measurement of inflammatory markers in serum and bronchio-alveolar lavages were performed. Lungs were then transplanted after 2 hours of cold ischaemia. Assessment of oxygenation indices, pulmonary haemodynamics and inflammatory markers were performed again during first 2 hours of reperfusion in vivo. **Results:** [means] Brain stem death and characteristic haemodynamic profiles were demonstrated in all BD and MP ***donors***. Sham operated animals retained normal cardiovascular and brain stem functions. Oxygenation indices remained stable in Shams, but deteriorated markedly within 4 hours in BD donors. [25.1 vs 45.0 (Kpa); p=0.008], but improved decidedly in MP treated donors after BD [50.7 Kpa; p= 0.011]. Alveolar lavage cellularity increased and neutrophil activation markers were up-regulated in the BD group but remained low in MP group in donors compared with Shams prior to transplantation [CD 11b and 18; p = 0.013 and 0.004]. ***After transplantation***, oxygenation indices in the BD group measured during early reperfusion remained low, but improved in shams and MP group [14.0 Kpa vs. 26.0 vs. 20.6 respectively; p= 0.0158]. Pulmonary haemodynamics [flow and vascular resistance (TPVR)] improved likewise in the MP pre-treated group.



Conclusions: Our data provide first evidence that inflammatory insult after BD results in acute donor lung injury. This has been modulated with methyl-prednisolone. Such pre-treated grafts have reduced reperfusion induced dysfunction in this rat model of lung transplantation. This may prove to be a feasible approach to reduce donor injury and recipient EGD in the clinical setting.

O3

KIDNEY TRANSPLANTATION FROM NON-HEART BEATING DONORS: SERIOUS ADVERSE EFFECTS OF A CHANGING CONSENT POLICY

R Elwell, R Taylor, SA White, PS Veitch, ML Nicholson, Department of Surgery, University of Leicester, UK

Introduction

One of the requirements of a successful non-heart beating donor (NHBD) programme is rapid in situ cooling of the kidneys. This is usually achieved by placing an intra aortic catheter via a femoral artery cut down in the groin. A number of centres perform this procedure before the consent of the family has been given and the ethical acceptability of this protocol has been questioned. The aim of this study was to assess the effects of a change in consent policy on the outcome of referrals for NHBD.

Methods

Prior to 2001 the local coroner had given consent for NHBD in situ renal perfusion without prior consent from the donor family. In 2001 this ruling was reversed and it then became a requirement to obtain written consent from the next of kin before in situ organ perfusion could be commenced. The NHBD programme was prospectively audited in two 6 month periods before and after this change in consent policy.

Results (see table)

Whilst there was no difference in the number of referrals for NHBD during the two periods, the perfusion catheter was inserted less often and fewer retrieval operations and transplants were performed in the period in which consent for in situ perfusion was required.

	2000	2001	P value (χ^2)
Referrals from A&E	19	19	NS
Relatives present	13	15	NS
Perfusion catheter introduced	14	8	0.09
Consent given	12	8	NS
Retrieval operations	9	3	0.08
Kidneys transplanted	11	0	<0.001

Conclusions

Donor families need a significant amount of time to give consent for in situ perfusion and if this period is too prolonged then the opportunity for organ retrieval is lost. If NHBD kidney transplantation is to become more widely adopted in the UK, then national legalisation will be required to allow preservation of kidneys prior to family consent.

O4

IDENTIFYING AND CHARACTERISING ALLOSPECIFIC T CELLS IN KIDNEY TRANSPLANT RECIPIENTS

CJ Pigott[1], NT Young[1], N Rust[1], MFC Callan[2] and SEF Marshall[1]
[1]Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford
[2]Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford

Introduction. In the normal physiological setting MHC molecules present a repertoire of self and/or foreign peptides. This multitude of different MHC/peptide conformations is likely to be responsible for the observed magnitude of acute T cell alloreactivity via direct presentation. However the contribution of specific peptide to direct alloreactivity remains a matter for debate. MHC tetramers have been useful in understanding self-restricted T cell mediated immunity to chronic and acute viral infections. These relatively novel reagents have recently been shown to identify allospecific CD8 T cells in an *in vitro* system. Here we have identified allospecific T cells, in renal allograft recipients presensitised to HLA-A2, using an HLA-A2 tetramer presenting viral CMV peptide.

Method. Three HLA-A2 negative renal transplant recipients (LDO, JBU and BOP) who were known to have been exposed to HLA-A2 through transplantation with an HLA-A2 positive graft were selected for this study. Two possessed an existing HLA-A2 positive, mismatched graft while the third had IgG antibodies to HLA-A2, in response to a failed graft. CD3 and CD8 positive lymphocytes from each patient were cell-sorted on the basis of positive staining with HLA-A2/NLVPMVATV (CMVpp65 peptide) tetramer. Sorted cells were cloned and assessed for killing capability at various effector:target ratios. Targets were HLA-A2 positive T2 cells unloaded or loaded with exogenous CMV or EBV peptide. Killing was assessed by ^{51}Cr release. Clones were also assessed for staining capability of the above tetramer and phenotyped for a variety of effector/memory antigens. Anti HLA-A2 CTLp frequency estimation was carried out by limiting dilution analysis.

Results. Clones were generated from all three patient samples: LDO generated four clones, two of which were used in the killing assay. JBU and BOP each generated one usable clone. All clones were assessed for tetramer staining and memory/effector phenotype. Between 0.01% and 0.05% CD8 positive CD3 positive *ex vivo* lymphocytes stained with tetramer in the different samples and CTLp frequencies varied between 3 and 24 per million. Two clones from the same patient sample were assessed in a killing assay: one was capable of killing T2 (HLA-A2+) targets both unloaded or loaded with exogenous viral peptides, whereas the second clone was unable to lyse any target cells.

Discussion. This study was based on the assumption that a T cell repertoire is made up of cross reactive T cell clones and some of these may therefore recognise an allogeneic HLA tetrameric complex presenting an otherwise "irrelevant" viral peptide. We have identified these allospecific cells, although they were present at very low frequency. Nonetheless, our results show that tetramer positive cells, sorted and cloned from the same individual possessed different functional characteristics, which correlated with effector phenotype. All clones generated contained cells capable of restaining with tetramer and exhibited variable memory/effector phenotypes.

O5

ADULT LIVING DONOR LIVER TRANSPLANTATION: INITIAL UK EXPERIENCE

[1,2]R. Williams, [1]A. Alisa, [1,3]J. Karani, [1,3]M. Rela, [1,3]N. Heaton. [1]Liver Unit Cromwell Hospital, [2]Institute of Hepatology, University College London Hospitals and [3]Institute of Liver Studies, King's College Hospital.

Adult-to-Adult living donor liver transplantation (aLDLT) can be dramatically successful for both recipient and donor and over the past two years, the procedure has been widely adopted in the USA and mainland Europe, fuelled by increasing waiting lists for Cadaver organs. The present report describes the first experience in the UK with the procedure when used for overseas patients who have the lowest priority for Cadaver organ allocation. Fourteen patients were transplanted over the period November 1998 to June 2001. Ages ranged from 22-65 years. The indication for transplantation was end stage cirrhosis from HCV/HBV (11 cases) with single instances of cryptogenic cirrhosis, secondary biliary cirrhosis and alcoholic liver disease. The grafts were left lobe in the first two and right lobe in subsequent 12 recipients as donated by 8 sons/daughters and 6 brothers/sisters. Over the same period of time, 5 potential recipients were excluded on account of medical unsuitability as were four donors - 3 because of steatosis and 1 on account of a previously undetected cryptogenic cirrhosis on liver biopsy.

10 of the 14 recipients did well and were discharged home. The 4 recipients who died had recurrent sepsis, (two following an arterial occlusion) and in three major surgical factors were present pre-transplant. Serial CT measurement in the survivors showed regeneration of the grafted lobe with final volumes reaching in each instance the calculated standard liver volume for their size. No major complications were experienced in the donors and liver function tests had returned to normal by day 7-14. Serial liver volume measurements showed rapid regeneration by week 2-4, although in only three did the final size attained come close to the pre-donation CT estimated size.

Although a complex procedure, the results with aLDLT overall both for recipient and donor are good. As with cadaver grafts the outcome in the recipient – if the larger right lobe is used – is dependent on the severity of clinical decompensation pre-transplant and other surgical risk factors. The recovery period of the donor was short but measures to protect their safety remain the main concern.

O6

CD4+CD25+ CELLS SUPPRESS THE ALLORESPONSE IN A SELF-HLA RESTRICTED MANNER

D.S. Game and R.I. Lechler, Department of Immunology, Imperial College Faculty of Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN

CD4+CD25+ T cells have been shown to be important in preventing autoimmune disease in animal models. In vitro studies using human blood have shown that CD4+CD25+ T cells are a naturally occurring population with suppressive properties. There is considerable debate about how these cells mediate suppression: some groups advocate release of suppressive cytokines while others favour cell-contact dependent mechanisms. Furthermore, the specificity of the CD4+CD25+ regulatory cells is uncertain, in particular are they self HLA-restricted? Our working hypothesis is that they are specific for widely expressed self-peptides, presented by self HLA class II molecules. Having recently demonstrated the ability of CD4+CD25+ cells to inhibit alloresponses against foreign HLA molecules, we have designed experiments to reveal self HLA restriction by the CD4+CD25+ regulatory cells. Using responder cells of known HLA type, mixed leucocyte reaction cultures were performed using stimulator cells that expressed either 2, 1, and where possible, no HLA-DR mismatches. Our prediction was that the regulatory cells would be most efficient in the combinations in which a given HLA polymorphism was expressed by both the stimulator and responder cells (i.e. 0 or 1 mismatched). The influence of depleting the responder population of CD4+CD25+ cells was assessed in these allogeneic combinations. The amount of proliferation and IL2 secretion after depletion of the CD4+CD25+ cells divided by that before depletion gives an indication of the degree of suppression mediated by these cells.

We have shown that, indeed, the degree of suppression follows the trend $2<1<0$ in every experiment where depletion of CD4+CD25+ cells caused an increased proliferation (8/11). This finding matched the pattern of IL2 secretion in the majority of cases (6/8). In a few experiments (3/11) there was no increase in proliferation in any combination after depletion and therefore no trend was found.

These results support the concept that this population of regulatory cells is indeed self HLA-restricted and have important implications in the design of strategies to maximise their potential to regulate unwanted immune responses, such as those against transplanted tissues and self.

Parallel Session 1(a)

Organ Allocation and Transplant Outcomes

Wednesday 17 April 2002

16:00 – 17:30

O7

COMPARISON OF MORTALITY IN PATIENTS LISTED FOR RENAL TRANSPLANTATION: REDUCED RISK FOR TRANSPLANTATION VS. DIALYSIS

G.C. Oniscu[1], H. Brown[2], J.L.R. Forsythe[1]
[1]The Transplant Unit, Royal Infirmary of Edinburgh, [2]Information and Statistics Division, Scottish National Health Service

AIM: To assess the magnitude of the survival benefit of renal transplantation compared with dialysis in patients selected for transplantation in Scotland (UK) (a country of 5.2 million people).

METHODS: Longitudinal study of survival and mortality risk in all adult patients (1732) who were listed for a first transplant between 1.01.1989 and 31.12.1999 in Scotland. Of these, 1076 patients were transplanted by 12.31.2000. A time-dependant Cox regression analysis adjusted for comorbid conditions, age, gender, social deprivation, distance from patients' home to the transplant centre, primary renal disease, time from first dialysis to listing and year of listing compared the risk of death for patients receiving a first transplant versus all patients on dialysis listed for transplantation.

RESULTS: The mortality rate for patients who received a transplant was 4.05 per 100 patient-years, significantly lower than mortality rates for patients on dialysis (9.45 per 100 patient-years). After adjustment for the covariates, the relative risk of death during the first 45 days following transplantation was 0.93 (95% CI: 0.29-2.94), compared with patients on dialysis (p=0.90). However, the long term relative risk (beyond 45 days) for the transplant recipients was 0.27 (95% CI: 0.19-0.37) significantly lower (p<0.0001) when compared with patients on dialysis. This lower long-term risk is significant in all age groups (p<0.001), with the highest benefit in the youngest patients. Irrespective of the cause of end stage renal disease, transplant recipients have a lower risk of death compared with dialysis patients. The effect was most pronounced among patients with glomerulonephritis (RR, 013), while diabetic recipients had a risk of death of 0.3 (95%CI, 0.17-0.74) compared with diabetic waitlisted dialysis patients as shown in the table.

CONCLUSIONS: Long term survival in patients who undergo transplantation is significantly better compared with patients who are listed but remained on dialysis. In this cohort of patients there was no initially increased mortality risk following transplantation as reported by other studies.

		Relative risk > 45 days (95%CI)
Age group	***18-39 years***	0.08 (0.01-0.59)
	40-49 years	0.23 (0.10-0.50)
	50-59 years	0.14 (0.06-0.29)
	60-64 years	0.21 (0.05-0.88)
	>65 years	0.30 (0.13-0.70)
Primary renal disease	Glomerulonephritis	0.13 (0.05-0.32)
	Interstitial nephritis	0.24 (0.12-0.51)
	Multisystem disease	0.16 (0.07-0.40)
	Diabetes	0.35 (0.17-0.74)

Table 1. Relative risk of death for transplant recipients compared with patients listed for transplantation on maintenance dialysis (waiting list patients on dialysis = reference group)

O8

FACTORS INFLUENCING THE WAITING TIME TO LIVER TRANSPLANT IN THE UK AND REPUBLIC OF IRELAND

Mr A J Hudson, Mrs F M Seeney, Mr C J Rudge UK Transplant, Bristol on behalf of the UK Transplant Liver Advisory Group

Background: Patients accepted for liver transplantation face an uncertain wait and the duration of their wait is dependent on a variety of factors. This study aimed to identify recipient factors that influence a patient's waiting time to a cadaveric routine first liver transplant and to estimate the effect of these factors on a patient's waiting time.

Methods: Data on 801 adult Group 1 patients listed for a routine first liver transplant in one of the 8 transplant centres in the UK and Republic of Ireland (IRL) during the period 1999 to 2000 were analysed. All UK and IRL patients are registered on the UK National Transplant Database (NTxD) at listing and deaths on the list, removals from the list and transplants are also notified to the NTxD. Patient characteristics that would either be known or easily measured at the time of listing were considered: blood group, gender, primary diagnosis, CMV status, height, weight, body mass index, age, year of registration.

A multifactorial Cox regression model was used to identify those characteristics that influenced the waiting time to transplant for adult patients. The model was stratified by transplant centre to allow for inherent differences between centres. The analysis considered transplants as events and censored deaths on or removals from the list prior to transplant. Median waiting times to liver transplant were calculated univariately using Kaplan-Meier estimates.

Results: The chance of a transplant for adult recipients was significantly affected by their weight, blood group, gender, primary diagnosis and year of registration. The chance increased by 1% for every 1kg increase in weight; patients under 65kg waited a median 95 days compared with those over 85kg who waited 49 days. Patients of blood groups AB and A had around twice the chance of a transplant than group O patients; they waited a median 38, 49 and 70 days, respectively. Men had a higher chance of receiving a transplant than women; median waiting times were 55 days compared with 69. Patients with cancer had a better chance of receiving a transplant than those with cirrhosis; median waiting times were 31 and 60 days, respectively. Patients registered in 2000 waited a median 54 days and had a higher chance of a transplant than those registered in 1999 who waited a median 65 days.

Conclusion: Patient characteristics that influenced waiting time to liver transplant were identified. For adult Group 1 patients the lowest median waiting times to a routine cadaveric first liver transplant were associated with heavier patients, blood group A or AB patients, those registered in 2000, males and patients with hepatocellular carcinoma.

O9

DONOR ACTIVITY IN THE UK AND REPUBLIC OF IRELAND 1990 - 1999

Miss KM Burbidge, Mr MA Belger, Miss J Mahler, Mr CJ Rudge
UK Transplant, Bristol

Introduction: This review of organ donors in the UK and Republic of Ireland covers all cadaveric and living solid organ donors in the ten year period from 1 January 1990 to 31 December 1999. It looks back on organ donation during the 1990s, hence providing information to assess the success of future donor initiatives.

Data: Data on cadaveric heart-beating solid organ donors and cadaveric non-heart-beating and living kidney donors in the UK and Republic of Ireland from 1 January 1990 to 31 December 1999 were obtained from the National Transplant Database.

Results: The main findings from the ten years studied can be summarised as:

- A 20% fall in the total number of cadaveric solid organ donors. The percentage of multi-organ donors increased from 53% in 1990 to 88% in 1999. There was also a 75% increase in the number of liver donors, however, the number of cadaveric kidney donors and cardiothoracic donors decreased by 22% and 25%, respectively.
- Variations existed with regard to donation rates per million population across the UK and Republic of Ireland. England and Scotland tended to have lower rates than Wales, Northern Ireland and the Republic of Ireland.
- More organs were offered for donation from donors, but for a variety of reasons, more offers were declined. "Organ unsuitable" was the main reason given for kidneys not being offered for donation. Livers were not offered for donation because permission was refused. Cardiothoracic organs were not offered for donation either because the family refused or the organ was unsuitable or the donor was of an unsuitable age.
- No seasonal trend was observed in the monthly donation rates during the ten year period. Each year organs were procured from over 200 hospitals, the majority of which provided fewer than ten donors per year, with approximately 1% of donor hospitals providing 20 or more donors per year.
- There was a decrease in the number of road traffic accident (RTA) donors. This was associated with the fall in deaths due to RTA. In the early 1990s, the number of donors from cerebral vascular accident deaths increased. This was correlated with an increase in the mean age of donors.
- The number of living kidney donors rose from 101 in 1990 to 269 in 1999. As with cadaveric donation, these numbers varied considerably across the country.
- Two hundred and fifty three (253) non-heart-beating donors resulted in 412 kidney transplants. The majority of these donors (78%) came from just three centres.

Future work: From the Quinquennial review, UK Transplant has been given the remit to support initiatives in increasing the number of solid organ transplants undertaken in the UK and Republic of Ireland. The National Transplant Database will be used to monitor these initiatives and to look at the effect they have on organ allocation and ultimately the outcome of the transplant.

O10

FACTORS AFFECTING ACCESS TO THE UK RENAL TRANSPLANT WAITING LIST

Armstrong S[1], Johnson RJ[1], Ansell D[2], Briggs JD[1], Belger MA[1], Sadek S[2], Rudge CJ[1], Feest TG[2] [1]UK Transplant, Bristol, [2]UK Renal Registry, Southmead Hospital, Bristol

Background: The databases of UK Transplant and the UK Renal Registry have been linked to obtain a unique dataset comprising data for 4944 adult renal dialysis patients and their associated listing dates where applicable. Using these data, the aim of this study was to investigate factors that influence whether a patient is listed for transplant on the national waiting list.

Methods: The Renal Registry, covering approximately 50% of the UK, reported 4944 patients (2602 <65 years) starting their first renal replacement therapy during 1998 or 1999. Patients were from 34 dialysis units, 17 of which have associated transplant units. Factors available for analysis were age at start of dialysis, primary disease, gender, ethnicity and dialysis unit. Separate multifactorial logistic models were developed for patients aged less than 65 years and patients aged 65 years or older for the binary outcome listed/not listed.

Results: Of the 2602 patients (<65 years) who started dialysis in 1998 or 1999, 1406 (54%) had been listed for transplant by June 2001. Factors that significantly affected whether a patient was listed for transplant were: primary disease ($p<0.0001$), age ($p<0.0001$) and whether the dialysis hospital also has a transplant unit ($p=0.03$). Gender and ethnicity of the patient were not found to have a significant effect. Age-adjusted results for the primary disease groups were:

Primary disease	No. of patients starting dialysis	Relative chance of listing	95% confidence interval	p
Aetiology uncertain/GN not proven (baseline)	470	1.0		
Glomerulonephritis (GN)	349	1.7	1.2 – 2.3	0.002
Pyelonephritis	242	1.3	0.9 – 1.9	0.1
Diabetes	540	0.3	0.2 - 0.4	<0.0001
Renal Vascular Disease	78	0.3	0.2 – 0.6	0.0001
Hypertension	122	1.4	0.9 – 2.1	0.2
Polycystic kidneys	237	3.0	2.0 – 4.3	<0.0001
Other	360	0.3	0.2 - 0.4	<0.0001
Not reported	204	0.4	0.2 - 0.6	0.0001

Multifactorial analysis of the 2342 patients aged 65 or older showed primary disease and age to be significant. Of these older patients, 108 (5%) had been listed for transplant by June 2001.

Conclusion: The chance of a patient (<65 years) on dialysis being listed for transplant varied significantly between primary renal disease groups; significantly decreased with increasing age and was also significantly less for patients attending a dialysis unit not attached to a transplant unit. The significance of this final factor was linked to different rates of pre-emptive listing for transplant across dialysis units.

O11

BLOOD GROUP INEQUALITIES IN ACCESS TO KIDNEY TRANSPLANTATION

Johnson RJ, O'Neill J, Belger MA, Rudge CJ, Fuggle SV, Bird SM
On behalf of the UK Transplant Kidney and Pancreas Advisory Group

Background: The allocation of kidneys for transplant is based primarily on blood group and HLA matching which differ between donor and waiting list patients. This analysis investigates factors affecting the waiting time to kidney transplant once listed and considers how inequalities could be somewhat redressed.

Methods: Patients registered on the national kidney transplant waiting list in 1998 or 1999 (n=3630) were included in a multifactorial model for waiting time to transplant. Cox regression was carried out with transplantation as the event of interest. Patients not transplanted at the time of analysis were censored as were patients who died on, or were removed from, the waiting list. Factors previously found to affect waiting times were adjusted for, but the main effects of interest for this analysis were three inter-related factors: blood group, ethnic origin and matchability.

Results: Blood group inequalities between donors (O 48%, A 40%, B 9%, AB 3% - based on 2722 donors) and waiting list patients (O 49%, A 32%, B 16%, AB 3%) resulted in different chances of transplant in the four blood groups:

Blood group	Number newly registered on waiting list in 1998 or 1999	Relative chance of transplant	95% CI	p
O	1689	1.0	(baseline)	
A	1351	1.6	1.5-1.8	<0.0001
B	454	0.7	0.6-0.8	<0.0001
AB	136	1.9	1.5-2.5	<0.0001

Results for patient matchability score (1-10 based on difficulty to HLA match as 000 or favourable) were also highly significant, as expected. Moreover, ethnic group was a significant factor in the analysis of waiting time to transplant, with Asians and other ethnic minority groups each having a relative chance of transplant of 0.6 (95% CI 0.5-0.8) compared with White patients. Further modelling revealed a significant interaction term for blood group and ethnic origin, suggesting that the effect of blood group on waiting time to transplant differs between ethnic origin groups.

Conclusion: The chance of receiving a transplant once listed is significantly influenced by blood group, matchability and ethnic origin of the patient. These inequalities in access to transplantation are difficult to resolve given that there are differences between donor and waiting list pools and that HLA matching currently forms the basis of kidney allocation. Computer simulations of a slightly modified Kidney Allocation Scheme suggest that allowing some blood group O kidneys to be allocated to blood group B patients may at least address the inequality in access to transplantation between these two groups.

O12

THE FIRST THREE YEARS OF A REVISED SCHEME FOR ALLOCATING CADAVER KIDNEYS IN THE UK

Johnson RJ, Belger MA, Hodge RA, Briggs JD, Fuggle SV, Rudge CJ
On behalf of the UK Transplant Kidney and Pancreas Advisory Group

A revised Kidney Allocation Scheme was introduced in the UK in July 1998 based on HLA matching at three levels: 000 mismatches, favourable matches (i.e. 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 also since July 2000, priority has been given to HLA-DR homozygous patients when the donor is HLA-DR homozygous. A further change to the Scheme in July 2000 was to give priority to favourably matched paediatric patients anywhere in the country over local favourably matched adults for the second kidney from an adult donor. 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 transplant centre import and export balance.

To assess the effectiveness of the revised scheme, results of the first three 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 ($p<0.0001$ and $p=0.0006$, respectively) and significant improvement for adult regrafts ($p=0.0004$). HLA matching levels for paediatric recipients are now comparable with those of adults. The proportion of 000 mismatched grafts has increased from 7% to 14% for adults and from 5% to 12% for paediatric recipients. There has also been an increase in the number of transplants for highly sensitised patients and during the first three years of the Scheme 60% of these transplants were 000 mismatched. For those kidneys allocated to adults through the national Scheme there have been some changes relevant to the use of 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 have received proportionally more transplants at the expense of those who are easiest to match ($p=0.002$).

In conclusion, the new UK Kidney Allocation Scheme has been associated with significantly improved HLA matching for all adult and paediatric patients and for adult regraft recipients. No change has occurred in age at transplant and there has been a decrease in donor-recipient age differences. Matchability points scoring may have helped to achieve an increase in the number of transplants for patients who are moderately difficult to HLA match and 60% of transplants in highly sensitised patients are now 000 mismatched. Finally, HLA-DR homozygous patients are achieving significantly better HLA matched transplants than previously.

O13

BENEFITS OF RENAL ALLIANCES

Miss JC Kiln, Mrs RJ Johnson, Mr MA Belger, Mr CJ Rudge UK Transplant, Bristol

Introduction: A renal sharing alliance can be formed by combining the waiting lists a several centres. When a kidney donor becomes available at one of the alliance centres the UK Transplant Duty Office carries out national and local matching runs. After the kidneys have been offered to patients in the prioritised groups from the national rules, the Duty Office will send the local matching runs to assist allocation locally. All alliances have a well-defined local algorithm for sorting patients into priority order to meet the needs of the alliance centres. Several of the alliances use local schemes based on the national scheme and all are based on HLA matching and other factors such as waiting time.

The first alliance scheme started in January 1996 in North Thames and by the end of 2001 six alliances operated in the United Kingdom, with only three renal transplant centres not associated with an alliance. An Audit was performed on retrieval, transplant and waiting list activity for the period 1 January 1990 to 31 December 1999, and incorporated the first five alliances.

Results: As at 31 December 1999, 65% of the active national kidney waiting list was associated with alliance centres.

The changes in the National Kidney Allocation Scheme resulted in an increase in 000 and favourably matched transplants. However, the alliances generally exhibited a statistically significant greater increase in well HLA matched transplants compared to the non-alliance centres for comparative time periods.

There was no statistically significant change in any alliance in the mean age of recipients receiving transplants after alliance formation. Generally, there was no change in the distribution of either recipient blood group or recipient gender after alliance formation.

Most alliances showed a slight increase in the proportion of regrafts undertaken after alliance formation.

Alliance formation resulted in a reduction in the proportion of transplants undertaken with kidneys retrieved by the local centre, but an increase in transplants undertaken with kidneys from other centres within the alliance group.

Conclusion: The main benefits of renal alliance formation are considered to be:

- Increase in well-matched transplants
- Greater sharing of local organs amongst the centres of the alliance
- Consolidation of renal transplant services
- Increased communication between centres (collaborative research, audit and discussion)

Accountability, provided by an objective and defendable allocation scheme, which can be explained to patients.

O14

REGIONAL VARIATION IN DONATION

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Introduction: This is a summary of the variation in retrieval rates and the differences observed in the demographic characteristics of donors (age, gender, blood group, ethnic origin, cause of death and type of donor) across the 22 kidney retrieval areas.

Data: All cadaveric heart-beating solid organ donors in the UK and Republic of Ireland between 1 January 2000 and 30 June 2001 were analysed.

Results: There were 1199 donors in this period, the proportion of donors from each kidney retrieval area ranged from 1.2% to 11.6%. The donation rate per million population (pmp) ranged from 6.2 to 23.3, with an overall donor rate pmp of 12.7.

The mean donor age varied between kidney retrieval areas from 35.4 years to 49.9 years and the overall mean donor age was 42.1 years. There was a statistically significant difference observed between the kidney retrieval areas in mean donor age, $p<0.001$.

The proportion of male donors in the kidney retrieval areas ranged from 35.7% to 75% compared with 54.8% overall. Only six kidney retrieval areas had a higher proportion of females than males. However, there was enough of a difference observed between the kidney retrieval areas in the proportion of males and females to show a statistically significant result, $p=0.029$.

The blood group distribution of donors varied between kidney retrieval areas. Eight areas had no blood group AB donors and two of these also had no blood group B donors. Overall, 95.3% of donors were white and 2.3% were specified ethnic minority donors from only ten kidney retrieval areas.

Donor cause of death was categorised as cerebrovascular accident (CVA), road traffic accident (RTA), other trauma and natural causes. Overall, 62.6% of donors died from CVA and 13.5% from RTA. Some kidney retrieval areas only had donors from two or three of the four cause of death categories.

Overall, 86.3% of cadaveric heart-beating organ donors during this period donated a kidney and at least one other solid organ, with kidney retrieval area proportions ranging from 67.8% to 100%. Only 8.8% were kidney only donors with proportions from each kidney retrieval area ranging from 0% to 32.1%.

Comment: Donation campaigns could be run in the kidney retrieval areas where organ donation is generally low and in some areas specific ethnic minorities could also be targeted to raise awareness and try to develop und

O15

KIDNEY TRANSPLANTS USING OLDER DONORS

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Information: This is a review of first adult kidney transplants using older donors (aged 60 years and over) compared with younger donors (aged between 18 and 59 years) in the UK and Republic of Ireland. The report covers all adult (18 years and over) cadaveric heart-beating kidney donors in the ten year period from 1 January 1990 to 31 December 1999.

Methods: Kaplan-Meier transplant survival estimates and the Cox proportional hazards regression method were used to identify differences in transplant outcome between older and younger donors. The multifactorial models incorporated various factors found to be significantly related to the outcome of transplant survival at the 5% significance level. The factors influencing transplant survival were also evaluated in distinct post-transplant epochs, <1 month, 1 month to 1 year and > 1 year post transplant. The analyses performed were based on: all donors; epochs for all donors; younger donors; older donors; and epochs for younger and older donors.

Results: There were 11571 transplants over the ten year period, 1552 (13%) were from older donors. One year Kaplan-Meier transplant survival estimates were calculated by donor age for a total of 10815 transplants with valid follow-up. The results from this univariate analysis showed that transplants using kidneys from older donors had significantly worse survival than those using kidneys from younger donors, at the 5% level; 75% compared with 85%, respectively (Log-rank test $p<0.001$).

A multifactorial analysis for all donors showed that donor age was a statistically significant factor in transplant survival after having adjusted for all other factors found to be related to transplant survival, with transplants from older donors having significantly worse (at the 5% level) transplant survival. The other factors found to be related to transplant survival were graft year, recipient age, HLA matchgrade, local or exchanged organ, donor cause of death and primary disease.

The epoch analysis for all donors showed that older donors had significantly worse (at the 5% level) outcome throughout the three post-transplant epochs. Factors that differed over the epochs included a cause of death of RTA and an exchanged organ.

Multifactorial analyses performed separately for both older and younger kidney donors showed that for younger donors non-favourable HLA matchgrade transplants had statistically significantly poorer outcome, but this was not observed for older donors.

The epoch analysis for younger donors gave similar results to that for all donors. The analysis for older donors showed HLA matchgrade was a statistically significant factor for the first epoch although it was not significant in the epoch analysis for all donors.

O16

HLA MATCHING IN PAEDIATRIC KIDNEY TRANSPLANTATION IN THE UK

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On behalf of the UK Transplant Kidney and Pancreas Advisory Group

Background: Children with renal failure face a lifetime of renal failure management and often require more than one kidney graft. It is therefore particularly important that they receive well-matched grafts. Changes to the national Kidney Allocation Scheme over recent years have resulted in significantly improved HLA matching in paediatric transplantation. This has been achieved through increased access to kidneys from adult donors and extension of the use of compatible blood group matching. Computer simulations suggest, however, that HLA matching could be further improved through the consideration of patient matchability scores.

Methods: Matchability score measures the difficulty with which a well-matched kidney can be found for an individual given their tissue type and unacceptable antigens. The score is calculated for each patient on the national kidney transplant waiting list and indicates which patients are easiest to match (1 - easy, 10 - difficult). Computer simulations of the Allocation Scheme were run to investigate what improvements could result from consideration of matchability scores in identifying children who would benefit from waiting for a 000 or favourably matched kidney transplant.

Results: Computer simulation results show that HLA matching for children could be further improved if non-favourably matched grafts were not accepted for the half of patients who are easiest to HLA match (scores 1-5). The indication is that for a small increase in waiting time (from three to four months on average), approximately 10% more paediatric grafts could be favourably rather than non-favourably matched. This would represent a further significant improvement in HLA matching in paediatric transplants, 39% of which were non-favourably matched in the year ending 30 June 2001.

Conclusion: The matchability score of each patient is readily available on UK Transplant waiting list reports. Paediatricians on the Kidney and Pancreas Advisory Group have encouraged their colleagues to use matchability score in deciding whether or not to accept an offer of a non-favourably matched kidney for each child on their waiting list. If centres adopt this policy, and evidence suggests that it has not yet been widely implemented, then HLA matching in children should improve still further.

O17

A MULTICENTRE ANALYSIS OF THE RESULTS OF KIDNEY TRANSPLANTATION FROM NON HEART-BEATING DONORS IN THE UK

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Introduction: Non heart-beating donors (NHBD) have been shown to be a useful additional source of transplant organs. Nonetheless, there continue to be concerns about the effects of prolonged warm ischaemic injury on long-term results. The aim of this multicentre study was to determine the outcome of kidney transplantation from NHBD in the UK.

Methods: Prospectively collected data from 4 centres (Leicester, South Thames, Newcastle and Cambridge) performing NHBD kidney transplants during the period 1991-2001 was collated. 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: 285 NHBD kidney transplants were performed in the study period. 51% of donors were controlled and 49% uncontrolled. The mean (range) donor age was 44 (6-74) yrs. The transplanted kidneys suffered a mean (range) first warm ischaemic time of 27 (10-75) minutes. The mean HLA A,B and DR mis-matches were 1.1,1.3, and 0.8. The rates of PNF, DGF and IF were 15, 68 and 17% respectively. Acute rejection occurred in 41% of patients in the first year post-transplant . Allograft function and survival rates are shown in the table.

Time post-transplant (mth)	Serum creatinine (µmol/l)	Allograft survival (%)
3	195 (92)	82
12	179 (88)	79
36	177 (73)	75
60	197 (101)	69

Conclusions: This audit presents the largest analysis of NHBD kidneys transplants from the UK. Renal transplants from NHBD yield acceptable renal function and allograft survival rates over a five-year period. This audit provides further evidence to support the more widespread introduction of NHBD kidney transplantation.

Parallel Session 1(b)

Alloimmunity I

Wednesday 17 April 2002

16:00 – 17:30

O18

IL-15 AND INTRATUBULAR T CELL PROLIFERATION IN RENAL ALLOGRAFT REJECTION : IN SITU AND IN VITRO STUDIES

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Background: Numerous studies have shown that there is transitory expression of IL-2 during acute renal rejection. In spite of this, recipient T cells that cross the tubular basement membrane to lie beneath and between tubular epithelial cells (TEC) persist and are often present in the "resolving/recovery" phase after acute rejection. Furthermore such T cells are capable of proliferating in the intratubular site. Recently IL-15, known to be produced by renal epithelial cells, has been identified as an important cytokine in controlling proliferation and maintenance of CD8+ T cells.

Methods: In this study proliferating intratubular T cells (ITL:CD3+/Ki67+) were detected and enumerated in diagnostic acute rejection biopsy sections with a dual-colour immunolabelling procedure and light microscopy. A semiquantitative immunofluorescence method with scanning laser confocal microscopy was used to show IL-15 in TEC both in untransplanted non-heart beating donor (NHBD) kidneys and in acute rejection biopsies. Biopsies were also dual-labelled to detect CD8 and IL-15Rα. IL-15 production by IFNγ-stimulated primary renal epithelial cells in culture was measured by ELISA.

Results: The proportion of ITL in active cell cycle at the time of biopsy did not vary significantly (range 7 to 17%; $p>0.08$), irrespective of the rejection status of the graft. Around 10% of tubular epithelium expressed IL-15 in NHBD kidneys but this distribution increased significantly ($p<0.01$) to reach a peak in biopsies showing moderate rejection. Of the tubules that expressed IL-15, intensity of expression showed only a small, but significant ($p<0.05$), increase between NHBD tissue and samples from grafts in any of the rejection categories including in the 'post-acute' recovery phase. These data are consistent with the results of the *in vitro* study which showed that resting renal epithelial cells produce IL-15 but that stimulation by IFNγ, known to be present during rejection, increased IL-15 expression to a small but significant extent ($p<0.01$).

Conclusions: The tubular microenvironment can actively support the proliferation and persistence of intratubular T cells, even after the resolution of acute rejection. It is possible that IL-15 expression by tubular epithelial cells promotes survival of CD8+ intratubular cells via anti-apoptotic activity and support for proliferation. Such cells may be long-lived memory CD8+ T cells that could play a role in the evolution of chronic changes within the tubulointerstitial compartment.

O19

THE EXPRESSION OF mRNA for IL-2, GRANZYME B AND PERFORIN IN TWO-WAY MIXED LYMPHOCYTE REACTIONS

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Although important for the interpretation of data in clinical transplantation, the kinetics and levels of expression of genes relevant for maturation (IL-2 mRNA) and effector function of cytotoxic T lymphocytes (Granzyme B and perforin) in alloantigen driven immune responses remain largely unexplored both in vivo and in vitro. We have recently demonstrated that pre-perfusion kidney core biopsy samples from some cadaveric donors predominantly express alternatively spliced variants of Interleukin-2 mRNA (IL-2δ2 mRNA-missing exon-2 and IL-2δ3 mRNA-missing exon-3). Alloantigen stimulated PBMC in two way MLR, however, predominantly express IL-2L mRNA. Majority of IL-2δ2 and IL-2δ3 mRNA positive pre- and post-perfusion kidney biopsy samples coexpressed Granzyme B but only one quarter of analysed biopsies expressed mRNA for perforin. In spite of immunsuppression, IL-2δ2 and IL-2δ3 and GrB mRNA could be detected 3, 6 and 12 months post transplantation.

It remains unclear how the interaction between pre-existing kidney donor T-cells and recipients T-cells and IL-2 generated as a result of that interaction determines the kinetics, level and longevity of expression of Granzyme- B and perforin mRNA. The aim of this study was to follow the kinetics of the expression of GrB and perforin in a two-way MLR and compare them with the expression of IL-2L mRNA.

Equal aliquots of the non-irradiated PBMC from two donors were mixed (1x10e7 each) and incubated with or without 20U/ml of recombinant IL-2 for 1, 2, 3, 6, 7, 10 or 14 days. To analyse the effect of IL-2 deprivation on the expression of genes, 200ng/ml of Cyclosporin A (CyA) was added on day 0, kept in cultures throughout the incubation period or kept for two days when the cells were washed and cultured without CyA for another day (day 3) or 4 more days (day 6). In some cultures CyA was added on day 3, to allow the initiation of the immune response, and left in cultures for the remaining period of MLR. SYBR Green I ® based real-time quantitative RT-PCR assays were developed to measure IL-2L mRNA, GrB mRNA and perforin mRNA.

In several MLR experiments the IL-2L mRNA was upregulated on day 1, peaked on day 3 (range 1.5 x10e3-1.8 x10e5 copies/50ng RNA) fell on day 4 and peaked again on day 5 or 6 and 7. On day 10, samples still expressed around 10e3 copies/25ng RNA. In IL-2 treated cultures the observed peaks for native IL-2L mRNA, were shifted to day 1 and were 5-10 fold higher than on day 3 of the normal MLR. Baseline expression of Granzyme B mRNA (0 point) in different PBMC donor combinations varied considerably from several hundred to several thousand copies per 50ng RNA. The significant enhancement of GrB mRNA was seen on days 3 and 4, reaching from day 5 to day 7 high plateau levels of several hundred-thousand copies/50 ng RNA. Even on day 14 the GrB mRNA levels were higher than background. Recombinant IL-2 accelerated while CyA added either on day 0 or day 3 inhibited levels of GrB mRNA indicating that IL-2 significantly affects GrB expression. Baseline expression of perforin mRNA (0 point) in different PBMC donor combinations varied considerably from several hundred to several thousand copies per 50ng RNA. As in vivo, where the expression of perforin mRNA did not always correlate with acute rejection, the allo-antigen stimulation in some two-way MLR donor combinations failed to significantly enhance levels of perforin mRNA. In MLR combinations, which showed significant upregulation of gene for perforin, mRNA levels peaked on day 5, but were always lower than GrB mRNA. Recombinant IL-2 accelerated and enhanced while CyA slightly inhibited expression of perforin mRNA.

We found that GrB and perforin mRNA levels successfully mirror patterns of expression in clinical kidney transplantation and confirmed that IL-2 is essential for the expression of GrB gene and to some extent perforin mRNA.

O20

EXPRESSION OF CXCR3 ON CIRCULATING AND TISSUE INFILTRATING T CELLS IN HUMAN RENAL TRANSPLANT RECIPIENTS

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Introduction. CXCR3 is a key receptor for the directed migration of activated T cells to tissue sites and may represent an attractive therapeutic target. The objective of this study was to analyze CXCR3 expression on circulating and tissue infiltrating T cells in renal transplant recipients to assess a role in human acute renal allograft rejection.
Methods. In 14 patients (8 male: 6 female, median age 46.5 years range 16-68years), receiving standard cyclosporine based triple therapy, with no antibody induction, triple labelled FACS was used to study expression of CXCR3, CD4, CD8, CD25 and CD45RO on lymphocytes in whole blood serial samples for the first 6 weeks post transplant. For tissue analysis the expression of CXCR3, CD3 and CD25 was studied by serial section and colocalisation immunohistochemistry in renal transplant biopsies subsequently obtained for early graft dysfunction using post perfusion biopsies as an internal control.
Results. By mean fluorescent intensity, the level of CXCR3 expression on circulating T cells was increased by day 3 post transplant, relative to the pre-transplant level, in those patients who developed AAR and this preceded clinical evidence of graft dysfunction. This was particularly marked in the CD4+ cell subset ($p<0.005$ by ANOVA). CXCR3 levels fell immediately after treatment of acute rejection with high dose steroids. Patients without AAR had a sustained reduction in CXCR3 expression. Tissue analysis showed low numbers of CXCR3+ cells immediately post-perfusion compared with acute allograft rejection (2.2/hpf vs 49.5/hpf; $p<0.0001$) with CXCR3 expression colocalising with CD3, CD25 and CD45RO.
Conclusion. The kinetics of CXCR3 expression on circulating T cells and subsequent colocalisation in tissue with infiltrating cells of an activated and memory phenotype indicate a central role for this molecule in the development of acute allograft rejection in human renal transplantation. These results are consistent with data from animal models where blocking CXCR3, or its ligating chemokines, prevents the development of allograft rejection.

O21

PIRFENIDONE- A NOVEL PHARMACOLOGICAL APPROACH TO THE PREVENTION OF ALLOGRAFT VASCULOPATHY?

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Aims: Intimal hyperplasia, a central feature of allograft vasculopathy, is mediated by vascular smooth muscle cell proliferation, migration and deposition of extracellular matrix proteins (ECM) resulting in luminal narrowing and end-organ ischaemia. Pro-fibrotic cytokines, matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are pivotal mediators in this process. Secretion of metalloproteinases (MMPs) facilitates ECM degradation and migration, whilst tissue inhibitors of metalloproteinases (TIMPs) favour the accumulation of ECM. This study investigates the effect of the novel anti-fibrotic agent Pirfenidone on the development of neointima.

Methods: Male Sprague-Dawley rats were assigned to receive either standard diet or diet supplemented with powdered pirfenidone (250, 500, 1000mg/kg/day). Animals underwent left common carotid balloon angioplasty and were explanted at 4, 8 and 14 days post arterial. Morphometric analysis was performed on representative transverse sections and intima medial ratios calculated using computer-aided planimetry. Extracellular matrix accumulation was quantified using picro-sirius red staining. Pro-fibrotic gene expression was measured with competitive RT-PCR and metalloproteinase activity assessed using gelatin zymography.

Results: Neointimal thickness was significantly reduced in a dose-dependent manner; Control 1.63(1.44-1.99), Pirfenidone 250mg/kg 0.73(0.65-1.26)-P<0.005, Pirfenidone 500mg/kg 0.48(0.12-0.85)-P<0.001, Pirfenidone 1g/kg 0.009(0-0.28)-P<0.001. Expression of MMP-2, MMP-9, TIMP-1, collagen III and TGF-beta were all significantly inhibited at 14 days (see table). Furthermore, Pirfenidone significantly inhibited the enzymatic activity of MMP-2 and 9 at 4 and 8 days. Pirfenidone significantly attenuated neointimal extracellular matrix deposition at all time points; median value 15.35 (range 12.05-19.11) compared with controls, 8.77 (6.85-11.63), P<0.002.

Gene	Control	Pirfenidone	P-Value
MMP-2	1.50 (0.76-2.75)	1.11 (1.02-1.37)	P< 0.001
MMP-9	1.51 (0.71-3.16)	0.58 (0.39-0.65)	P< 0.001
TIMP-1	1.04 (0.62-1.77)	0.91 (0.77-1)	P< 0.001
Collagen III	2.20 (1.09-4.29)	0.8 (0.69-1.04)	P< 0.001

Conclusion: Pirfenidone reduces expression of MMPs governing smooth muscle cell proliferation and migration (MMP-2 & 9), and genes favouring ECM accumulation (TIMP-1 & collagen III) and offers a potential therapeutic approach to attenuate the development of chronic rejection in solid organ allografts.

O22

HEPATOCYTE MINICHROMOSOME MAINTENANCE PROTEIN 2 EXPRESSION PREDICTS GRAFT FIBROSIS IN RECURRENT HCV INFECTION FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION.

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Background: Hepatitis C virus – related cirrhosis is a leading indication for orthotopic liver transplantation in the UK. One year survival rates are comparable to other indications but graft re-infection is virtually universal. Progression of graft fibrosis is accelerated as the median time to reach cirrhosis is 5-7 years post transplant compared with 20-30 years pre transplant. Some transplant patients may develop cirrhosis within 2 years yet others remain fibrosis free and there are no reliable markers available to predict damage before it occurs. Previous work has shown a correlation between hepatocyte expression of minichromosome maintenance protein 2 (MCM2) and fibrosis stage in the non-transplant population. MCM proteins are involved in DNA replication and are sensitive and specific markers for the cell division cycle.
Methods: Standard immunohistochemical techniques were used to detect hepatocyte MCM2 expression in serial liver biopsies from patients with recurrent HCV and no or minimal fibrosis up to 8.5 years post transplant and patients with recurrent HCV and development of cirrhosis within 2 years. All samples had no histological diagnosis other than recurrent HCV and no patient had hepatocellular carcinoma prior to or after transplantation. Normal liver served as a negative control.
Results: Hepatocyte MCM2 expression in early post transplant liver biopsies differed between the two groups as summarised in the table below. In normal liver MCM2 is present in <0.1% of hepatocytes.

Group	HCV recurrence, progressive fibrosis	HCV recurrence, no or minimal fibrosis
%MCM positive mean	35.3	2.0
%MCM positive range	22-48	0.28-5
Interval between transplant and biopsy	2-6 months	2-72 months

In those patients who subsequently developed cirrhosis MCM2 expression remained elevated compared to the non-fibrotic group.
Conclusion: In patients with recurrent HCV following orthotopic liver transplantation, increased hepatocyte expression of MCM2 is associated with subsequent graft fibrosis. Use of this method to predict fibrosis progression would allow optimisation of immunosuppressive therapy and targeting of antiviral treatment, thus preventing graft damage.

O23

TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES IN RENAL ALLOGRAFT NEPHROPATHY.

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Background: The renal extracellular matrix (ECM) is in a continual state of turnover with a tight balance between synthesis and degradation rates. ECM breakdown occurs predominantly through the matrix metalloproteinase (MMPs) system, which is regulated by Tissue inhibitors of MMP (TIMPs). Over expression of TIMPs causes decreased breakdown of ECM that contributes to the ECM accumulation characteristic of fibrogenesis. However, it remains to be determined if TIMPs play a role in elevating the ECM levels characteristic of allograft nephropathy. Here, we use sequential renal biopsies from graft recipients (including implantation) to determine the expression of the two most abundant renal TIMP's, TIMP 2 & TIMP 3, in the development of allograft nephropathy.
Patients and Methods: 25 cadaveric renal allograft recipients were studied retrospectively. These had an implantation biopsy followed by repeat biopsies taken within (early) and after (late) the first year as clinically indicated. TIMPs 2 & 3 were localised within the renal allografts by immunohistochemistry and evaluated by point counting. To compare TIMP staining with the degree of interstitial fibrosis, sections were further evaluated by Masson's Trichrome staining and immunostaining for collagen IV and alpha smooth muscle actin.
Results: Analysis of implant, early and late allograft biopsies showed a steady progression in Massons Trichrome, Collagen IV and SMAα staining indicating progressive fibrosis and allograft nephropathy in these patients with levels for each increasing approximately 3 fold in early and 7 fold in late biopsies ($p<0.05$).
In normal tissue taken from the healthy pole of kidneys resected due to hypernephroma, minimal staining of both TIMP 2 (1.42±0.76%) and TIMP 3 (0.09 $\pm$ 0.03%) was seen. In comparison, in biopsies taken during implantation both TIMP 2 (12.9 $\pm$ 3.9%) and TIMP 3 (17.45 $\pm$ 3.7%) were significantly increased ($p<0.01$). These increases occurred predominantly within the proximal and distal tubules, however this appeared to remain within the tubule cell with little extracellular or interstitial staining. Some glomerular staining was evident within the endothelial cells and Bowman's capsule. In early transplant biopsies, both TIMP 2 (10.24% $\pm$ 1.9%) and TIMP 3 (14.5 $\pm$ 2.0%) showed reduced expression in comparison to the implant levels ($p<0.01$), however they were both still significantly higher than normal kidney levels ($p<0.01$). In late biopsies, TIMP 2 levels rose to almost twice that at implantation (26.3 $\pm$ 14.1%, $p<0.01$), while TIMP 3 levels continued to decrease from early biopsy levels (5.2 + 2.7%, $p<0.01$).
Conclusions: TIMP 2 and TIMP 3 expression is increased in transplanted kidneys. The high expression in implantation biopsies indicates this may be instigated by a pre transplant event. However, while lower than at implantation, high TIMP 2 and 3 levels persist early post transplantation with TIMP 2 levels increasing in late biopsies. Elevated TIMP 2 & 3 therefore have the potential to inhibit MMP activity in the allograft and may therefore play a role in the ECM accumulation seen in allograft nephropathy.

O24

CYSTATIN C AS A GUIDE TO GLOMERULAR FILTRATION RATE (GFR) IN RENAL TRANSPLANT RECIPIENTS WITH GRAFT DYSFUNCTION SECONDARY TO CHRONIC ALLOGRAFT NEPHROPATHY (CAN)

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Background. Serum creatinine is used as a guide to GFR in clinical practice, but there are disadvantages in terms of its poor sensitivity and the effect of non-renal factors on both the serum concentration of creatinine and the performance of laboratory creatinine assays. Furthermore, renal tubular secretion of creatinine may lead to a significant overestimation of GFR when excretory function is impaired. More accurate methods for determining GFR are too time-consuming and expensive for routine use. Cystatin C, a 13 kD protein that inhibits cysteine protease, is synthesised by all nucleated cells at a constant rate. The serum concentration of cystatin C is a more sensitive endogenous marker of mild renal impairment than serum creatinine. We have examined the reliability of serum cystatin C in an adult renal transplant population.
Methods. Serum creatinine (alkaline picrate assay) and cystatin C (particle-enhanced nephelometry (PENIA), Dade Behring, Germany) were measured in blood samples obtained from 25 patients with CAN (9 female, GFR range 15 to 48 mL/min/1.73m^2) immediately prior to GFR measurement (99mTcDTPA-GFR, corrected for body surface area using the DuBois equation). Tests of correlation between 1/creatinine, 1/cystatin C and 99mTcDTPA-GFR were performed, and an established GFR prediction formula based on cystatin C (Cyst-GFR) was assessed for bias and scatter.
Results. There was a strong correlation between the three measures of transplant function at 0 and 6 months (1/cystatin C vs 99mTcDTPA-GFR, r = 0.79 and 0.82, p<0.001; 1/creatinine vs 99mTcDTPA-GFR, r = 0.75 and 0.70, p<0.001; 1/creatinine vs 1/cystatin C, r = 0.84 and 0.86, p<0.001). The calculated Cys-GFR values tended to exceed the corresponding 99mTcDTPA-GFR measurements, with a bias (median difference in values) of +10.9 mL/min/1.73m^2 and scatter (median absolute difference in values) of 11.1mL/min/1.73m^2.
Discussion. Cystatin C correlates more closely with 99mTcDTPA-GFR than does serum creatinine and can therefore be regarded as a more reliable marker of GFR in adult renal transplant recipients. The Cys-GFR formula overestimates GFR as measured by a reference method and so its use in this setting would be inappropriate.

O25

CYTOKINE PRODUCTION BY NATURAL KILLER CELL SUBSETS DURING ALLOGRAFT REJECTION

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BACKGROUND: Natural killer (NK) cells have been implicated in the rejection of allografts though the precise mechanisms remain poorly understood. Their activity is controlled by their repertoire of inhibitory and stimulatory receptors (e.g. Ly49 A – W). Here we examine the effect of a solid organ allograft on Ly49 expression and the production of cytokines by subsets of NK cells expressing different repertoires of Ly49 receptors.

METHODS: Fully allogeneic donor CBA ($H2^k$) cardiac allografts were transplanted into C57BL/6 ($H2^b$) recipient mice. Graft infiltrating and splenic NK cells were stained with anti- CD49b-PE monoclonal antibody (mAb), a pan-NK cell marker and changes in NK receptor expression were studied by staining NK cells with FITC conjugated mAb to Ly49A, D, C and G2. Analysis was performed using 2 colour flow cytometry. Intracellular cytokine production in subsets of NK cells expressing different repertoires of Ly49 receptors was assessed using Cy-Chrome labelled mAb against different cytokines.

RESULTS: All Ly49 receptors that are known to bind to donor $H2^k$ class 1 MHC molecules were down regulated in recipient splenic NK cells 1 day following transplantation but the effect was most marked in the inhibitory receptors: expression of the inhibitory receptors Ly49A and Ly49C were down regulated by 89 and 68% respectively while the stimulatory receptor Ly49D was only down regulated by 45%. Both graft infiltrating and splenic NK cell express IL2, IL12 and IFNγ during early rejection. The production of these cytokines was analysed in NK cell subsets according to their expression of the stimulatory NK receptor Ly49D. We found that the production of IL2 and IL12 was mainly from the $Ly49D^+$ NK cells (73% of IL2 producing NK cells were $Ly49D^+$, 79% of IL12 producing NK cells were $Ly49D^+$). IFNγ production was not dependent on the expression of Ly49D receptors with 60% of IFNγ positive NK cells not expressing Ly49D.

CONCLUSIONS: NK cells infiltrate cardiac allografts and this is followed by a marked down regulation of their Ly49 inhibitory receptors. We hypothesis that during allograft rejection, NK cells calibrate the expression of their receptors in order to promote inflammatory cytokine production as part of the rejection process. Whether this calibration is a result of cell death of those cells expressing redundant receptors or by modulation of the transcription of these receptor genes requires further investigation. These findings do however indicate that the Ly49D receptors may play a major role in the control of NK cell activity during allograft rejection.

O26

IS CLINICAL CARDIAC TRANSPLANTATION ASSOCIATED WITH ENDOTHELIAL ACTIVATION?

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Background: Brain death affects the right ventricle (RV) more than the left ventricle (LV). Furthermore, RV allograft failure is a common cause of peri-operative morbidity and mortality. Our objectives were: 1) To describe the pattern of endothelial cell activation (ECA) in the RV and LV of the human heart across clinical transplantation. 2) To correlate ECA with cardiac function before and after implantation.
Methods: 49 donor hearts had trucut biopsies from the RV and LV at: initial assessment of the donor, end of implantation and after 10 min. of reperfusion. Follow up RV biopsies during rejection surveillance at 1 week, 1 month and 3 months postoperatively were also included. Six of the patients were cystic fibrosis domino donors (free of brain stem death). Six hearts from brain-dead donors were turned down on account of poor function, in the absence of any structural disease. P-selectin, VCAM-1, E-selectin and thrombomodulin (Thr) were examined with immuno-histology. Post-operative biopsies showing rejection were excluded from the analysis. Nine patients undergoing routine cardiac surgery served as controls.
Results: There was no differences between the RV and the LV at any of the intraoperative time points, but important time-dependent variations were seen. P-selectin, and VCAM-1 (but not E-selectin) were up-regulated in brain-dead and in domino donors. Thr. was reduced at baseline in all hearts used for transplantation, and the depletion accentuated post-operatively. P-selectin was present in 85% of vessels throughout transplantation and decreases to 60% post-transplant ($p<0.001$). VCAM-1 was present in 20% of vessels initially, decreasing to 5% during storage (this fall was inversely correlated to ischaemic time), increased to 47% at reperfusion and gradually decreased thereafter ($p<0.001$). E-selectin expression increased progressively from 15% initially to 45% at reperfusion to gradually decrease post-operatively ($p=0.001$). Unused donor hearts showed up-regulation of P-selectin and VCAM-1 too, but significantly less than usable organs. There was also a trend towards further Thr. depletion. Patients with donor organ failure did not have a specific pattern of ECA, but had a trend towards accumulation of clinical risk factors. Recipients of aprotinin had reduced expression of E-selectin and VCAM-1 in the LV at reperfusion.
Conclusion: Cardiac transplantation (including domino) is associated with marked type I and type II ECA, with no inter ventricular differences. Noted changes reached a peak at reperfusion and persisted in the post-operative period even in the absence of rejection. Expression of ECA markers was influenced by administration of aprotinin but was not predictive of donor organ failure. Strategies to modify the acute inflammatory response may have a favourable impact on ECA expression improving the outcome of cardiac transplantation.

O27

THE EFFECT OF INTRAPORTAL INFUSION OF PROSTAGLANDIN E1 ON SERUM LEVELS AND HEPATIC EXPRESSION OF ICAM-1 IN WARM ISCHAEMIA REPERFUSION INJURY

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Prostaglandin E1 (PgE1) may have a role in ameliorating hepatic ischaemia-reperfusion injury. However, many studies administered PgE1 intravenously where it is rapidly metabolised, and usually in the post-reperfusion period when the cascade of ischaemia-reperfusion injury is already well established. This study has investigated the cytoprotective effect of PgE1 administered intraportally. In addition, since leukocyte-endothelial cell adhesion has been strongly implicated in the initiation of ischaemia-reperfusion injury, we also studied intracellular adhesion molcule-1 (ICAM-1) expression to assess if PgE1 acted through this pathway.
Methods: 20 mongrel canine livers were subjected to warm hepatic ischaemia by 60 min of in situ inflow obstruction with continuous intraportal infusion of PgE1 1 mg\kg\hr in lactated Ringers solution (n=10) or equal volumes of lactated Ringers solution (controls, n=10). The infusion was administered before the initiation of ischaemia, during the ischaemic phase, and for 30 minutes of the reperfusion phase. Hepatic venous blood and liver biopsies were taken at baseline, at 60 min ischaemia and at 30 min reperfusion. The blood samples were assessed for liver enzymes, TNF-a, IL1b, sICAM-1, while the liver biopsies were assessed histologically for ischaemia-reperfusion injury and immunohistochemically for ICAM-1 expression.
Results: PgE1 treatment significantly reduced the rise in AST, ALT, TNF-a and serum ICAM-1 following ischaemia and reperfusion ($P<0.05$). It did not reduce IL-1b. Biopsies from the PgE1 treated group displayed that PgE1 reduced the degree of hepatocyte necrosis, sinusoidal congestion during both the warm ischaemia and reperfusion phase when compared with controls ($P<0.05$). These changes were associated with significant down regulation of ICAM-1 ($P<0.05$).
Conclusion: This study demonstrates that intraportal PgE1 infusion prior, during and immediately following warm hepatic ischaemia ameliorates ischaemia-reperfusion injury, and inhibits ICAM-1 expression and TNF-a secretion. We suggest that one of the mechanisms by which this cytoprotection is attained may be through ICAM-1 inhibition.

O28

STRESS PROTEIN PRE-CONDITIONING: IMPLICATIONS FOR HEPATOCYTE ACUTE PHASE PROTEIN PRODUCTION AND CELLULAR METABOLISM.

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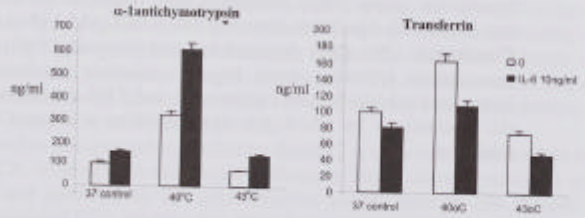
Introduction: Heat shock preconditioning offers theoretical potential to improve resistance of cells to ischaemia reperfusion injury. One limitation of this cell survival response is a temporary loss of differentiated cell function.
Aims: This study investigated the effect of heat shock pre-conditioning of hepatocytes on their production of secreted acute phase proteins and on cellular ATP stores.
Methods: Human Hep G2 cells were heated at 40 and 43°C for 45 minutes and allowed to recover at 37°C for variable periods of time. Cells were pulse-labelled with ^{35}S methionine and allowed to recover for 12 hours following heat treatment. Supernatants and cell lysates were harvested and trichloroacetic acid-precipitable ^{35}S methionine was counted by liquid scintillation and samples were run on SDS PAGE gels. Other cells treated in identical manner were harvested on ice and adenosine triphosphate (ATP) concentrations measured using a luciferase assay. Mitochondrial activity of cells was estimated using the Alamar blue reaction following 2 hour incubation. Acute phase proteins were measured in cell lysates by ELISA and probes were constructed for northern analysis of acute phase mRNA.
Results: Heat shock at 43°C was associated with decreased secreted and increased intracellular precipitable ^{35}S methionine counts. By contrast a febrile-range heat stress of 40°C was associated with increased secreted and intracellular protein compared with 37°C control cells. Changes in secreted acute phase proteins were consistent with the general effects on protein synthesis (fig). Heat shock was associated with increased mitochondrial activity and ATP depletion which was reversible by inhibitors of transcription or ribosomal translocation.



Conclusions: Exposure of hepatocytes to heat shock is associated with reduced APP production while at febrile range temperature APP secretion is augmented. Heat shocked cells may adopt a defensive phenotype sacrificing normal differentiated cell function in favour of elaboration of molecular chaperones (heat shock proteins) to correct protein miss-folding. Pre-conditioning stimuli which utilize lesser or adjunctive chaotropes may preserve differentiated cell function and may offer more benefit in the context of transplantation.

Medawar Medal Papers

Thursday 18th April 2002

09:00 – 11:00

O29

OUTCOME OF *EX SITU* SPLIT LIVER TRANSPLANTATION

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Background
Split liver transplantation (SpLT) has become an accepted method of increasing the number of available grafts. The best technique of splitting the liver, however, remains debatable.

Methods
The outcome of 154 consecutive SpLTs (100 children, 54 adults) performed at our centre between 1991 and 2001 was analysed. The median age and weight at transplantation was 9 years (range, 5 days to 68 years) and 23.5 kg. (range, 2.3 to 100 kg.) respectively. Twenty-five (16.2%) transplants were performed for acute liver failure (ALF) and 14(9.1%) were retransplants. A total of 81 livers were split and *ex situ* splitting was used in all but 4 grafts. Fifteen grafts were exported (14 right lobes, RL and one left lateral segment, LLS) and 7 imported (3 RL, 4 LLS). The median post transplant follow up was 40 months (range 4 to 123 months).

Results
The Kaplan-Meier patient survival rates for the entire cohort at one, 3 and 5 years were 89.4%, 81.9% and 80.6% and the corresponding graft survival rates were 86%, 77.7% and 74.8% respectively. Nine (5.8%) patients (8 children, one adult) required retransplantation, 4 for hepatic artery thrombosis (HAT), 2 for chronic rejection and one each for biliary sepsis, arterio-portal fistula and de novo autoimmune hepatitis. Two of these retransplantations were performed with further SpLT. The patient and graft survival rates by subgroup are tabulated below.

Patient survival rates				
	RL	LLS	Children	Adults
1 year	86.9	91.5	92.7	83.2
3 year	79.1	84.3	87	72.7
5 year	76.4	84.3	85	72.7

Graft survival rates				
	RL	LLS	Children	Adults
1 year	85.6	86.5	87.6	83.2
3 year	74.2	81.1	82.1	69.7
5 year	71.6	77.9	77.5	69.7

There were 25 (16.2%) deaths (12 adults and 13 children). Fourteen deaths occurred in RL recipients. Vascular complications occurred in 14 patients including HAT in 7 (4.5%; 5 RL and 2 LLS) and portal venous thrombosis in 4 (2.6%; all LLS). Biliary complications occurred in 16 (10.4%) patients (10 RL, 6 LLS).

Conclusions
These results, which represent the experience of a single institute over the last 11 years, indicate that ex situ split liver transplantation can be performed in both children and adults with good overall outcome and acceptable morbidity.

O30

CHARACTERISATION OF GENE EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM RENAL TRANSPLANT RECIPIENTS WHO DEVELOPED STEROID WITHDRAWAL SYNDROME

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We have previously reported that late steroid withdrawal is not associated with a higher incidence of acute rejection, chronic allograft nephropathy (CAN), or graft loss at one year. However, we found that 8 of 44 (~20 %) of patients develop polyarthralgia termed "steroid withdrawal syndrome" (SWS). As assessed by cortisol response to synthetic ACTH (short Synacthen test) SWS is associated with biochemical hypoadrenalism.
Our original hypothesis was that those individuals who develop SWS would have an associated up-regulation of pro-inflammatory cytokines. Therefore, we have investigated peripheral blood mononuclear cells (PBMCs) from 5 patients with SWS and 2 patients who did not develop symptoms following steroid withdrawal using cytokine gene arrays to assess the expression of 268 cytokines, cytokine receptors and growth factors.
Unexpectedly, patients with SWS showed predominant downregulation of gene expression (Fig. 1) as 9/10 genes showed changes in >3 (of 5 investigated) patients were down >2 fold in respect to the pre-withdrawal levels. The majority of down-regulated genes in SWS are growth factors or their receptors (macrophage colony-stimulating factor I receptor, osteoblast specific factor 1 (OSF1), axl oncogene, nerve growth factor receptor, insulin-like growth factor-binding protein acid labile, TRK-T3 oncoprotein). In contrast, the main finding in patients with no symptoms is up-regulation of gene expression after steroid withdrawal (Fig. 1), as IL-1β, IL-8, TNFβ, stem cell tyrosine kinase 1 and cytokine receptor EBI3 were all upregulated >2 fold when compared to their levels of expression during steroid treatment.
In conclusion, painful joints and muscles and general malaise in SWS are unlikely to be caused by increased levels of cytokines. Furthermore, the physiological levels of steroids seem to be important in regulating expression of various growth factors and thus may have positive effects on metabolism and activity of osteoblasts, myocytes and other cell types. This is in contrast with the effects of high, pharmacological doses of steroids that cause bone loss. Paradoxically, sub-physiological levels of steroids, such as in SWS, also seem to cause imbalance in osteoblast/osteoclast activity and polyarthralgia symptoms.

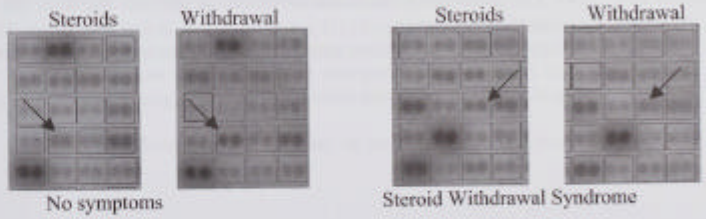

Figure 1. Representative sections of gene arrays show changes in gene expression after steroid withdrawal in patients without symptoms, up-regulation of IL-1β, and in SWS, down-regulation of OSF1 (arrows).

O31

GENETIC SUSCEPTIBILITY FACTORS FOR CICLOSPORIN INDUCED GINGIVAL OVERGROWTH IN RENAL TRANSPLANT PATIENTS.

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Introduction: Gingival overgrowth is a side effect experienced by at least 30% of renal transplant patients receiving ciclosporin. This immunosuppressive drug alters the production of cytokines involved in the fibrotic process, including TGFβ1, IL-10 and IL-6. Genotypes associated with polymorphic sites present in these cytokine genes have been shown to correlate with differential protein expression. CYP3A4 is involved in ciclosporin metabolism and although levels of this enzyme have been shown to vary up to forty fold between individuals, little is known about the functional importance of polymorphisms within the gene.

Hypothesis: Patients taking ciclosporin post transplant are at risk of developing overgrowth if they carry particular genetic variation on the molecules involved in a) fibrotic processes and b) ciclosporin metabolism.

Methods: Renal transplant recipients (152 consecutive cases) on ciclosporin monotherapy were genotyped for the following polymorphisms: TGFβ1-codon 10 (Leucine/Proline) and codon 25 (Arginine/ Proline), IL-10 (–1082, -819 and –592), IL-6 (–174) and CYP3A4 (–392). CYP3A4 gene screening was performed using WAVE Maker technology (Transgenomics, UK). PCR-SSP methods were developed and patient and control populations genotyped for all polymorphisms tested. Each subject underwent a dental examination at 12 months post transplant and severity of overgrowth was scored from plaster models. Patients with equivocal overgrowth were excluded from the study (n=23).

Results: The TGFß1 C allele at codon 25 (p=0.05), and the GC genotype at IL-6 –174 were significantly increased (p= 0.00057) in patients with overgrowth (n=62), compared to patients with no overgrowth (n=67). Three previously unreported single nucleotide polymorphisms were detected in Exon 4, Intron 6 and Intron 10 of the CYP4A4 gene at positions 11451 (A→G), 14316 (G→C), and 20230 (G→A) respectively. The GA genotype at position 20230 was significantly decreased (p=0.03) in the patients with gingival overgrowth compared to the patients with no overgrowth.

Conclusion: We have identified genetic markers that significantly influence susceptibility to ciclosporin-induced gingival overgrowth.

- The presence of proline at codon 25 of the TGFß1 gene and the IL-6 GC genotype increase susceptibility.
- The presence of the A allelic variant at position 20230 of the CYP3A4 gene confers protection.

Screening individuals for these genetic markers will enable identification of the patients most at risk, prior to ciclosporin treatment. This would allow these individuals the option of immunosuppression tailoring or an oral hygienist treatment programme.

This work was supported by the Sir Jules Thorn Charitable Trust

O32

REGULATORY T CELLS GENERATED BY ANTI-CD154 mAb THERAPY SUPPRESS $CD8^+$ T CELL-MEDIATED REJECTION.

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Introduction. CD40 Ligand (CD154) delivers a co-stimulatory signal pivotal to T cell-dependent immune responses. Blockade of CD40-CD154 interactions using an anti-CD154 mAb facilitates long-term allograft survival in several rodent and primate models. Although there is compelling evidence that $CD8^+$ T cells are resistant to CD154-blockade, it has recently been shown that following transplantation, anti-CD154 mAb therapy enables the generation of regulatory/suppressor T cells. The aim of this study was to determine whether regulatory cells that develop in anti-CD154 mAb-treated recipients can inhibit $CD8^+$ T cell mediated rejection.

Results. We have previously demonstrated that fully MHC mismatched C57BL/10 (B10; $H2^b$) cardiac allografts survive indefinitely (> 100 days) in CBA/Ca (CBA; $H2^k$) recipients following administration of an anti-CD154 mAb at the time of transplantation (MR1; 500µg i.p. on day 0,2,4). Adoptive transfer of $50x10^6$ unsorted splenocytes, from anti-CD154 mAb-treated CBA recipients that had accepted B10 cardiac allografts for over 100 days into naïve secondary syngeneic CBA recipients prolonged the survival of donor-type B10 cardiac allografts (MST >100 days; n=9) but not NZW ($H2^z$) grafts (MST 8 days; n=4).

Next we investigated whether these donor alloantigen-specific regulatory cells had any influence on $CD8^+$ T cell-mediated rejection using an *in vivo* skin allograft model, where rejection is mediated solely by $CD8^+$ T cells specific for MHC class I alloantigen $H2K^b$. (MST 12 days; n=5). When regulatory cells from anti-CD154 mAb–treated recipients were co-transferred with the $H2K^b$-specific $CD8^+$ T cells, B10 skin graft rejection was prevented (MST >50 days; n=3). Cells with the capacity to prevent $CD8^+$ T cell-mediated skin allograft rejection were confined to the $CD4^+$ T cell population; no evidence of suppressive activity was found in the $CD8^+$ T cell subset.

Conclusion. These data demonstrate that following transplantation, anti-CD154 mAb therapy generated a population of $CD4^+$ regulatory T cells specific for donor alloantigen that can suppress the rejection response initiated by naïve $CD8^+$ T cells. The ability of $CD4^+$ regulatory T cells to control anti-CD154-resistant allo-aggressive $CD8^+$ T cells has important implications for the design of CD154-mAb-based protocols.

O33

ANTI-CD40L ANTIBODY EFFECTS ARE FC REGION DEPENDENT: SELECTIVE DEPLETION OF ACTIVATED T CELLS RATHER THAN COSTIMULATION BLOCKADE

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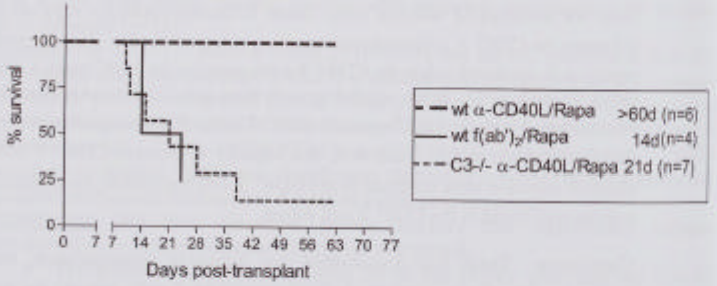
Although the underlying mechanisms are not well understood, it is generally believed that antigen recognition by T cells in the absence of costimulation may alter the immune response, resulting in non-responsiveness. In animal models of autoimmunity and transplantation, treatments based on costimulation blockade, in particular anti-CD40L Ab, have been highly effective, lending further support to this concept. In this study we have investigated the mechanisms of action of anti-CD40L Ab and provide evidence that its effects are Fc region dependent. We transplanted tail skin from BALB/c to C57BL/6 mice and recipients were treated with 250µg of anti-CD40L Ab on days 0, 2, 4, 7 and 10 in addition to 3mg/kg of Rapamycin on days 0-13. This treatment prolonged allograft survival (MST >60 days, n=6) compared to untreated recipients (MST=8 days), figure 1. When anti-CD40L Ab was replaced with $f(ab')_2$ fragment and used in combination with Rapamycin, allograft survival reduced dramatically (MST=14 days, n=4). Importantly, anti-CD40L Ab and Rapamycin treatment of C3 deficient recipients also showed decreased allograft survival (MST=21 days, n=7). These data provide further evidence to suggest that anti-CD40L Ab is acting via Fc-mediated mechanisms that are complement dependent and/or involve ADCC.



To verify the role of C'-mediated mechanisms we assessed the ability of the hamster anti-mouse CD40L Ab to fix mouse C' and trigger deposition of C' components on the surface of CD40L-expressing cells. Both C3c and C3d were deposited on these cells in the presence of anti-CD40L Ab. Importantly, the $f(ab')_2$ fragment did not fix C'. These data imply that anti-CD40L Ab acts through Fc-dependent mechanisms suggesting the selective depletion of activated T cells rather than by costimulation blockade, as currently postulated. This finding opens new avenues for the treatment of immune disorders based on the selective targeting of activated T cells.

O34

COMPARISON OF ALLOGENEIC AND XENOGENEIC IN VITRO T-CELL PROLIFERATIVE REPSONSES OF SENSITISED PATIENTS AWAITING KIDNEY TRANSPLANTATION

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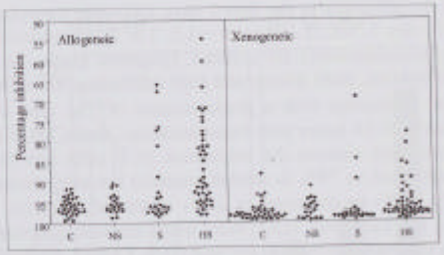
Introduction: Patients who have broadly reactive HLA-specific antibodies (HLA-Ab) have a low probability of receiving a cross match negative human organ donor transplant and might be considered as candidates for future xenotransplant clinical trials. Human and porcine MHC share a high degree of structural homology, giving rise to the possibility of inter-species crossreactivity. We have investigated cyclosporin (CyA) resistant proliferative T-cell responses of non-sensitised and sensitised patients awaiting kidney transplantation against human and porcine lymphocytes.

Methods: *In vitro* T-cell proliferation responses of 6 non-sensitised (NS), 7 sensitised (S) (IgG PRA 11-84%), 14 highly sensitised (HS) patients (IgG PRA>84%) and 12 normal human controls (C) were tested in an MLR against stimulator cells from 3 humans (allogeneic MLR) and 3 pigs (xenogeneic MLR). Cultures were performed in the presence and absence of CyA (100ng/ml and 500ng/ml) to identify secondary responses that are refractory to CyA immunosuppression.

Results:



There was no difference between allogeneic and xenogeneic MLR responses between controls, non-sensitised patients and sensitised patients in the absence of CyA. In the presence of 100 ng/ml CyA, all allogeneic MLR responses of controls (N=30) and non-sensitised patients (N=18) were inhibited by >90% (Fig.). In contrast, allogeneic responses of 15/21 (71%) sensitised and 21/42 (50%) highly sensitised patients were inhibited by >90% (C/NS vs. S/HS $p<0,01$). For xenogeneic MLRs, 35/36 (97%) of controls, 18/18 (100%) non-sensitised, 18/21 (86%) sensitised and 36/42 (86%) highly sensitised patient responses were inhibited by >90%.

Conclusion: Lymphocytes from sensitised patients show CyA resistant *in vitro* proliferation to allo-antigens. In contrast, proliferative T-cell responses to xenogeneic stimuli were more easily suppressed by CyA, suggesting that sensitised patients may not be at increased risk of T-cell mediated xenograft rejection.

O35

NATURAL KILLER CELL ACTIVITY AGAINST ALLOGRAFTS: MODULATION BY CO-STIMULATORY BLOCKADE

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BACKGROUND: Natural Killer (NK) cells are emerging as an important component in the rejection process. They do not express CD4 or CD8 surface markers and are therefore unlikely to be affected by monoclonal antibody (mAb) treatment against these two molecules. In common with T cells, they express surface molecules involved in the co-stimulation pathway. Here we investigated the effects of co-stimulatory blockade on NK cell activity against fully vascularised cardiac allografts.
METHODS: Donor CBA ($H2^k$) heart grafts were transplanted into C57BL/6 ($H2^b$) recipient mice. Kinetics of lymphocyte infiltration into the grafts were examined by 2-colour flow cytometry using anti-CD49b (Clone DX5, a pan-NK cell marker), anti-CD4 and CD8 mAb. Intracellular cytokine staining for IFNγ, IL2, IL4, IL12 and TNFα was performed on splenic and graft infiltrating NK cells. T cell suppression was achieved by administering 2 x 100ug doses of anti-CD4 and CD8 mAb 27 and 28 days before transplantation in combination with $5x10^6$ donor bone marrow cells (BMC). Co-stimulation was blocked by intra-peritoneal administration (500ug) of anti-CD154 mAb (MR1) on day of transplant. Lymphocytic infiltration into the graft and their production of cytokines were compared in treated vs naive or control antibody treated animals.
RESULTS: A higher proportion of NK cells compared to CD4+ and CD8+ T cells infiltrated the allografts in the first 3 days following transplantation with a reversal of this ratio by day 7. NK: T cell ratio = 1.5, 1.4, 2, 0.5 respectively at days 1, 3, 5,and 7 days after transplantation). By contrast, syngeneic grafts did not show any significant NK cell infiltration. Both splenic and graft infiltrating NK cells showed a similar pattern of cytokine expression with a predominance of Th1 type cytokines (IFNγ, IL2 and IL12) as early as 24 hours post transplantation. Anti-CD4, CD8 mAb and donor bone marrow treatment reduced the infiltration of T cells into the allografts dramatically (73% in untreated vs 79% in treated animals) but only decreased NK cell infiltration moderately (5.8% in untreated vs 2.2% in treated animals). However, following co-stimulatory blockade with MR1, there was a marked reduction of both NK and T cells infiltrating the grafts (0.3% NK cell and 0.6% T cells). Intracellular cytokine staining of NK cells was also significantly reduced following MR1 treatment but not after anti-CD4, CD8 mAb and donor BMC. (e.g. percent of NK cells staining positive for IL12 was 32% in naive animals compared with 8.2% in MR1 treated and 29% in anti CD4, CD8 and donor BMC treated mice).
CONCLUSIONS: NK cells infiltrate allografts earlier than T cells and can secret Th1 type cytokines which may have an important effect on subsequent T cell alloreactivity. The activity of NK cells is only moderately influenced by anti T cell treatment but co-stimulatory blockade with anti CD154 mAb had a profound effect on NK cell infiltration and cytokine production. This may offer a potential avenue for therapeutic intervention against NK cell alloreactivity.

O36

CHRONIC ALLOGRAFT REJECTION: GENOTYPE/PHENOTYPE CORRELATES FOR IL1β BIOACTIVITY.

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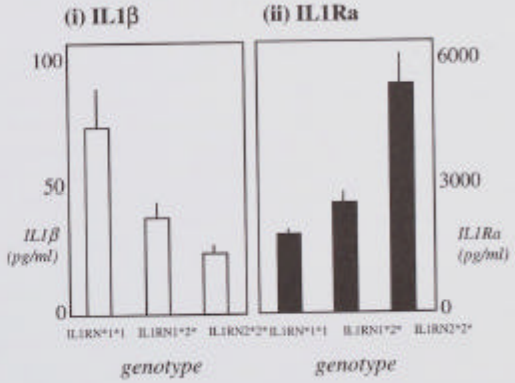
Introduction. Interleukin 1 beta (IL1β) is a proinflammatory cytokine involved in chronic rejection (CR) of organ allografts. Multiple mechanisms regulate IL1 bioactivity and we have demonstrated an association between CR and a VNTR* polymorphism of the IL1 receptor antagonist gene (*IL1RN*) in clinical heart graft recipients. To test this association further, we looked for genotype-phenotype correlates in IL1β bioactivity.
Methods: Peripheral blood mononuclear cells from 50 volunteers were genotyped for two single nucleotide polymorphisms (SNPs) in the *IL1β* gene, and one VNTR polymorphism of the *IL1RN* gene. Phenotypic responses to LPS were identified by FACS analysis of intracellular IL1β and IL1 receptor antagonist, and by quantifying secreted IL1β and IL1 receptor antagonist protein levels by ELISA.
Results: The IL1β+3953/IL1RN VNTR haplotype proved a strong, independent predictor of systemic IL1β levels; this haplotype was identical to that which correlated with CR in heart graft recipients. In vitro, the IL1RN*1 allele of the IL1RN VNTR polymorphism was consistently associated with lower levels of IL1 receptor antagonist and IL1Ra/IL1β ratios in an allelic dose dependent manner. Fig 1 below shows levels of (i) IL1 and (ii) IL1Ra measured in the supernatants of cells taken from individuals, according to their IL1RN genotype.



Conclusion: The IL1RN genotype emerged as the principle genetic regulator of IL1β bioactivity, influencing (a) constitutive IL1β and IL1Ra release and (b) the positive feedback loop of IL1β synthesis. The resultant modulation of the IL1β/IL1Ra ratio differed according to stage of monocytoc differentiation. This is the first report of genetic regulation of IL1β lying outside the IL1β gene. ** VNTR : variable number tandem repeat*

Parallel Session 2(a)

Immunosuppressive Therapy

Thursday 18 April

16:00 – 17:30

O37

VARIABILITY OF CYCLOSPORIN (CsA) EXPOSURE AND ITS RELEVANCE TO CHRONIC ALLOGRAFT NEPHROPATHY (CAN): A CASE-CONTROL STUDY

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Background. The effect on late graft loss of CsA-based immunosuppression has been limited in comparison to the substantial improvements in short-term outcome that were seen at the time of its introduction. It may be that CsA has both beneficial and detrimental effects which tend to balance out, or that CsA fails to prevent CAN, a major cause of late graft loss. For individual patients however, there is some evidence that the degree and variability of CsA exposure may be of prognostic importance. We have performed a case-control study to examine further the contribution of these and other factors to the development of CAN in renal transplant recipients receiving follow-up care in our unit.

Methods. The electronic record was interrogated to identify adult CsA-treated renal transplant recipients with chronic progressive renal allograft dysfunction secondary to CAN (group A, n = 35), and CsA-treated controls with stable graft function over at least 4 years post-transplantation and a serum creatinine of less than 200 umol/l (group B, n = 67). Study exclusion criteria included non-compliance with immunosuppressive medication (n = 3). Age at transplant, gender, years post-transplant, donor source and age, kidney preservation time, HLA match, occurrence of delayed graft function, immunosuppressive regimen, weight-adjusted maintenance CsA dose and coefficient of variation ($CvarC_0$) of dose-adjusted CsA trough blood levels ($C_0/dose$) were recorded. $CvarC_0$ was calculated using the formula:

$CvarC_0$ = (SD[$C_0/dose$]/ mean[$C_0/dose$]) x 100

Chi square and unpaired t-tests were used to compare variables in A and B. In addition, a binary logistic regression analysis was performed, using a forward conditional sequential (stepwise) method of entering study variables into a computerised model (SPSS version 9.0).

Results. A total of 102 adult renal transplant recipients were included in the study. Recipient age (A<B, p<0.001), $CvarC_0$ (A>B, p<0.01) and weight-adjusted CsA dose (A>B, p<0.02) were significantly different by univariate analysis. Only low recipient age (p<0.001) and a high $CvarC_0$ (p<0.02) were independent predictors of CAN according to the regression model.

Discussion. Our data suggest that younger renal transplant recipients and those with variable CsA exposure are at greater risk of developing CAN. It may be helpful to identify such patients with a view to employing alternative immunosuppression strategies.

O38

THE EFFECT OF CYCLOSPORINE AND RENAL WARM ISCHEMIA ON VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION AND ITS RECEPTORS VEGF-R_1 AND VEGF-R_2

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Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen involved in physiological and pathological angiogenesis. VEGF has potent effects on vascular permeability and regulates angiogenesis through stimulating endothelial cell proliferation. The response to VEGF is mediated by binding to type III tyrosine kinase receptors termed VEGF-R_1 (formerly known as Flt_1) and VEGF-R_2 (formerly known as Flk_1). At present, little information is available concerning the effect of cyclosporine (CsA) or renal warm ischemia (RWI) on the expression of VEGF and its receptors in the kidney.

The left renal pedicle of male Sprague-Dawley rats (200-300g) was clamped for either 30 min or 60 min under halothane anaesthesia and kidney tissue removed after 30 days (n=6 per group). The study was repeated in animals receiving CsA 15mg/kg/day ip (n=6 per group). The expression of VEGF and its receptors were determined by immunohistochemistry in the glomerular and tubulointerstitial (TI) spaces using the following antibodies VEGF (C-1) mouse monoclonal IgG_{2a}, Flk-1(A-3) mouse monoclonal IgG_1, Flt-1(H-225) rabbit polyclonal IgG (Santa Cruz, California). The results are expressed as mean ± sem and compared by the non-parametric Mann-Whitney test.

VEGF expression was significantly reduced by treatment with CsA 15 mg/kg/day in both the TI (CsA: 17.1 ± 1.7% vs Control: 28.8 ± 3.4%, p <0.05) and the glomerular (14.1 ± 1.1% vs 7.5 ± 0.6%, p< 0.05) spaces compared to vehicle-treated animals. Kidneys subjected to RWI for either 30 or 60 min also showed a decrease in the TI and glomerular expression of VEGF. The combination of RWI and CsA had an effect comparable but not additive to either intervention.

The expression of VEGF-R_1 was also significantly reduced by treatment with CsA 15mg/kg/day in both the TI (CsA: 7.5 ± 1.7% vs Control: 11.8 ± 0.9%, p< 0.05) and glomerular (5.1±1.7% vs 9.6±1.1 %) spaces. However, in kidneys subjected to RWI, the expression of VEGF-R_1 did not decrease until the duration of the ischaemic insult was increased from 30 to 60 min. In rats treated with CsA 15 mg/kg/day, RWI 60 min was also associated with a decrease in the expression of VEGF-R_1, while interestingly, RWI 30 min was able to prevent the inhibitory effect of CsA administration. Similar results were obtained for the expression of the VEGF-R_2 receptor.

These findings suggest that cyclosporine can down-regulate the renal expression of both VEGF and its cell membrane receptors. Although VEGF and its receptors can also be down-regulated by renal warm ischaemia, this effect appears to be a function of the duration of the ischaemic insult.

O39

PRIMARY IMMUNOSUPPRESSION WITH TACROLIMUS IS ASSOCIATED WITH A REDUCTION IN RENAL ALLOGRAFT FIBROSIS COMPARED WITH NEORAL THERAPY

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Aims: Long-term patient and allograft survival is influenced by multiple aetiological factors that can accelerate the progression of chronic allograft nephropathy (CAN) and cardiovascular disease. This study aims to evaluate the results of a single centre randomised prospective trial comparing the safety profile of Tacrolimus versus Neoral based primary immunosuppression one year following renal transplantation and the association of these risk factors on renal allograft fibrosis.

Methods: 102 consecutive renal transplant patients were randomised to receive microemulsion cyclosporin (Neoral) or Tacrolimus based immunosuppression in conjunction with steroids. Patients were assessed for one year for delayed graft function, acute rejection glomerular filtration rate, lipid profiles, hypertension and glucose intolerance. All patients were invited for a renal biopsy at one year to quantify fibrosis using picro sirius red staining.

Results: There was no significant difference in the incidence of acute rejection or steroid resistant rejection between the two agents in this study (Tacrolimus 34.8% versus Neoral 31.9%, P=0.77). Tacrolimus was associated with a significant reduction in the duration (15 days versus 21 days in the Neoral group, P<0.035) but not the frequency of delayed graft function (P=0.83). The incidence of post transplant diabetes was higher in the Tacrolimus group but this was not significant (Tacrolimus 6.4% versus Neoral 3.2%, P=0.5). Neoral was associated with a significant increase in total cholesterol (P<0.03) and LDL levels (P<0.02). There were no significant differences in blood pressure between the two groups. However, Tacrolimus was associated with a significant reduction in extracelluar matrix deposition, mean value 14.18 (range 6.73-28.57) versus Neoral, 23.50 (6.3-59.81) at one year-P<0.002.

Conclusion: Despite different side effect profiles, Tacrolimus is associated with decreased allograft fibrosis at one year. Long-term prospective randomised controlled trials are warranted to assess if Tacrolimus should be established as primary immunosuppression to prolong long-term allograft survival.

O40

RESULTS OF RENAL TRANSPLANTATION USING VERY SHORT STEROID TREATMENT WITH TACROLIMUS AND MMF

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The use of the combination of steroids with Tacrolimus is associated with high levels of *de novo* diabetes in renal transplant recipients. Previous data from Chicago using short course steroids with Tacrolimus and MMF have shown good results in terms of patient and graft survival with a low incidence of *de novo* diabetes. We have started using a short-course initial steroid regime (1mg/kg Prednisolone tapered to zero over 1 week), combined with Tacrolimus and MMF. The first 60 renal allograft recipients under this regime (16 live donor, 44 cadaveric, 57 first graft, 3 second or subsequent graft) were prospectively analysed in terms of patient and graft survival, graft function, rejection rate and grade, weight gain, and *de novo* diabetes. Current mean follow-up time is 8 months (range 1 to 17).

Results:
Patient and graft survival remains 100%.

The overall rejection rate is currently 18%. The rejection rate at 6 months (in the 40 patients on whom 6 month data is available) was 17.7% and at one year (in the 11 patients with 1 year follow-up) was 18.1%. Of the 11 total rejection episodes 2 were classified as mild or borderline, 7 as tubulointerstitial, and 2 as vascular by Banff criteria. All rejection episodes were successfully reversed with IV pulsed methyl-prednisolone followed by re-introduction of oral steroids to the immunosuppressive regime.

Estimated mean GFR (by Cockroft & Gault formula) was 61.9 ml/min at 6 months (95%CI 53 to 70) and 66.7 at one year (95%CI 45 to 88). Trough Tacrolimus levels averaged 9.8 ng/ml at 1 month, 9.9 ng/ml at 6 months, and 9.8 ng/ml at 12 months.

Patient weights did not increase significantly following transplantation at six months (mean weight gain 1.4kg 95%CI 6.2 to −3.4kg) or 12 months (mean weight gain 1.15 kg 95%CI 6.5 to −4.2).

The incidence of *de novo* diabetes was very low with 0/11 new diabetics in the patients with 1 year follow up and 1/40 at 6 months.

Conclusions: The use of a very short-course steroid regime in combination with Tacrolimus and MMF appears to allow renal transplantation with excellent results in terms of graft and patient survival without the disadvantages of long-term steroid usage or the need to use very high Tacrolimus dosing to achieve low rejection rates.

O41

SYNERGISTIC EFFECTS OF RAPAMYCIN AND TACROLIMUS ARE PREFERENTIAL TO ITS COMBINATION WITH CYCLOSPORIN IN THE DEVELOPMENT OF INTIMAL HYPERPLASIA

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Aims: Allograft vasculopathy, a central feature of chronic rejection in all solid organ allografts, remains the leading cause of late graft failure following heart transplantation. The anti-proliferative drug sirolimus has been shown to inhibit the development of intimal hyperplasia, a central feature of allograft vasculopathy. This study aims to assess the efficacy of dual combination of sirolimus and calcineurin inhibition on the development of intimal hyperplasia, reflective of modern clinical immunosuppressive practice.

Methods: Male Sprague-Dawley rats were assigned to receive sirolimus (0.05mg/kg/day) and either tacrolimus (0.1mg/kg/day) or cyclosporin (5mg/kg/day) and compared to an untreated control group. All animals underwent left common carotid artery balloon angioplasty and were explanted at 2 weeks. Morphometric analysis was performed on representative transverse sections and intima medial ratios calculated using computer-aided planimetry. Pro-fibrotic gene expression was assessed with competitive RT-PCR at 2 weeks for metalloproteinase-2, metalloproteinase-9, TIMP-1, collagen III and TGF-β. Sections were stained with picro-sirius red and extracellular matrix deposition was quantified using an image intensifier.

Results: Sirolimus in combination tacrolimus was associated with the greatest reduction in intimal thickening, median intima medial ratio 0.68 (range 0.36-0.94) compared to both cyclosporin, 1.12 (range 1.06-1.68, P<0.032) and untreated controls, 1.47 (1.02-2.04, P<0.005). Both drug regimes significantly reduced the expression of pro-fibrotic growth factors. Both treatment groups significantly increased the accumulation of intimal extracellular matrix proteins compared to untreated controls. However treatment with sirolimus and tacrolimus significantly attenuated extracellular matrix deposition compared to sirolimus and cyclosporin (P<0.023).

Conclusion: The synergistic benefits of sirolimus in combination with tacrolimus are preferential over those observed with cyclosporin. Further experimental and clinical evidence is required to assess if tacrolimus should be the primary calcineurin-inhibitor of choice when used in combination with sirolimus.

O42

DIFFERENTIAL EFFECTS OF CALCINEURIN-INHIBITORS AND RAPAMYCIN ON THE DEVELOPMENT OF INTIMAL HYPERPLASIA

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Aims: Modern immunosuppressive agents such as tacrolimus and rapamycin are claimed to be associated with a reduction in vascular narrowing, a central feature of chronic rejection. This study assesses the effect of cyclosporin, tacrolimus and rapamycin on intimal thickening, fibrosis-associated genes and deposition of extracellular matrix in a model of intimal hyperplasia.

Methods: Male Sprague-Daley rats were a
Assigned to receive no treatment, cyclosporin 5mg/kg/day, tacrolimus 0.1mg/kg/day or rapamycin 0.05mg/kg daily. Animals underwent left common carotid balloon angioplasty and were explanted at 14 and 28 days post arterial injury. Morphometric analysis was performed on representative transverse sections and intima medial ratios calculated using computer-aided planimetry. Extracellular matrix accumulation was quantified using picro-sirius red staining. Pro-fibrotic gene expression was measured with competitive RT-PCR and metalloproteinase activity assessed using gelatin zympgraphy.

Results: Cyclosporin was associated with increased intimal thickening compared to controls (P<0.004). Tacrolimus had no effect on intimal thickening, whilst rapamycin significantly inhibited intimal thickening at 14 and 28 days (P<0.004 and P<0.026 respectively). All groups significantly inhibited MMP-2, MMP-9, TIMP-1, TGF-β and collagen III expression at 14 days (P<0.001) but increased ECM deposition. However rapamycin marginally reduced ECM deposition compared to cyclosporin (P<0.06).

Fibrotic Gene Expression	Control	Cyclosporin	Tacrolimus	Rapamycin	P-value
MMP-2	2.4 (2.1-2.7)	1.3 (1.1-1.6)	1.1 (0.9-1.4)	1.2 (0.8-1.4)	P<0.001
MMP-9	1.4 (1.0-1.6)	0.6 (0.4-0.7)	0.5 (0.3-0.6)	0.4 (0.3-0.6)	P<0.001
TIMP-1	1.6 (1.2-1.9)	0.7 (0.4-0.9)	0.6 (0.4-0.9)	0.6 (0.3-0.8)	P<0.001
TGF-β	1.8 (1.6-2.1)	0.6 (0.4-0.7)	0.6 (0.4-0.6)	0.6 (0.5-0.7)	P<0.001
Collagen III	1.5 (1.2-1.8)	0.6 (0.5-0.8)	0.7 (0.5-0.9)	0.7 (0.4-0.6)	P<0.001

Conclusion: Treatment with cyclosporin was associated with worsening of vascular narrowing, whilst rapamycin showed a beneficial reduction in intimal thickening. Tacrolimus had no effect on vascular narrowing compared to controls. Treatment with all immunosuppressive agents resulted in increased ECM deposition. Rapamycin may halt the rate of progression of vascular narrowing compared to both cyclosporin and tacrolimus.

O43

INTERLEUKIN-2 RECEPTOR MONOCLONAL ANTIBODIES IN RENAL TRANSPLANTATION: META-ANALYSIS OF RANDOMISED TRIALS

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Objective: To study the efficacy of interleukin (IL)-2 receptor monoclonal antibodies on rates of biopsy proven acute rejection, allograft loss and complications in renal transplantation.
Methods: We identified 8 randomised controlled studies involving 1816 patients who were followed for a minimum of 3 months. All studies were performed with patients who received IL-2 alpha receptor monoclonal antibodies (anti-TAC, BT563, Basiliximab or Daclizumab) in addition to standard cyclosporin A (CsA)(Neoral) based immunosuppression compared to patients treated with standard CsA based immunosuppression alone. A meta-analysis was performed to compare the following outcomes: incidence of acute rejection at 6 months following transplantation, graft survival, incidence of infections (including cytomegalovirus infections), malignancies and patient mortality.
Results: The addition of IL-2 receptor monoclonal antibodies to standard immunosuppression significantly reduced the risk of acute rejection (odds ratio (OR) 0.51: 95% Confidence Interval (CI) 0.42-0.62) (2P<0.00001). There was some reduction in graft loss at one year (OR 0.78: CI 0.58-1.05), although this was not significant (2P=0.09), and there was no difference in patient mortality (OR 0.75: CI 0.46-1.22) (2P=0.3). There was no difference in the overall incidence of infections (OR 0.97: CI 0.77-1.23) (2P=0.8) and a slight but insignificant reduction in Cytomegalovirus infections (OR 0.72: CI 0.64-1.05) (2P=0.1). There was no difference in the risk of lymphoma or other malignancies (OR 0.88: CI 0.43-1.82) (2P=0.7). The effect size of the reduction in graft loss was comparable in patients treated with CsA and prednisolone, with CsA, prednisolone and azathioprine, or with CsA, prednisolone and mycophenolate mofetil.
Conclusions: The addition of IL-2 receptor antibodies to standard CsA based immunosuppression results in a significant reduction in acute rejection at 6 months by 49%, and also reduces graft losses at one year by 22%, although this was not significant. This was achieved without any increase in infective or other complications or of death. Follow-up studies are needed to confirm whether IL-2 receptor antibodies improve long-term graft and patient survival.

O44

LONG TERM PROSPECTS FOR IMMUNOSUPPRESSION WITHDRAWAL AFTER SUCCESSFUL AUXILIARY TRANSPLANTATION FOR ACUTE LIVER FAILURE.

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Introduction: Auxiliary partial orthotopic liver transplantation (APOLT) has potential advantages over conventional transplantation in the treatment of patients with acute liver failure. The ability to withdraw immunosuppression after the native liver has recovered is perhaps the most important advantage for these patients.
Patients and methods: Twenty one patients (median age 14 .1 years; range 1-32) underwent APOLT and survived for greater than one month post-transplant. Of these, 10 were female, and the indications for transplantation included seronegative hepatitis in 8, paracetamol overdose in 7, drug induced in 2, hepatitis B in 2, autoimmune in 1 and mushroom poisoning in one. The decision to proceed with APOLT was based on several factors including the status of the donor liver, recipient age, clinical condition and macroscopic and histological assessment of the native liver. Twelve patients underwent APOLT with right lobe, 5 with left lateral segment, 2 with left lobe grafts and two using whole grafts. The median cold ischaemia time was 11.2 hours (range 6.3-17.2).
Results: Twenty one patients surviving more than 1 month after APOLT have a median follow-up of 32 months (range 1-91). Sixteen patients have a follow-up of greater than 20 months. One adult patient was retransplanted at 3 months for chronic rejection of the auxiliary graft. Fifteen patients have evidence of native liver regeneration and, of these, 8 have been withdrawn completely from immunosuppression over a period of 6-24 months post-transplant. One patient stopped taking immunosuppression abruptly and infarcted the auxiliary graft necessitating surgical excision. Grafts have atrophied in the other 7 patients following gradual immunosuppression withdrawal and there have been no other complications. Seven other patients are currently having their immunosuppression tapered and are well with stable liver function. One patient transplanted a month ago is well and is awaiting a protocol biopsy of the native liver to assess regeneration. Four patients have shown minimal or no regeneration of the native liver and are dependent on their graft and receive standard tacrolimus based immunosuppression.
Conclusion: APOLT allows for native liver regeneration and eventual immunosuppression withdrawal in the majority of patients surviving long term. APOLT is an important potential treatment for a selected group of patients with acute liver failure. Immunosuppression should be withdrawn gradually to allow the graft to atrophy. Surgical excision of the auxiliary graft is seldom required.

O45

MEASUREMENT OF COMPLIANCE POST RENAL TRANSPLANTATION USING ELECTRONIC MONITORING – THE EFFECT OF FEEDBACK AND TIME

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Non-compliance is a problem in all medical specialities and leads to apparent therapeutic failure. In transplantation it is thought to be responsible for the majority of late acute rejection episodes and up to one-third of cases of chronic graft dysfunction. This in turn leads to reduced graft survival and the patients are returned to the transplant waiting list.

Measurement of non-compliance is not easy due to its covert nature and most commonly used methods tend to under estimate the true extent. Electronic monitoring is the most accurate approach.

100 stable renal transplant patients (> 1 year post transplant) were approached and asked if they would use a "Smart Top" pill bottle (Aardex Switzerland) for their regular azathioprine/prednisolone for a 12 month period. The tops contain a microprocessor which records the date and time on each occasion the bottle is opened. This information can be downloaded onto a computer database via a special modem at their regular outpatient clinic visits.

The patients were randomly placed into two groups. Group 1 received feedback at their first outpatient visit and group 2 received no feedback throughout the course of the study.

Results are available on 48 of the patients, 23 patients received feedback and 25 did not. Overall over the 12 month period 40 (83%) missed at least one dose, 26 (54%) took extra doses and 11 (23%) missed consecutive doses. Only 3 (6%) patients were 100% compliant for the whole period.

Of the 23 patients who received feedback, 6 (26%) then improved, 9 (39%) worsened and 8 (35%) were no different after feedback.

In the group of 25 that did not receive any feedback, 5 (20%) improved, 10 (40%) worsened and 10 (40%) were no different after their first outpatient visit.

These patients were willing volunteers and were monitored on once daily medication. They therefore most likely represent our most compliant patients taking the regime with the best compliance rates. This may explain why there is no significant improvement in the group which received feedback.

In the subsequent monitored period there was a tendency for both groups to worsen their compliance the further both groups were from the study period. 48% (11) of the feedback group and 52% (13) of the non feedback group had poorer compliance than during the study period. This suggests that whilst they know they are being watched their compliance is better.

Parallel Session 2(b)

Alloimmunity II

Thursday 18 April 2002

16:00 – 17:30

O46

ANTIGEN PRESENTATION BY MOUSE CD4+ T CELLS AFTER ACQUISITION OF MHC: PEPTIDE COMPLEXES: A NOVEL MECHANISMS OF T CELL-MEDIATED REGULATION

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Antigen presentation by activated T cells to activated T cells has long been known to induce unresponsiveness in human and rat T cells. This may provide a mechanism limiting T cell clonal expansion during the course of an in vivo immune response. It has been assumed that no parallel mechanism operates in mice due to the inability of mouse T cells to synthesise MHC class II molecules. However, in this study we have observed extensive acquisition of MHC class II:peptide complexes by activated mouse $CD4^+$ T cells from antigen-presenting cells (APC). Using the 3A9 T cell hybridoma (specific for HEL $_{46\text{-}61}$ peptide/H2-A^k complexes) we observed the acquisition of HEL/H2-A^k complexes after exposure to peptide-pulsed H2-A^k-transfected cell lines, using the C4H3 antibody which specifically recognises these complexes. Parallel observations were made with T cell lines established from D011.10 mice (specific for OVA $_{323\text{-}339}$/H2-A^d complexes) and peptide-pulsed dendritic cells. Most importantly the 3A9 and D011.10 T cells that had acquired peptide:MHC complexes were competent APC for 3A9 or resting DO11.10 T cells. In keeping with the human and rat data bi-directional T:T antigen presentation by the antigen:MHC class II expressing DO11.10 T cells led to hyporesponsiveness to subsequent re-challenge. These findings raise the possibility that T:T antigen presentation may have immunoregulatory effects in vivo in mice.

O47

REGULATORY TOLERANCE IS ASSOCIATED WITH INDUCTION OF C-KIT, THE RECEPTOR FOR STEM CELL FACTOR.

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Introduction:
Regulatory tolerance to fully mismatched murine heart grafts (BALB/c to CBA) can be induced by a short course of non-depleting antibody therapy to block CD4 and CD8 co-receptor functions. Once tolerant, CD4+ve spleen cells from recipient mice show specific, dominant, and infectious tolerance *in vivo*. Analyses of these spleen cells *ex vivo* has demonstrated differences in signal transduction pathways compared to the rejection response.

Aim and Methods:
In order to identify critical determinants of tolerance, *versus* rejection, we have measured c-Kit, the receptor for stem cell factor (SCF), during tolerogenic responses of CBA (BALB/c-tolerant) spleen cells to irradiated donor-type (BALB/c) spleen cells. The kinetic profile of c-Kit induction was measured in parallel to that of STAT3, a downstream signal transduction factor responsive to SCF/c-Kit activation. Base line levels of c-Kit and STAT3 were measured at the time of stimulation with irradiated donor-type spleen cells (time zero): thereafter samples were prepared for measurement of STAT 3 and c-Kit at 16h; 24h; 48h; and then after restimulation with a further aliquot of irradiated responder cells (at 120h) at 120h (new baseline), 121h, 123h, 126h and 134h. A parallel kinetic profile was repeated for CBA spleen cells primed to reject a BALB/c skin allograft .

Results:
In contrast to rejecting cultures, those of tolerant cells responding to BALB/c donor antigen showed upregulation of both c-Kit and STAT3. This was specific for donor antigen: third party (C57Bl10) allo-antigen had little effect on the STAT3 and c-Kit levels in CBA spleen cells from BALB/c tolerant mice.

Conclusions:
This is the first report of regulatory tolerance being associated with signal transduction through the SCF - c-Kit - STAT3 pathway.

O48

CD40 COSTIMULATES A HUMAN CD4$^+$ T CELL ALLOPROLIFERATIVE RESPONSE IN THE ABSENCE OF B7

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Optimal proliferation of T cells, although initiated via ligation of the CD3/TCR complex, requires additional costimulatory signals. A large body of *in vivo* data clearly demonstrates prolongation of allograft survival by inhibiting both the B7/CD28 and CD40/CD40L pathways. However, whether CD40 can function as an independent costimulatory molecule is less clear. We have studied the role of CD40/CD40L by generating a panel of human fibroblasts expressing DR1 supratransfected with either human CD80, CD86 or CD40. These transfectants have been tested in functional assays with allogeneic CD4$^+$ T cells as responders. We demonstrated that CD40 is capable of costimulating allogeneic CD4$^+$ T cell proliferation and cytokine release in the absence of B7. On day 3 the dominant costimulation was provided by CD40, by day 5 this was overshadowed by costimulation provided by CD80 and CD86. Despite decreased proliferation by day 5 however, the provision of costimulation by CD40 was enough to expand a functional allogeneic T cell line. After 3 days co-culture with the three transfectants, CD4 cells demonstrated different surface receptor expression profiles for CD28 (high on cells co-cultured with CD80), CTLA4 (high on cells co-cultured with CD86 & CD40), and CD40L (high on cells co-cultured with CD40), CD25 and CD69 were upregulated in each case. Thus, CD40 is capable of functioning independently (in the absence of B7) as a costimulatory molecule both in terms of proliferation and cytokine release, this raises questions concerning the consequences of antigen presentation by tissue parenchymal cells.

O49

REGULATORY EFFECTS OF OX40/OX40L BLOCKADE IN THE ALLOIMMUNE RESPONSE.

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OX40 and OX40 Ligand are a pair of co-stimulatory molecules believed to play a role in late stage T cell - APC interactions, sustaining T cell activation and the generation of memory. Expression of OX40 is thought to be limited to antigen-reactive T cells, which are thereby marked as targets for possible therapeutic intervention in autoimmune disease and allograft rejection. Blockade of OX40/OX40L has been shown to reduce disease severity in murine experimental models of EAE, GvHD, IBD and Leishmaniasis. We have investigated whether OX40/OX40L blockade influences alloimmune responses *in vitro* and allograft survival in a mouse model of heterotopic heart transplantation, using major HC (BALB/c to CBA) and minor HC (B10.BR to CBA) disparate strain combinations.

Addition of OX40 fusion protein to allogeneic lymphocyte co-cultures reduced proliferative responses only when added from 72 hrs, in contrast to CTLA4 fusion protein which inhibited proliferation from 24 hrs but had little effect when added later. These results were substantiated by FACS analysis, which showed that although CD69 and CD25 were expressed early during the MLR, expression of OX40 was maximal at 96 hrs. Analysis by ELISA of *in vitro* cytokine production showed a reduction in both Th1- (IFN-γ, IL-2) and Th2- (IL-10) associated cytokines corresponding with the reduced proliferative responses observed. These findings were supported by intracellular cytokine staining of alloantigen-responsive T cells. Immunohistology of rejecting mouse heart allografts confirmed the presence of OX40-positive T cells in the accumulating perivascular and interstitial mononuclear cell infiltrate.

Mouse heart allograft recipients were treated i.p. with 150 µg OX40-Ig, either alone or in combination with CTLA4-Ig administered daily or on alternate days until day 15 post transplantation. This treatment succeeded in prolonging heart graft survival to >100 days in 72% of recipients mismatched at only minor HC locus antigens but failed to influence graft survival in the major HC mismatched strain combination. Current strategies based on the *in vitro* data involve treating graft recipients with alternative immune modulation during the first 72 hrs post transplant followed by daily administration of OX40-Ig.

These findings indicate that alloantigen-specific T cells may be successfully targeted with immunomodulatory agents to diminish the downstream events following T cell engagement with alloantigen, and blockade of OX40/OX40L may prevent allograft rejection.

O50

DISTINCT EFFECTS OF CD86-MEDIATED COSTIMULATION ON RESTING VERSUS ACTIVATED HUMAN CD4$^+$ T CELLS.

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For T cells to respond to a given antigenic stimulus, multiple activation signals are required, namely the ligation of TCR/CD3 complex (signal 1) and the provision of costimulation (signal 2). Whilst much data has been published demonstrating the importance of the B7 family of costimulatory molecules in T cell activation, the individual contributions of CD80 and CD86 remain unclear. We have studied the independent roles of CD80 and CD86 on the provision of costimulation for naïve and activated human CD4 T cells using a panel of human fibroblasts expressing DR1 supratransfected with either CD80, CD86. These transfectants have been tested in functional assays with either allogeneic CD4$^+$ T cells (resting) or DR1 restricted T cell clones (activated) as responders. Proliferation was measured on day 5 for allogeneic assays and day 2 for T cell clones by incorporation of thymidine. IL-2 was measured by CTLL-2 bioassay. We demonstrated that whilst the capacity of CD86 to costimulate a primary allogeneic response was similar to CD80, when using DR1 restricted T cell clones, CD86 was either inhibitory or ineffective. Furthermore, proliferative responses of the clones to triple transfectants expressing DR1, CD80 and CD86 showed enhanced proliferation (>50%) in the presence of an anti-CD86 blocking antibody. Flow cytometry demonstrated elevated levels of CTLA4 on clones cultured with the CD86-expressing transfectants compared to those expressing CD80. In parallel, different surface expression profiles of CTLA4 and CD28 were observed following 3 days culture of allogeneic CD4 T cells with either the CD80 or CD86 expressing transfectants: CD28 was elevated and CTLA4 was not expressed following co-culture with CD80, whilst in contrast, CTLA4 was elevated and CD28 down-regulated following co-culture with CD86. Expression of the costimulatory molecules themselves was also detected at day 3, with high levels of CD80 or CD86 on CD4 cells co-cultured with the CD80 or CD86-expressing transfectants respectively. Experiments are underway to determine whether this is due to upregulation or acquisition. In conclusion, CD86-mediated costimulation differs in its efficiency between resting and activated human CD4 T cells.

O51

HUMAN CD4+CD25+ T REGULATORY CELLS ARE POTENT INHIBITORS OF CD8+ T CELL FUNCTION.

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CD4+CD25+ T cells represent a unique population with suppressive activity, mainly responsible for the prevention of autoreactive T cells. Recently, we described the existence of this population in man, demonstrating great similarity to the original murine subset. CD4+CD25+ T cells are hyporesponsive to conventional T cell stimuli and inhibit CD4+CD25- T cell proliferation by a cell:cell contact-dependent mechanism. In this study, we investigated whether this population also regulates CD8+ T cell function in man. CFSE staining was used to measure proliferation, gated on CD8+ and PI- population. Intracellular staining was used to detect cytokines. Confocal microscopy was performed to study T cell activation. CFSE-labelled CD8+ T cells proliferated less in the presence of CD4+CD25+ T cells by a cell-cell contact mechanism, this could be partially reverted by exogenous IL-2. CD8+ T cells that had been co-cultured with CD4+CD25+ T cells were less responsive to subsequent rechallenge compared with those that had been co-cultured with CD4+CD25- T cells. Furthermore, CD4+CD25+ T cells decreased the production of IL-2, perforin and IFN-gamma by CD8+ T cells in co-culture. Calcium activation studies examined by confocal microscopy revealed that CD8+ T cells were less activated when in contact with CD4+CD25+ T cells when compared with CD4+CD25- T cells. The regulatory activity was more pronounced on the memory subset CD8+ T cell population. These results demonstrate a new immunoregulatory role played by CD4+CD25+ T cells, with relevance to the generation of effector CD8+ T cells.

O52

INDUCTION OF TOLERANCE IN T CELLS WITH INDIRECT PATHWAY ALLOSPECIFICITY

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The relative contributions of direct and indirect pathway T cell activation to transplant rejection have been a subject of longstanding interest. The aim of this study was to promote tolerance in T cells with indirect allospecificity by non-immunogenic (costimulation deficient) dendritic cells that share MHC-antigens with the donor.

Methods. Dendritic cells (DCs) were purified from bone marrow of (LEW x AUG)F1 rats and were cultured in the presence of Dexamethasone (DEX) to prevent their maturation. The phenotype of these cells was tested by FACS analysis. T cell proliferation assay or a two-step anergy culture were used in vitro to study the capacity of immature DCs to stimulate an allogenic T cell response or to induce anergy. To investigate tolerance induction in vivo, T cells were purified from LEW rats pre-immunised with (LEW x AUG)F1 DCs and the responsiveness of the direct and indirect pathway allospecific T cells was studied in vitro.

Results. Rat bone marrow-derived DCs cultured in the presence of DEX retain an immature phenotype characterized by low expression of MHC class II and of costimulatory molecules (CD80, CD86). These cells caused minimal proliferation by LEW T cells compared to mature DC. Moreover, they could induce AUG-specific anergy in LEW T cells, such that on re-challenge, LEW T cells previously exposed to immature (LEWxAUG)F1 DCs remained non-responsive to AUG antigens, but not to third party stimulators. T cells purified from LEW rats that have been pre-immunized with immature (LEWxAUG)F1 DCs were non-responsive when re-challenged in vitro with LEW DCs that had been pulsed with AUG cellular proteins, but they could mount a secondary proliferative response when re-challenged with AUG splenocytes. The capacity of the unresponsive T cells to respond to AUG cellular proteins restricted by LEW MHC class II was restored after depletion of CD25+ T cells.

Conclusions. DEX treatment "freezes" rat DCs in an immature state, those immature DCs do not stimulate a proliferative response from allogenic T cells and they can efficiently induce anergy in vitro. Moreover, immature rat DCs induced tolerance in vivo in T cells with indirect allospecificity. A proliferative response of the anergic T cells with indirect allospecificity was restored after depletion of CD25+ cells.

O53

REGULATION OF ALLOREACTIVE T CELL FUNCTION BY TRANSMIGRATION ACROSS MHC CLASS II EXPRESSING ENDOTHELIAL CELLS

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It is known that MHC class II positive human endothelial cells (EC) stimulate activation of resting CD4+, CD45RO+ B7-independent T cells, as measured by uptake of 3H-thymidine or release of Interleukin-2. The biological significance of activation, as measured in vitro, is unclear. It has been suggested that cognate interaction between CD4+ T cells and MHC class II positive EC results in a stop signal, preventing migration of antigen specific T cells through the ECs expressing that antigen.

Here we have investigated the effect of MHC class II EC on rate of migration and function of DR11 and DR13 (EC specific) or DR4 and DR7 (third-party) reactive T cells. CD4+ T cell clones and lines were raised against DR11, DR13, DR4, DR7 using PBMC stimulators. The T cells were migrated through the endothelial cell line Eahy.926, either untreated (MHC class II negative) or after transfection with the CIITA construct (Eahy.926/CIITA), resulting in expression of MHC class II DR11 and DR13. T cells were added to the upper chamber of a two chambered well, separated by an insert on which a confluent EC monolayer has been grown. Rate of migration was measured either by counting cells in the lower chamber from 2 to 24hrs, or by CFSE labelling one of two populations of EC specific and third party T cell lines and migrating them simultaneously through EC. Function was assessed by measuring the ability of T cells to proliferate to Eahy.926/CIITA and PBMC bearing the same alloantigens prior to and after migration for 24 hrs. In addition, frequency of IL-2 producing T cells to Eahy.926/CIITA prior to migration was compared to the frequency of migrated cells after 24 hrs. Responses and frequencies of non-migrated but cocultured cells from the upper chambers were also measured.

Results: The rate of migration of EC specific T cell clones was significantly reduced through Eahy.926/CIITA compared to third party T cell clones, at 2, 8 and 12 hours (8.8% specific T cells migrated compared to 29.5% at 12 hrs, $p=0.0039$, $n=3$). The rate of migration of EC specific T cell lines across Eahy.926/CIITA was either retarded (5/7 expts) or unchanged compared to migration of third party lines. However, the ability of EC specific T cell lines to proliferate to Eahy926/CIITA was always significantly reduced ($p<0.007$, $n>3$) 24 hrs after transmigration, but response to PMBC bearing the same alloantigen was unchanged. Meanwhile the response of non-migrated cells from the upper chamber remained unaffected. These results suggest that migration through, and not mere contact, reduces T cell proliferation to endothelial specific DR11 or DR13 molecules. This was confirmed by the observation that frequencies of EC specific T cells was significantly reduced after migration through Eahy.926/CIITA but not class II negative Eahy.926. (from 1/351 to <1/8000 after migration through Eahy.926 or Eahy.926/CIITA, respectively, $n=4$).

Conclusions: Results suggest that not only rate, but also the function of EC reactive T cells have changed after transmigration through MHC class II EC. There is also possibility that these T cells have died, or remain adhered to the EC or filter, which is being explored.

O54

NATURAL KILLER CELLS IN HUMAN RENAL TRANSPLANTION: AN UNRECOGNISED ENEMY?

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Introduction: The role of Natural Killer (NK) cells had been overlooked in allogeneic transplantation. They use their inhibitory receptors (e.g. Killer Ig-like receptors, KIR) which bind to self class I MHC molecules to inhibit killing of autologous cells. Mismatched allografts can therefore, be potential targets for NK cell killing. The genes for these inhibitory killer receptors are found in Leukocyte Receptor and NK gene complex on chromosomes 19 and 12 respectively. They are therefore inherited independently of HLA (chromosome 6). Our living related/unrelated renal transplantation programme with varying degree of HLA and KIR mismatch provides an unique opportunity to study the role of NK cells in human renal transplantation and to investigate if sharing of HLA and/or KIR genes with donor results in decreased activation of NK cells post transplantation.

Methods: NK cells were purified from recipient peripheral blood mononucleocytes (PBMC) by negative magnetic beads sort and used in a 4hr cytotoxicity assay against living related/unrelated donor PBMC at different effector:target (E:T) cell ratios 2 days before, on the day of transplant (recipients had been loaded with cyclosporin (CyA) for 2 days) and 3 days after transplantation (with addition of, azathioprine, steroid and Simulect) (Fig 1).

Results: Mean NK cell cytotoxicity against donor target cells was 14.9% (at E:T ratio of 25:1) 2 days before transplantation and was unaltered after 2 days treatment with CyA (12.5% n=15, p=0.57). However, it rose significantly in all but 5 patients 3 days after transplantation to 22%, P= 0.015, Fig 2). Recipient KIR genotyping was performed and correlated to donor HLA expression based on our current knowledge of HLA binding to specific KIRs. Our preliminary data showed that recipients exhibiting increased NK cytotoxicity against donor after transplantation express fewer number of inhibitory NK receptors but more stimulatory receptors that recognise donor class I MHC molecules when compared with those who did not demonstrate an increase. Furthermore, recipient NK cell cytotoxicity against 1st degree relative donor target cells is lower when compared with unrelated 3rd party individuals (Fig 1), suggesting that recipients sharing HLA and/or KIR genes with donors resulted in decreased NK cell cytotoxicity.

Conclusion: NK cell activity can be activated after transplantation despite quadruple immunosuppression, suggesting that recipient NK cell cytotoxicity against donor may be a previously unnoticed area of the rejection process:- the unrecognised enemy, especially in poorly matched donor/recipient pairs where the recipient may not express the correct repertoire of inhibitory receptors to prevent killing of donor cells.

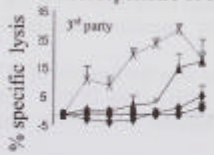

Fig 1: Example of NK cells cytotoxicity against donor (a 1st degree relative) PBMC at different E:T cell ratios before and after transplantation (solid line). NK cytotoxicity was generally lower in related individuals compared with unrelated 3rd party (dotted line).

Fig 2: NK cell activity (at a effector:target ratio of 25:1) against donor PBMC is unaffected by 2 days treatment by CyA but rose significantly in all but 5 patients (shown as dotted lines) 3 days after transplantation (n=15).

Parallel Session 3(a)

Organ Procurement and Surgical Technique

Friday 19 April 2002

08:45 – 10:30

O55

A COMPARSION OF RENAL ALLOGRAFT FIBROSIS FOLLOWING TRANSPLANTATION FROM HEART BEATING AND NON-HEART BEATING DONORS

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Introduction

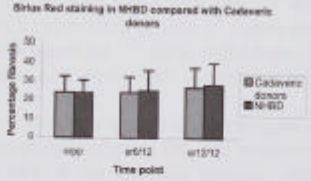
Although kidney transplants from non-heart beating donors (NHBD) yield acceptable renal function and allograft survival rates in the medium term, the long-term results are less certain and there is a paucity of information relating to the development of chronic allograft nephropathy. The aim of this study was to compare the development of renal allograft fibrosis in kidneys transplanted from NHBD and conventional heart beating cadaveric donors (HBD).

Methods

A series of 36 NHBD and 74 HBD renal transplants were studied. The populations were well matched in terms of donor and recipient characteristics, although mean donor age was higher in the NHBD group. Protocol needle core renal transplant biopsies were performed pre-perfusion and at 6 and 12 months post transplant. Biopsy sections (4μm) were stained with Sirius red to determine the level of interstitial extracellular matrix deposition. Renal allograft fibrosis was quantified using a computerised image analysis system.

Results (see graph)

The mean warm ischaemic time for NHBD kidneys was 25 minutes. Delayed graft function (DGF) occurred in 24% of HBD and 87% of NHBD. There were no significant differences in the level of renal allograft fibrosis between NHBD and HBD kidneys at any time point. The level of renal fibrosis in 6 month biopsies was positively correlated with serum creatinine levels at 12 and 24 months post transplant (P=0.005).



Conclusions

Despite sustaining a prolonged period of warm ischaemic injury leading to high rates of DGF, NHBD kidneys do not appear to be more susceptible to the development of renal allograft fibrosis. This study supports the growing body of evidence that NHBDs are a practical and viable alternative to HBD kidney transplants.

O56

IS THERE A CORRELATION BETWEEN GST AND ALA-AP LEVELS DETERMINED DURING MACHINE PERFUSION AND THE BEST CREATININE CLEARANCE AFTER TRANSPLANT WITH KIDNEYS FROM NHBD

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Introduction

NHBDs are increasingly being used to help curtail the kidney donor shortage. Viability screening is crucial to disqualify poor kidneys from being transplanted. Machine perfusion with cold preservation solution enables viability to be quantified by perfusion parameters and enzyme analysis of kidney effluent.
Glutathione S-transferase (GST), an established biomarker of renal tubular damage, has been previously used to predict warm ischaemic damage, development of ATN, and short term transplant outcome. High levels of GST, beyond a pre-determined critical value, have been correlated with DGF and PNF. We have looked at renal function in NHBD renal transplant in relation to GST emission of kidney effluent during machine preservation.

Materials and methods

55 renal transplants obtained from 49 NHBD's, which had been machine perfused were evaluated retrospectively. 48.5 % of these were obtained from uncontrolled donors.
Kidney effluent samples were assayed for biomarkers, total GST and alanine aminopeptidase (Ala-AP). The best serum creatinine and creatinine clearance (calculated from Cockcroft-Gault formula) in the post operative period was determined and collated. A scatter plot of serum creatinine versus total GST and Ala-AP, and total GST and Ala-AP were plotted and their respective correlation coefficients determined.

Results

Survival rate at one year	
Kidney	90.5 %
Patient	91.8 %
Renal function	
Serum creatinine at 1 year	177.0 ± 18.9 μmol/l
Creatinine clearance at 1 year	49.7 ± 4.4 mls/min

	Correlation
Maximum GST versus best serum creatinine clearance	$r^2 = 0.09$
Maximum Ala-AP versus best serum creatinine clearance	$r^2 = 0.05$

Conclusion

There is no correlation between maximum GST or Ala-AP at machine perfusion with the best creatinine clearance.

O57

WARM EX-VIVO KIDNEY PERFUSION PERMITS PRE-TRANSPLANT EVALUATION OF RENAL FUNCTION

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Introduction: Renal preservation by normothermic machine preservation of kidneys may permit a pre-transplant measurement of organ function, and therefore viability, during preservation, and may offer the prospect of improved organ preservation. The aims of this study were to compare the efficacy of preservation by normothermic ex-vivo perfusion with conventional cold techniques, and to compare ex-vivo and post-transplant renal function.

Methods: A left nephrectomy was performed on pigs, having clamped the pedicle for a specified warm ischaemic time (WIT), 0 or 30 minutes. Preservation was by either static cold storage (UW solution, 4^{0}C), or cold perfusion (Belzer's II MPS, RM3, 3-8^{0}C, 60mmHg systolic) or warm perfusion (n=5 for each group). The warm perfusion apparatus was a modified RM3 system, adapted to incorporate a heat and gas exchanger unit, (32^{0}C, 120mmHg). The perfusate used was a tissue culture fluid/ perflourodecalin emulsion (12%w/v) to increase oxygen capacity. 24 hours later, the kidney was transplanted into the same animal, and a right nephrectomy performed. Survival for 14 days post transplant was the primary end point, renal functions of surviving animals in the 0 WIT group are compared by calculating the area under the curve for serum creatinine against time. Ex-vivo renal function during warm perfusion was measured by creatinine clearance. Results are presented as median (range).

Results:

Survival	0 WIT	P Value	30 WIT	P Value	AUC [Cr]	P Value
Warm Perfusion	3/5		1/5		11062 (6117)	
Cold Perfusion	4/5	0.7	1/5	1.0	9247 (8493)	0.2
Cold Storage	4/5		1/5		11458 (5877)	

Ex-vivo renal function	0 minutes WIT	60 minutes WIT	P value
Creatinine clearance (ml/min)	6.6 (5.3)	1.3 (1.4)	0.01

Discussion: This study demonstrates for the first time that good ex-vivo function is associated with high rates of post-transplant renal viability, the converse also being true. A further new finding is that warm perfusion is of equivalent efficacy for renal preservation as conventional hypothermic techniques.

O58

CARDIOVASCULAR INSTABILITY IN DONOR IS NOT A MAJOR DETERMINANT OF OUTCOME IN LIVER TRANSPLANTATION

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Background: Cardiovascular instability and prior cardiac arrest were determinants of primary liver allograft non-function (PNF). In this era of severe organ shortage, improved donor management and organ procurement, we re examine these factors, which traditionally render potential donors marginal.
Aim: To determine the effects of donor cardiac instability (cardiac arrest or severe hypotension) on patient and graft outcome in OLT.
Methods: Prospectively collected data of 556 primary adult OLTs performed between Jan 1995 and Dec 1999 were analysed. The OLT recipients were divided into three groups according to the severity of the donor's cardiovascular instability.
Group 1 (n=41) received livers from donors who had suffered a cardiac arrest with a median of 30 (6-90) minutes. Group 2 (n=194) recipients' donors suffered a prolonged period of hypotension (mean BP <80 mm Hg for at least 1hour). This group was further subjected to a sub-analysis according to the duration of hypotension (1, 2-4, >4 hrs). Group 3 (n=321) donors were haemodynamically stable.
Results: Donor and recipient demographics (age, sex, and cause of disease) did not differ significantly between the groups. Cardiac arrest resulted in a significant rise in mean donor AST levels ($p<0.001$) and reduction in mean serum sodium ($p=0.04$). There was a trend for an increased incidence of PNF in G1 ($p=0.1$). Patient ($p=0.3$) and graft survival ($p=0.6$) at 1 year were lower in G1 but did not differ significantly. Postoperatively, a significantly higher INR and lower platelets count was noted in G1. There was no significant difference between the incidence of hepatic artery thrombosis, biliary complications, the incidence and severity of acute or chronic rejection between the study groups. Sub-analysis of G2 revealed significantly lower post transplant AST levels using livers from donor suffering prolonged (>4 hrs) hypotension which may indicate ischaemic preconditioning.
Conclusions: Although donor cardiac arrest history was associated with a trend towards an slightly higher incidence of PNF. Donor circulatory instability without cardiac arrest is not a significant determinant of allograft outcome.

O59

A PROSPECTIVE COMPARISON OF OPEN AND LAPAROSCOPIC LIVE DONOR NEPHRECTOMY

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Introduction

Laparoscopic donor nephrectomy (LDN) has the potential to remove some of the disincentives to live kidney donation. The aim of this study was to compare donor morbidity, postoperative donor recovery and early transplant outcome following LDN and conventional open nephrectomy (ODN) with (Rib+) or without (Rib-) rib resection.

Methods

A consecutive series of 55 live donor nephrectomies (LDN n=20; ODN Rib+ n=20; ODN Rib- n=15) performed during the period 1996-2000 were studied. All patients were managed with a patient controlled analgesia system (PCAS). Donor and recipient outcomes were recorded using a prospective database.

Results (see table)

In-patient stay and postoperative analgesia requirements were lower and return to normal activities was quicker following LDN when compared to either form of ODN (table). ODN Rib- was also associated with less postoperative pain and a speedier return to normal activities than ODN Rib+. There were no differences in donor morbidity rates or early recipient outcome variables.

	ODN (Rib +) (n=20)	ODN (Rib -) (n=15)	LDN (n=20)
Total dose morphine (mg)	185±98	116 ±56*	69±52**
Return to work (weeks)	12.6±5.9	10.2±4.4	6.5±2.1**
Driving (weeks)	5.9±3.9	3.8±1.0*	2.5±1.7**
In-patient stay (days)	6±1.8	5.9±2.3	4.1±1**

Mean ± SD * $P < 0.05$ vs ODN Rib + ** $P < 0.01$ vs ODN Rib + and ODN Rib -

Conclusions

ODN Rib- is a less traumatic operation than ODN Rib+ and it is difficult to justify the use of rib resection. LDN leads to substantial improvements in donor recovery when compared with either type of open nephrectomy. More experience is required to establish the exact place of laparoscopic donor nephrectomy.

O60

LIVE DONOR LAPAROSCOPIC NEPHRECTOMY: LOWER POLE DISSECTION FIRST, A TECHNICAL MODIFICATION.

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Since the introduction of laparoscopic nephrectomy for live renal donation by the Baltimore group in 1995, the technique has gained widespread acceptance within the transplant community. Ratner and colleagues described the technique where the first step is to mobilise the upper pole after ligation of the adrenal vein. It is during this part of the procedure that the upper pole of the kidney and adjacent spleen can be easily damaged. This was followed by ligation of the gonadal vein and subsequent mobilisation of the main renal vasculature.

We report a modification to the technique where the lower pole is dissected first. The first structure to identify is the renal vein. Having done this, the adrenal and gonadal veins are identified, ligated and divided. The ureter and periureteric adventitia is then mobilised from the medial side of the gonadal vein down to the pelvic brim. The ureter is not ligated/clipped at this point. The ureter is traced back up to the lower pole of the kidney. The attachments of the lower pole are divided, from medial to lateral, deep to the lower pole. Having performed this manoeuvre it becomes possible to elevate the lower pole enabling easier visualisation of the lumbar tributary of the renal vein. It can now be ligated and divided safely. Mobilisation of the main renal vessels can now be completed ready for subsequent cross clamping. The final stage is to divide the lienorenal ligament from its lateral aspect following it round the upper pole and into the cleft between the adrenal gland. Performing this step last means that the kidney stays in an anatomical position during renal vein and artery mobilisation. Once the stump of the adrenal vein is reached the kidney should be free and ready for vessel clamping and retrieval.

In our hands, this modification has further refined laparoscopic donor nephrectomy. It reduces the risk of damaging the kidney (particularly adjacent to the upper pole) and also allows for full visualisation of the renal vessels and their tributaries prior to their safe ligation and division. This presentation will include video coverage of the procedure described.

O61

ANTERIOR DONOR NEPHRECTOMY – A SAFE AND VIABLE ALTERNATIVE.

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Introduction:
The advent of laparoscopic live donor nephrectomy has led to a re-evaluation of the open surgical method. The latter is usually performed using a 12th rib resecting loin approach, with its attendant complications of pneumothorax, nerve injury and wound pain. We have addressed this by undertaking a minimally invasive anterior abdominal, non-rib resecting extraperitoneal approach and evaluate the outcome here.

Material & Methods:
Since June 1999, 20 donors have undergone this operation following the standard work-up for all living donors. The time taken to perform the operation, blood loss incurred, requirement for parenteral analgesia, need for blood transfusions, incidence of complications and the impact on kidney function, if any, was assessed. The return to normal mobility & work and any long-term problems were also studied.

Results:
All 20 donors underwent a left nephrectomy. The mean age of the donors was 46.25 years with a range of 22 to 67 years. There were 13 males and 7 females. The average length of the operation was 155 minutes (range 115-200 minutes), with the mean blood loss being 600 mls. (200- 1500 mls.). All donor kidneys functioned primarily in the recipient. One donor needed 2 units of blood transfusion. Two (10%) had antibiotic treatment for presumed chest infection. Parenteral analgesia was required for a median of 2 days (range 1-4 days). The mean post-operative stay was 6.5 days but most stayed in the hospital for non-medical reasons. No patient required readmission. The mean period of return to normal mobility was 3 days while the average time to return to work was 7.5 weeks (range 4- 13 weeks). The follow-up period ranges from 3 to 30 months. No donor developed incisional hernia or suffered nerve injury. The mean length of the incision was 9.7 cms. (range 7 –12cms.). All donors were very pleased with their scars and expressed their satisfaction with the operation.

Conclusion:
The anterior donor nephrectomy appears to be a safe and viable alternative to the classical and laparoscopic donor operations. It obviates the need for expensive equipment, has an acceptable rate of complications and avoids the potential risks of a trans-peritoneal laparoscopic approach. It achieves good cosmesis and high patient satisfaction.

O62

THE ROUTINE USE OF THE DOUBLE J STENTS IN RENAL TRANSPLANTATION – DOES IT MAKE A DIFFERENCE?

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INTRODUCTION: Routine stenting of ureterovescical anastomosis during renal transplantation is believed to reduce early urological complications. However randomised trials on this issue are not conclusive.

METHODS: Between November 1998 and October 2001, 372 cadaver and 54 living donor transplants were performed at our centre. 201 patients consented to enter this prospective randomised trial of stent or not. The incidence of early urological complications (urinary leak, obstruction and urinary tract infection) as well as the cost-benefit were compared. Main exclusion criteria were paediatric recipients, patients with urinary diversions and an intra-operative compromised ureteric/bladder appearance.

RESULTS: Randomisation led to 112 patients receiving a double J stent while 89 did not. 11 patients who were randomised to No stent group went on to have stent inserted because of intra operative findings; leaving 78 non stented patients for analysis. Mean time to stent removal was 74.3 days. Donor and recipient age, sex, first or regraft, cold ischemic time, primary renal disease and immunosuppression were comparable in both group.

Complication	Stent (112)	No stent (78)	
	%	%	Fisher's exact
Urinary leak	0.9	8.9	0.008
Hydronephrosis	0	7.7	0.004
Urinary Tract Infection	31.3	16.7	0.02
Transplant failure	8.0	3.8	non sig.

There were no urinary tract infection within the 11 patients excluded from 'No stent' group. But, one patient developed hydronephrosis, 1 week post transplant due to haematuria and another developed late hydronephrosis, 4 weeks following removal of stent. All urological complications were successfully managed. There were no deaths in either study group. The cost of managing the urological complications outways the cost of consumables.

CONCLUSIONS: Ureteric stent in renal transplantation significantly reduces urinary leak and obstruction but it does lead to a significantly higher incidence of urinary tract infection.

O63

A NEW TECHNIQUE FOR KIDNEY-PANCREAS TRANSPLANTATION

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Introduction: Kidney-pancreas transplantation with enteric drainage of the pancreatic graft is classically performed via a midline incision with consecutive implantation of the pancreas on the right and the kidney on the left side. This technique is associated with: a) extensive manipulation of the bowel, b) difficulty in controlling haemostasis following revascularisation of the grafts due to the presence around the grafts of many bowel loops, c) undue pressure applied on the pancreas during the kidney transplant because bowel retraction to the right d) direct impact on the kidney graft of any problem related to the pancreatic graft, e) a prolonged operative time which, in our experience (50 pancreatic transplants performed between January 1995 and January 2001), is (mean ± SD) 360 ± 85 minutes. Therefore, we introduced a simplified technique for synchronous implantation of the kidney and pancreatic grafts.

Methods: Two incisions are made simultaneously (Consultant and Registrar) on the right and the left lower part of the abdomen which allow a classic extraperitoneal dissection of the iliac vessels on both sides. The application of the Omnitract allows sufficient retraction for the synchronous implantation of the two grafts. After revascularisation it is easy to identify any bleeding points and, if any, control them quickly. Following that, the peritoneum on the side of the pancreas is opened along the graft and a side-to-side anastomosis of the graft duodenum to the ileum is done by using a GIA 80 surgical stapler.

Results: From February 2001 to November 2001 we have performed five operations with synchronous implantation of the two grafts. The operative time was (mean ± SD) 120 ± 15 minutes. There were no post-operative complications and there was 100% patient, pancreas and kidney graft survival (range of follow-up:1 to 10 months).

Conclusions: This technique allows a fast implantation of both grafts combined with minimal manipulation of the small and large bowel, no pressure on the pancreatic graft during the implantation of the kidney, easy application of haemostasis following revascularisation (especially of the pancreatic graft) and protection of the extraperitoneally implanted kidney from any problem related to the pancreatic graft.

O64

CURRENT PRACTICE REGARDING THE USE OF FATTY LIVERS: A TRANS-ATLANTIC SURVEY

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Introduction/Methods

In the present climate of evidence based medicine it is vital that clinical practice can be backed up with sound experimental and clinical verification. Many controversies exist regarding the transplantation of fatty livers due to use of inconsistent and often confusing nomenclature, as well as differences in practice between institutions based on subjective personnel experience. If meaningful audit of best practice is to be established with the aim of improving standards, then it is imperative that an accurate impression of current clinical practice within the international transplant community needs to be established. This survey was designed for this purpose, and to highlight major differences between the US and UK experience. A simple anonymous one-page, 10-question survey with tick-box answers was sent to every practicing liver transplant surgeon in the United Kingdom. This exact same proforma was sent via email to every liver transplant surgeon whose current email was listed in the American Society of Transplant Surgeon registry in the US. These results have been categorically compiled.

Results

In the UK, 16 of 19 surgeons polled responded (84.2%); this was considered representative. From the US, there were 78 respondents from 52 centers representing all 11 UNOS regions. Using data from the UNOS website based on 1999 statistics, these centers accounted for 82.6% of the liver transplants performed that year. **Space allows only 5 of the 10 questions in the survey to be shown in the table below.**

United Kingdom (n=16)

Over what level of macro-vesicular steatosis would you always reject a donor liver, irrespective of other variables?	20%-40%	40-60%	No maxim-um limit	N/A		
	6%	69%	19%	14%		
Under what circumstances do you seek histological appraisal of a donor liver?	Looks Bad	Risk Factors	Never	Always		
	38%	6%	50%	6%		
How many biopsies do you take ?	1	2	3	4	0	
	44%	6%	0%	0%	50%	
Do you consider microvesicular steatosis a risk factor for Primary Non-Function?	Yes	No	Unsure			
	38%	38%	24%			
Do you use CT/MRI/US to assess fatty change prior to retrieval?	Never	Sometimes	Always			
	94%	6% (LRD)	0%			

United States (n=78)

20%-40%	40-60%	No maxim-um limit	N/A		
59%	33%	2%	6%		
Looks Bad	Risk Factors	Never	Always	Both 1&2	
47%	27%	8%	14%	12%	
1	2	3	4	0	
78%	18%	3%	0%	0%	
Yes	No	Unsure			
27%	54%	19%			
Never	Sometimes	Always			
86%	14%	0%			

CONCLUSIONS

There is a marked international discrepancy in the use of biopsy to quantify steatosis, including histological staining technique, number and positions of biopsies. Radiological quantification of steatosis remains largely a research tool. There is clearly no consensus on the significance of microvesicular steatosis and a large percentage of surgeons whose hold opinions that are contrary to published scientific data. There is a great range in the degree of steatosis that surgeons will accept before rejecting the liver

Parallel Session 3(b)

Immunogenetics/Virology

Friday 19 April 2002

08:45 – 10:30

O65

THE USE OF IVIg FOR PRE-TRANSPLANT ANTIBODY REMOVAL IN HIGHLY SENSITISED PATIENTS.

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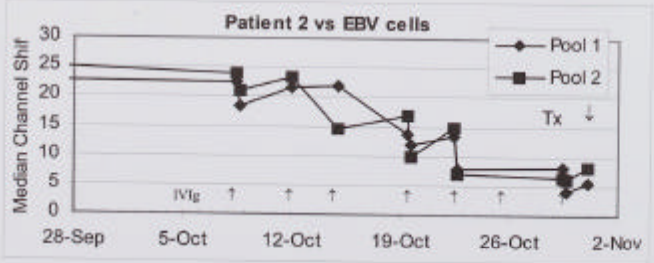
Intravenous Immunoglobulin (IVIg), prepared by pooling and concentrating IgG antibody from up to 10,000 donors, has been used for over 20 years. Initially developed to overcome primary and secondary immune deficiencies, IVIg is now successfully used to treat autoimmune diseases with a pathogenic antibody component, such as idiopathic thrombocytopenia purpura, and Kawasaki disease. IVIg has more recently been investigated as a method of reducing HLA specific antibodies, and has been used both as induction therapy and rejection rescue in the transplant setting.

We have recently devised a pre-transplant protocol to attempt to reduce the overall panel reactive HLA-specific antibody (PRA) of highly sensitised patients on the renal waiting list. 500mg/Kg IVIg (Octagam) is given twice weekly for 4 weeks, in the absence of any other immunotherapy. Further doses of IVIg would be given at monthly intervals until a suitable kidney was found.

We report the use of this protocol in two highly sensitised paediatric patients. There was a fall in panel reactive antibody using both cytotoxic tests against a random panel (table), and a flow cytometric screen using 2 pools of ten EBV cell lines (see graph for patient 2, where median channel shift is a measure of antibody binding). Patient 1 had the number of unacceptable antigens reduced post therapy and was offered a cadaver kidney from the national sharing scheme within 4 days. He was transplanted successfully. Patient 2 received a suitable kidney in the last week of therapy, and was also transplanted. Serum samples from both patients gave a positive crossmatch with their respective donor's cells at various times prior to IVIg therapy. This would normally preclude transplantation, but their crossmatches were negative after therapy. The PRA of both patients has remained low post transplant, and there has been no evidence of rejection.

	Cytotoxic PRA pre-IVIg	Cytotoxic PRA Post IVIg
Patient 1	70%	40%
Patient 2	90%	80%



O66

HLA-DP SPECIFIC ANTIBODIES IN RENAL TRANSPLANT RECIPIENTS.

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Introduction. HLA specific antibodies can be produced as result of HLA mismatches at transplant, pregnancy and blood transfusions. HLA antibody detection and definition prior to transplantation is important in preventing rejection and graft loss. It has recently been suggested that HLA-DP has a role in transplant outcome and that undetected DP specific antibodies may be implicated in situations where the reasons for graft loss remain unknown.
We have previously reported the development of an assay to detect antibodies to HLA–DP using FlowPRA beads[1]. Using this assay we have studied the production of HLA–DP specific antibodies in a cohort of well matched renal transplant recipients.
Methods. Sera from 75 patients with HLA-A, -B and –DR 000 mismatched transplants were tested for the presence of HLA-DP specific antibodies using FlowPRA Class II panel beads. These are microparticles coated with HLA Class II (HLA-DR, -DQ, -DP) antigens purified from a panel of 30 cell lines which cover all the frequently occurring and many of the less common HLA specificities. A pre-transplant sample and four post-transplant samples (at 1, 3, 6 and 12 months) were tested from each patient. A post-transplant increase in %PRA for a patient indicated the production of HLA Class II specific antibodies. The presence of DP specific antibodies was determined by pre-incubating the FlowPRA beads with a mouse anti-DP antibody to block antibody binding to DP epitopes. A reduction in antibody binding indicated the presence of DP specific antibodies. Further tests using FlowPRA specificity beads were performed on sera positive for DP specific antibodies to determine antibody specificity. Again DP blocking assays were used to measure the reduction in patient antibody binding to beads coated with HLA-DP antigen purified from cells of known genotype.
Results. 11 (14.7%) of the 75 patients tested showed the development of HLA-DP specific antibodies following transplantation. Positivity for DP antibodies did not correlate with rejection episodes in this cohort of patients (p=0.17).
Sera from 9 DP antibody positive patients were analysed to determine antibody specificity. We were unable link specificity results to HLA-DP genotype but our initial results indicate the presence of antibodies directed against discrete DP epitopes.
Discussion. Our results show that renal patients can produce HLA-DP specific antibodies following transplantation and that these antibodies are directed against discrete HLA-DP epitopes. Further studies to identify these epitopes and to link their specificities to HLA-DP genotype mismatches are in progress.

Reference. [1]MF Brennan, AJ Robson and S Martin (2001). *European Journal of Immunogenetics* **28**, 365.

O67

EVALUATION OF CDC CROSSMATCHING & B CELL CUT OFF VALUES USED IN FLOW CYTOMETRIC CROSSMATCHING.

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Historically, pre renal transplantation crossmatching has been performed using a complement dependent cytotoxicity (CDC) assay. However this technique has some disadvantages. It is less sensitive than flow cytometric crossmatching for the detection of IgG antibodies, it is unable to detect non-complement fixing IgG subclasses and the analysis of results is subjective. The flow cytometric crossmatch overcomes a these disadvantages and most laboratories now use a combination of CDC and flow cytometry in their crossmatching protocol. However, one perceived disadvantage of flow cytometric crossmatching is a relatively high proportion of positive B cell crossmatches, the clinical relevance of which has been questioned. One critical factor in determining the incidence of positive B cell crossmatches is the defined positive threshold. The aim of this study was to determine if this threshold could be raised without any adverse clinical effects. The previously defined cut off level in our laboratory is >1.5x the log MdX value of our negative AB/s control. An initial retrospective study of 87 local renal transplants, performed in 2 transplant centres, suggested that this value could be raised to 2x that of our negative control and that CDC crossmatching contributed no additional relevant information. A prospective, 6 month clinical trial was undertaken to further assess both of these issues. The trial was performed using two different crossmatching protocols. The protocol for Transplant Centre 1 was flow cytometric crossmatching (no CDC) using the existing B cell cut off value of 1.5x the negative control. For Transplant Centre 2 both flow cytometric and CDC crossmatching was performed using a B cell cut off of 2x the negative control. Using the split protocol a total of 33 crossmatches (14 centre 1, 19 centre 2) were performed. Thirteen out of the 19 Centre 2 crossmatches were negative, 3 of which would have been positive using a 1.5x. Two of these patients had no rejection. The third had mild parenchymal rejection which was successfully treated. Seven out of the 14 centre 1 patients had positive crossmatches. Three of these would have been negative if the B cell cut off was 2x that of the negative control. Non of these 3 patients had rejection. Of the 4 positive crossmatches >2x the negative control 2 were shown to be due to donor specific HLA antibodies and the patients were not transplanted and 2 were due to auto-antibodies and were successfully transplanted. In the retrospective arm of the study 10 patients had a CDC +ve / flow -ve crossmatch. Non of these patients had biopsy proven antibody mediated rejection. In the prospective study there were no CDC +ve / flow -ve crossmatches. CDC crossmatching therefore provided no clinically relevant information which was not obtained from the flow cytometric crossmatch results.

Using the combined data of the two trials, we have now adopted a common crossmatch protocol for both our transplant units. Only flow cytometric crossmatching is performed (no CDC) using a B cell cut off value of 2x that of our negative control. The data shows that this will reduce the incidence of B cell positive crossmatches and allow successful transplantation of a number of patients who might previously have been considered unsuitable for a specific donor.

O68

GENETIC DETERMINANTS OF DELAYED GRAFT FUNCTION AFTER KIDNEY TRANSPLANTATION

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Introduction There is currently no method available to identify individuals who have an inherently high probability of delayed graft function (DGF) prior to renal transplantation. Were such risk factors recognised, this could have significant clinical benefit as organs could be handled and transplanted at an appropriate level of urgency. Protection from the deleterious effects of free radicals is essential to the successful function of the transplanted organ. Enzymes such as glutathione S transferase (GST) and manganese superoxide dismutase (MnSOD) are major free radical scavengers. We hypothesised that genetic variation in these determinants of oxidative metabolism could influence the immediate function of a kidney, particularly after prolonged ischaemia when the capacity for free radical scavenging would be maximally saturated. To test this hypothesis, we examined the relationship between GST and MnSOD polymorphisms in donors and recipients of transplants with over 24 hours of cold ischaemia, and the subsequent requirement for haemodialysis in the first week after transplantation.

Methods Recipients and donors of first cadaveric kidney transplants between June 1985 and June 2000, who had a cold ischaemic time of >24 hours, and for whom DNA was available, were included in this study. PCR-SSP was performed to differentiate polymorphisms for MnSOD and three classes of GST enzymes: the mu (GSTM1), pi (GSTP1), and theta (GSTT1) classes. Phenotype, genotype and allele frequencies were determined for all polymorphisms and the data was analysed according to the presence or absence of delayed graft function, defined as the requirement of haemodialysis in the first week after transplantation. The Chi-squared test with Yates correction or Fisher's exact test was used, and a Bonferonni correction factor for multiple comparisons was applied.

Results Enzyme polymorphisms were defined for 229 recipients and 104 of their respective donors. Patients receiving a kidney from a donor who expressed GSTM1*B either alone or in combination with GSTM1*A experienced significantly lower rates of delayed graft function ($Pc<0.05$). When recipients results were analysed, no association was identified between delayed graft function and any of the examined polymorphisms.

Conclusions Polymorphisms in enzymes involved in free radical metabolism have previously been associated with susceptibility to a variety of diseases including different types of malignancy, asthma and multiple sclerosis. In this study we showed that the presence of the GSTM1B allele in the donor kidney was significantly associated with protection against delayed graft function after transplantation. The mechanism by which this protective effect is conferred has not been elucidated, but this raises the possibility of defining the inherent biological risk of DGF that an organ possesses. Furthermore, the implications of GST polymorphisms with differential roles in protecting tissues from ischaemic injury could have an impact on other diseases processes that involve reactive oxygen species, such as ischaemic heart disease, neurodegenerative diseases and aging.

O69

SCREENING FOR FACTOR V LEIDEN AND PROTHROMBIN G20210A MUTATIONS IN RENAL TRANSPLANT RECIPIENTS & DONORS: RESULTS OF A LARGE UK STUDY

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Aim: Several recent studies from European centres have suggested thrombophilia is a risk factor for early graft loss after renal transplantation. This UK study is the largest series of renal transplant recipients and donors screened for the two commonest known prothrombotic genotypes, the factor V Leiden (FVL) and prothrombin G20210A (PT G20210A) polymorphisms.

Methods: 562 renal transplant recipients and 457 kidney donors were genotyped for the FVL and PT G20210A mutations by PCR-RFLP assays. Data on graft survival at 30 days and at 1-year post transplantation were obtained from a prospectively collected database and causes of graft loss cross-referenced with pathology records. Survival analyses were performed using the Kaplan Meier product-limited method, and statistical significance assessed using the log rank test. Multiple logistic and Cox's proportional hazards regression models were used to determine factors contributing to graft loss.

Results: The prevalence of heterozygous FVL in transplant recipients was 3.4% (n=19/562) and 2.6% (n=12/457) in donors, and PT G20210A was 2% (n=11) and 1.1% (n=5) in recipients and donors respectively. The prevalence of thrombophilic mutations was similar to that of the general UK population. The 30-day and 1-year graft survival in recipients with thrombophilic mutations was 97% & 93%, compared to 88% & 82% in patients without these mutations (log rank P=0.34). Thrombophilia in the recipient OR 0.54, (CI 0.06 – 2.22), P=0.56, or in donors OR 1.45, (CI 0.26 – 5.43), P=0.48, did not correlate with graft loss at thirty days after transplantation. The presence of thrombophilic mutations in transplant recipients OR 0.34, (CI 0.04 – 1.40), P=0.20, or donors OR 2.27, (CI 0.40 – 8.62), P=0.19, did not appear to be a risk factor for graft loss at one-year following transplantation. Increasing age of the recipient, diabetes mellitus and peritoneal dialysis as the mode of pre-transplant renal replacement therapy were associated with poorer outcomes.

Conclusions: In contrast to recent reports, this study did not demonstrate an association between thrombophilia and renal allograft loss. There is little utility in these markers predicting graft outcomes after transplantation, and consequent to the low prevalence rates in the UK compared to the rest of Europe, routine screening is not recommended.

O70

GENETIC POLYMORPHISM ASSOCIATED WITH HEPATIC EXPRESSION OF CYTOCHROME P450 3A5, CORRELATES WITH DOSE REQUIREMENT FOR TACROLIMUS

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Tacrolimus is metabolised by the liver enzymes cytochrome P450 3A4 and 3A5 (CYP3A5). African (Black) patients are known to require larger doses of tacrolimus to achieve target blood trough concentrations than Caucasian patients. It has been suggested that this difference is due to different levels of expression of P-glycoprotein in the gut. However, the recent description of a genetic polymorphism in the CYP3AP1 pseudogene promotor (A/G_{44}), that was commoner in African Americans and strongly associated with levels of hepatic CYP3A5 activity, raised the possibility that this is also a factor in the heterogeneity of tacrolimus dose-requirement. We genotyped Black and Caucasian patients for this single nucleotide polymorphism and correlated this with tacrolimus dose-requirement.

Trough (pre-dose) blood concentrations of tacrolimus of Black and Caucasian renal transplant patients were measured using an Abbott IMX immunoassay. DNA was extracted from whole blood samples. Genotyping was performed by using polymerase chain reaction (PCR) followed by enzymatic digestion using AciI endonuclease and agarose gel electrophoresis. The local research ethics committee gave approval.

The blood concentrations of tacrolimus at three months after transplantation were related to dose by the concentration of drug (µg/L) achieved by administering one mg of drug per kg body weight (concentration/dose(mg/kg)). Black patients achieved significantly lower blood tacrolimus concentration per 1 mg/kg dose than Caucasians (p=0.001). Within the Caucasian group there was a significant difference between the patients with the AA genotype compared with the AG and GG genotypes. A similar pattern was found for Black patients but there were no statistically significant differences between the groups.

	Genotype: Number of patients (%)		
Race	**AA**	**AG**	**GG**
Black	6 (33%)	11 (61%)	1(6%)
Caucasian	57 (85%)	9 (13%)	1 (2%)

	Concentration/dose: median (inter-quartile range)			Mann-Whitney U test
	AA	**AG**	**GG**	(AA vs AG & GG)
All	88.8 (62.6-132.0)	37.5 (30.2-58.0)	23.6	P<0.0001
Black	59.0 (49.5-71.4)	39.8 (32.0-62.3)	23.6	P=0.10
Caucasian	90.2 (63.1-137.3)	34.6 (30.0-41.2)	23.7	P<0.0001

We conclude that patients with the AG or GG genotype require higher doses of tacrolimus to achieve a target blood concentration than patients with the AA genotype. The higher frequency of the G allele in the Black population, as compared to Caucasian patients, therefore defines, in part, the higher dose requirement in this group.

O71

CANDIDATE ENDOTHELIAL AUTOANTIGENS IN PATIENTS SUFFERING FROM TRANSPLANT ASSOCIATED CORONARY ARTERY DISEASE

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Introduction: Transplant associated coronary artery disease (TxCAD) is the most common and serious long-term complication following cardiac transplantation. At three years, approximately 44% of patients have angiographically detectable disease. Although a disease of multifactorial actiology, TxCAD is associated with a chronic humoral response to a variety of antigens, including possible autoantigens. In particular the presence of anti-endothelial cell antibodies (AECA) that react to a large number of proteins, the majority of which are in the 30 to 110 kDa weight range, have been described. Previous studies have identified candidate autoantigens by 2D-electrophoresis, however using screening of cDNA libraries from umbilical vein endothelial cells it has been shown it is possible to identify AECA antigens. The purpose of this study was to use a more physiologically relevant cDNA library and expression cloning to identify novel antigens recognised by sera from patients with TxCAD.

Methods: A human coronary artery endothelial cell cDNA library (Stratagene) was screened using sera from 3 patients suffering from TxCAD. Approximately $2.5x10^5$ pfu/patient were screened and positive plaques selected for further screening. After three rounds of screening, plasmids were extracted from the phage and sent for sequencing. Homology searches were performed using FASTA at EMBL.

Results: 13 plaques were identified and following three rounds of screening 9 plaques remained positive. Sequencing of the cDNA inserts yielded nine different genes (see Table).

Patient	Plaque	Identity
1	1	HBV pX associated protein 8α
	4	M-phase phosphoprotein
2	no positive plaques	
3	1	MEKK family kinase
	2	rabaptin
	4	neuropilin 2
	6	retinoblastoma-binding protein 1-like-1
	7	pKIAA1229
	8	interferon γ inducible protein 16
	9	mki67a (mouse antigen of monoclonal antibody Ki-67)

Conclusions: None of the candidate autoantigens we have identified have been previously described in TxCAD patients. The majority are intracellular proteins involved either in regulation of the cell cycle or cell signalling. Of particular interest is neuropilin 2, which is the VEGF receptor. These results suggest the existence of a great diversity of autoantibodies in TxCAD patients. Further studies will establish what autoantigen targets are predominant in TxCAD patients.

O72

LOW DOSE VALACICLOVIR PROPHYLAXIS AGAINST CYTOMEGALOVIRUS IN RENAL TRANSPLANT RECIPIENTS

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Background: Cytomegalovirus (CMV) is the most important pathogen affecting renal transplant recipients. High dose valaciclovir up to 8 grams/day adjusted to creatinine clearance has been shown to be effective in prophylaxis against CMV disease in a randomised control trial. We report our experience with low dose valaciclovir prophylaxis of up to 3 grams/day adjusted to creatinine clearance.

Methods: Seropositive Recipients receiving antilymphocyte therapy (R+) and Donor positive/Recipient negative (D+R-) not receiving any antilymphocyte therapy were included in the study group. A historical control group was used. D+R- patients receiving antilymphocyte therapy were excluded from analysis as most of these patients in the control group had received ganciclovir prophylaxis. Statistical analysis was undertaken utilising SPSS software. Chi square test and Fisher's exact test were used to compare categorical data and unpaired t test was used to compare continuous data.

Results:

Patient Characteristics

	Prophylaxis (n=35)	Control (n=35)	P Value
Age	42.4±12.243	43.8±12.202	0.546
Sex Male: Female	19:16	20:15	0.81
Transplant Type Living /cadaveric Donor	13/22	7/28	0.112
HLA mismatches mean	2.69	2.71	0.94
Immunosuppression			
Cya, Aza,Pred	28	33	0.151
Cya, MMF, Pred	6	2	
FK, Aza, Pred	1	0	

Outcome:

	Prophylaxis (n=35)	Control (n=35)	P Value
CMV Disease at 6 months	3 (8.5%)	13(37.1%)	0.004
Graft loss at 6 months	0	2(5.7%)	0.493
Mortality 6 Months	0	1(2.8%)	1.0
CMV R+(n=20)	1/20 (5%)	9/20 (45%)	0.003
CMV D+R- (n=15)	2/15 (13.3%)	4/15 (26.6%)	0.651

Low dose valaciclovir prophylaxis resulted in a statistically significant decrease in CMV disease in the study group at 6 months. On subgroup analysis the decrease was statistically significant only in R+ group but not in D+R- group.

Conclusion: Low dose valaciclovir prophylaxis seems to be adequate for R+ patients receiving antilymphocyte therapy. The role of low dose valaciclovir prophylaxis needs to be evaluated further in a large prospective trial.

O73

CYTOMEGALOVIRUS VIRAEMIA IN LIVER TRANSPLANT PATIENTS, WHO IS AT RISK?

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Background: Cytomegalovirus (CMV) infection following orthotopic liver transplantation (OLTx) has been associated with an increase in patient morbidity and mortality. In recent years the prevention of CMV infection has been attempted through the use of prophylactic regimes of oral ganciclovir targeted at high-risk groups, primarily, CMV IgG negative recipients receiving a graft from a CMV IgG positive donor.

Aim: This study aimed to identify other pre-OLT factors associated with the post-transplant development of a CMV viraemia (CMV, serum, PCR positive).

Methods: Over the period January 1st 1999 to August 31st 2000, 74 OLTxs were performed on 66 patients. Patient information (age, sex, actiology of OLTx, urgency of OLTx, donor CMV IgG status, recipient CMV IgG status, CMV PCR results, CMV prophylaxis, co-existing morbidities) was prospectively entered onto databases at the SLTU and the Edinburgh Clinical Virology Laboratories.

Results: The mean incidence of CMV viraemia was found to be 32.43% (24/74).

Pre-OLT Factors	Incidence of CMV/factor	Incidence of CMV viraemia (%)	Significance P=
CMV status of donor and recipient			
+ to –	10/16	62.5	0.010
+ to +	10/22	45.45	0.199
- to +	1/14	7.14	0.028
- to -	1/15	6.66	0.027
Other factors			
Diabetes Mellitus	7/9	77.78	0.004

Conclusion: Examination of Pre-OLTx factors revealed that, as previously documented, CMV IgG –ve patients receiving CMV +ve grafts where at increased risk. However, the most interesting finding is that patients with pre-existing diabetes mellitus experienced the highest incidence of CMV viraemia (77.8% p=0.004) a previously undocumented risk factor. This would suggest that the provision of anti-viral prophylaxis should be widened to include patients with pre-existing diabetes mellitus.

O74

THE RECONSTITUTION OF CMV-SPECIFIC CD8+ T-CELL CLONES AND THE GENERATION OF *DE-NOVO* CD8+ T-CELL CLONES, IN RECIPIENTS FOLLOWING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION.

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Reconstitution of CMV-specific CD8+ T cells plays an important role in preventing CMV disease following allogeneic haematopoietic stem cell transplantation (alloSCT). Previous techniques used to monitor CMV-specific immunity after alloSCT, have had insufficient resolution to identify the individual clones that comprise the CMV-specific CD8+ T-cell population, and do not distinguish between clones that are of donor or recipient in origin. By comparing the clonal composition of CMV (pp65 and IE1) and EBV (EBNA 3C) peptide-specific CD8+ T-cells in 9 healthy donors and their recipients, we have been able to provide new insights into human antigen-specific CD8+ T-cell reconstitution following alloSCT. We determined whether viral peptide-specific CD8+ T-cell clones generated in the recipient following alloSCT were of recipient or donor origin by analysis of DNA for gender and/or informative single nucleotide polymorphisms. T-cell receptor analysis (clonotype probing) of multiple independently obtained peptide-specific CD8+ T-cell clones was used to establish the reconstitution kinetics of individual T-cell clones within the recipient following alloSCT. All CD8+ virus-specific T-cell clones generated in alloSCT recipients contained DNA of donor origin, and a recipient clone generated prior to transplant was eradicated by the preparative regimen. We demonstrate that the immunodominant donor virus-specific clone expands, persists and retains CTL function in the seropositive recipient, and in some cases underwent substantial expansion. The immunodominant donor CD8+ T-cell clones remained detectable up to 6 years later. However, donor CMV-specific CD8+ T cell clones did not persist in 2 of 3 CMV seronegative recipients. In seropositive recipients of seronegative allografts, a primary virus-specific CTL response can arise in the recipient within 5 months, in cells of donor origin. Evidence from a recipient who received a Campath T-cell depleted alloSCT, suggests that the CD8+ T-cells participating in the primary response appear to originate from *de-novo* T-cell generation from donor-derived progenitor cells. Two CMV seropositive recipients developed a different clonal composition from their seropositive donors, with delayed persistent expansion of individual CMV-specific clones (containing donor DNA), that could not be detected in the donor and were absent in the recipient for the first 5 months after transplant; these may represent expansion of subdominant clones present at low levels in the donor, or expansion of novel clonotypes generated in the recipient from donor cells. The naïve CD28+ $CD45RA^{high}$ phenotype present in healthy subjects is applicable to reconstituted alloSCT recipients, and HCMV-specific CD8+ T-cells predominantly reside in the expanded CD28- population, and are CCR7-.
Our findings provide a rationale for immunisation of the seronegative donor before stem cell harvesting. Immunisation to boost antigen-specific CD8+ T-cell responses in the seropositive donor prior to harvesting, and of the recipient following allograft, also merit further investigation.

Clinical Posters

P1

EXPERIENCE WITH THE USE OF SIROLIMUS (RAPAMYCIN) IN HEART AND LUNG TRANSPLANTATION

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Background Sirolimus (SRL) is a potent new immunosuppressant, lacking the nephrotoxicity associated with calcineurin inhibitors. Its use is becoming established in renal transplantation, but there is currently little experience with heart and lung transplant recipients. Cyclosporin-induced acute and chronic renal dysfunction is a major factor limiting its use in this group of patients.

Methods Patients were enrolled between August 2000 and June 2001. All were taking cyclosporin (CyA) and had a serum creatinine over 150mcmol/l. CyA was reduced by 25mg/day to stop and SRL commenced with a 15mg loading dose, then 5mg daily, and a trough level at one week - target range 5-15ng/ml. The loading dose was later omitted due to side-effects associated with toxic drug levels. SRL was discontinued if unacceptable side-effects continued to occur despite levels within the target range. Patients were followed up with indices of renal function for three months and SRL levels were monitored monthly. Rejection in lung recipients was assessed by transbronchial biopsy: routinely performed at one month post transplant and subsequently on the basis of a reduction in spirometric values, x-ray changes or new symptoms.

Results Seventeen recipients were enrolled: 1 heart, 1 single-lung, 11 heart-lung, and 4 double-lung. Five had an acute deterioration in renal function following introduction of immunosuppression using our usual protocol (CyA, azathioprine, prednisolone, and Anti-Thymocyte globulin for lung recipients). In this group, the mean serum creatinine on entry was 311mcmol/l (range 230-483) and this fell in all five patients by an average of 56% (actual mean reduction 188mcmol/l – range 65-394). One patient died four months after heart-lung transplantation from pancytopenia and sepsis. No significant episodes of rejection occurred.
Eleven patients had chronic CyA-induced renal failure. The mean creatinine on entry was 253mcmol/l (range 189-335). Three discontinued the drug due to adverse effects (rash, mouth ulceration, anaemia) and one progressed to renal transplantation. Of the remaining eight, in six the creatinine fell by an average of 24mcmol/l, in one there was no change and one a small rise (3mcmol/l). No episodes of rejection occurred.

Conclusion SRL is effective and provides adequate immunosuppression in this group of patients with trough serum levels of 5 -15ng/ml, with minimal toxicity. In acute CyA-induced renal failure, use of SRL allows withdrawal of CyA, resulting in a significant improvement in renal function. In chronic renal dysfunction a levelling or small reduction in serum creatinine is seen. The side-effect profile differs from that of the calcineurin inhibitors and reactions are mainly minor and dose-related. However, bone marrow suppression may occur and can be severe. This appears to be precipitated by infection and may prove to be a serious factor limiting its use in some patients with lung transplants.

P2

SIGNIFICANCE OF RECURRENT AND *DE NOVO* RENAL DISEASES AFTER TRANSPLANTATION: A SCOTTISH EXPERIENCE

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We present a detailed analysis of graft survival in 789 adult renal transplants performed in a single centre in Scotland between January 1984 to December 1993. Thirty-seven patients were excluded because of primary non-function. During this period, 280 patients (37%) had biopsies of their allograft for proteinuria or deterioration in renal function. Review of biopsies showed that 33 patients had recurrent or *de novo* diseases. IgA nephropathy was the commonest recurrent disease (16 cases, 41%). The other recurrent diseases were FSGS (3 cases); Henoch-Schonlein nephritis (3 cases); Mesangio capillary GN (4 cases); Focal Glomerulonephritis (4 cases); Crescentic Glomerulonephritis (2 cases); and Haemolytic Uraemic Syndrome (1 case). This included *de novo* diseases in 2 cases of focal glomerulonephritis and 1 case of mesangio capillay GN. The mean follow-up period was 9.53 years (minimum 5.26 years, maximum 15.2 years). Graft loss due to recurrent disease was 3.8% (16 patients). Younger age, male gender and living-related transplants were found to be factors increasing the risk of the development of recurrent disease. The risk of recurrent disease at 5 years was 5.9%, increasing to 7.4% at 8 years. The actuarial allograft survival for patients developing recurrent or *de novo* disease was 8.59 years versus 10.1 years for all other patients, which was not statistically significant. We conclude that in our population, recurrent disease does not have a major adverse effect on allograft survival.

P3

A RENAL TRANSPLANT & RENAL-RELATED SURGERY BTS STANDARDS AUDIT PROJECT

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This Renal Transplant Surgery Standards Audit Project (commenced in September 2001) is funded as a NHS supra-district audit project. The aim is to assess performance against selected BTS standards. Initially we selected 17 BTS Standards for audit and grouped these into 7 broad categories. At this early stage we have investigated our past performance in 6 areas.

Our transplant unit has always prioritised allocation of cadaver donor kidneys on the basis of ABO compatibility (now identity) and minimal HLA mismatching. We have always participated in exchange schemes to help achieve this aim. Currently we offer kidneys through the National and North of England Renal Transplant Sharing Alliance prior to local patients. *(BTS Standard 2.5 – "all centres must participate in the sharing schemes which are designed to produce the best possible outcome for every donated organ which is available for transplant")*

From 1991 to October 2001 we performed 1473 renal transplants. Annual transplant activity ranged from 105 (1992) to 148 (1995) for cadaver donors and 4 (1991) to 21 (2000) for living donors. *(BTS Standard 4.3.1 – "Renal transplant units should be capable of achieving 75 transplants per annum")*

Kaplan-Meier survival estimates for 1329 cadaver donor kidney transplants during the period 1991 to October 2001 were:

	Patient survival	Transplant survival
One Year	95.4 (>90)	86.4 (>80)
Five year	87.3 (>80)	72.4 (>60)
Ten year	77.2 (>60)	58.8 (>45)

(BTS Standards 4.7.2 & 4.7.3 – "Patient survival should exceed 90% at 1 year, 80% at 5 years and 60% at 10 years" and "Graft survival should exceed 80% at 1 year, 60% at 5 years and 45% at 10 years")

From 1989-92, 35.8% of our patients received a "favourably" matched kidney (000, 100, 010 or 110 HLA-A, -B, -DR mismatches). This figure rose to 51.4% for years 1993-99 and for 2000-October 2001 the figure is 49.9%. *(BTS Standard 4.5.1 – "45% of recipients should receive a "favourably" matched kidney")*

In 1991 the mean cold storage time for cadaver donor kidneys was 27.6 hours (range 13-56 hours). This mean value consistently reduced year-on-year to 25.3 hours in 1995 and 22.8 hours in 2000. For the period up to October 2001, the mean cold storage time is currently 22.7 hours. *(BTS Standard 4.2.3 – "the kidney cold storage time should whenever possible be kept below 24 hours")*

We will also develop standards for renal transplant associated surgery (vascular access). This preliminary review of past performance is encouraging and outcomes and activity levels must be maintained. Data for performance against other BTS Standards is currently being collected on a prospective basis.

P4

THE CENTRE EFFECT: HIGH INTERCENTRE VARIABILITY IN RECOGNITION AND MANAGEMENT OF RISK FACTORS FOLLOWING RENAL TRANSPLANTATION

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The intercentre difference in renal allograft outcome, affecting both graft and patient survival, is well recognised and has been attributed to centre activity levels/size and case mix. A recent UKT analysis adjusting risk for immune and medical risk factors confirmed a significant difference in results between all UK centres. This observation could be explained by differences in case mix and the immunosuppressive regimen employed in the presence of various risk factors. These findings are significant as there is considerable historical evidence linking risk factors to adverse effects on outcome.

This Group defined immune (ethnicity, BMI, PRA, HLA, positive T/B X-match, CMV status, GI disease, compliance, lipids, cold ischaemia time (CIT) and, for ≥ 2nd transplant, previous graft loss and tolerability of immunosuppressive agents) and non-immune (ethnicity, CIT, dialysis time, malignancies, lipids, age, CMV status, compliance, BMI, smoking, anticoagulant use, glucose intolerance, urinary tract abnormalities, underlying renal disease, Hep B/C positive, cardiovascular disease (CVD)) risk factors generally accepted as influencing graft outcome and which could be used to distinguish between standard and high risk recipients. Case notes from 25 adults and 15 children recently transplanted at the participating centres were examined.

21/25 (84%) of adults had ≥ 1 immune and 25/25 (100%) had ≥ 1 non-immune risk factors. For children, the figures were 9/15 (60%) and 12/15 (80%) respectively. Amongst the adults, the mean number of immune and non-immune risk factors was 1.4 and 2.9; 20/25 (80%) had ≥ 3 risk factors; 5/25 (20%) were diabetic, 13/25 (52%) had pre-existing CVD, 5/25 (20%) were obese, 8/25 (32%) had hyperlipidaemia. The Group validated these observations by determining the prevalence of a limited number of risk factors in the LOTESS database of renal transplant recipients (n=4230). More than 60% of recipients had ≥ 1 risk factor in all years since 1994.

Additionally, an ad hoc telephone survey of 5 centres indicated agreement with the above risk factors and revealed a lack of uniformity in baseline immunosuppression. The importance of risk factors and flexibility of regimen in response to these was recognised. A questionnaire is being circulated to all UK Units to survey this aspect further.

It is proposed that the "centre effect", caused by non uniformity in approach to immunusuppressive regimens in the context of immune and non-immune risk factors could lead to differences in outcome between individual centres. Guidelines should be developed for standardised risk assessment in the UK, and the theme of tailoring immunosuppression to risk should be adopted.

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P5

A PROSPECTIVE, RANDOMIZED TRIAL OF TACROLIMUS/PREDNISONE WITH ZENAPAX INDUCTION VERSUS TACROLIMUS/ PREDNISONE/ MYCOPHENOLATE MOFETIL IN CADAVERIC KIDNEY RECIPIENTS

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Between June 1999 and December 1999, 60 adult patients undergoing their first or second cadaveric kidney transplantation were randomized to receive Tacrolimus/Prednisone with Zenapax induction (n=30) or Tacrolimus/Prednisone/Mycophenolate Mofetil (n=30) with the goal of reducing the incidence of rejection. There were 7 patients with primary nonfunction of the graft (4 in the Zenapax and 3 in the MMF group). These were excluded from analysis. The minimum follow up was 13 months. Patients underwent protocol allograft biopsies at 1 week, 1 month, and 1 year. The patient demographics were matched in both the groups and there were a large number of elderly recipients and donors.

Results The overall 1-year actuarial patient survival was 94.3%: the overall 1-year actuarial graft survival was 77.4% (Zenapax 92.3% and 69.2%; MMF 96.3% and 85.2%).

There were 26 patients <60 years of age and with no delayed graft function (Zenapax, n= 10; MMF, n= 16). In this group the 1- year actuarial patient survival was 96.2% (Zenapax 100%; MMF 81.3%), and the 1-year actuarial graft survival was 84.6% (Zenapax 90%; MMF 81.3%).

The incidence of delayed graft function was 24.5% (Zenapax 30.8%; MMF 18.5%). The incidence of cytomegalovirus was 17 % (Zenapax 11.5%; MMF 22.2%). The distribution of rejection (including protocol and clinically indicated biopsies) is shown below:

	Tacrolimus/Prednisone with Zenapax induction	Tacrolimus/Prednisone/ MMF	Overall
No rejection	4 (15.4%)	5 (18.5%)	9 (17%)
Borderline Only	11 (42.3%)	8 (29.6%)	19 (35.8%)
> Banff IA	11 (42.3%)	14 (51.9%)	25 (47.2%)
> Banff 2A	6 (23.1%)	7 (25.9%)	13 (24.5%)

Over 2/3 of the biopsies showing borderline histopathology, and nearly half of the biopsies showing Banff IA or worse tubulitis, represented sub clinical pathology, i.e. they were performed in patients without evidence of renal dysfunction.

The mean serum creatinine (mg/dl) in the patients with functioning grafts was 1.7 $\pm$ 0.1 (Zenapax 1.8 $\pm$ 0.2; MMF 1.6 $\pm$ 0.2). The mean Tacrolimus dose (mg/day) was 7.2 $\pm$ 0.5 (Zenapax 7.1 $\pm$ 0.1; MMF 7.3 $\pm$ 0.5), and the mean Tacrolimus level (ng/ml) was 9.4 $\pm$ 0.5 (Zenapax 9.7 $\pm$ 0.6; MMF 9.2 $\pm$ 0.8). There appeared to be no statistical difference between the two groups in terms of outcome. This study included a large number of older recipients and a substantial number of older donors, especially in the Zenapax group. The use of protocol biopsies uncovered a high incidence of subclinical rejection. In view of the high incidence of subclinical rejection, it is speculated that the combination of Tacrolimus, MMF, steroids and Zenapax may be worth investigating, to ascertain if it will be associated with a lower incidence of clinical and subclinical rejection.

P6

SPLIT LIVER TRANSPLANTS: IMPORTED OR LOCAL LOBES?

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Background: Splitting a liver can increase the number of transplants achieved by using two lobes from a single liver for two recipients thus achieving two split liver transplants. There is concern that recipients transplanted with cadaveric liver lobes donated from outside the transplant centre's retrieval zone have poorer outcomes than those transplanted with lobes donated from within the centre's retrieval zone. Lobes in the former group are termed imported and those from the latter, local. The aim of this study was to compare the outcome of transplants using local and imported lobes.

Methods: Data on split liver transplants performed in the UK from 1 January 1997 to 31 December 2000 were obtained from the National Transplant Database. Kaplan-Meier estimates of one year graft and patient survival after a split liver cadaveric first transplant were calculated for adult and paediatric recipients separately and compared for local and imported lobes.

Results: Cadaveric liver donors in the UK and Republic of Ireland provided 80 organs for a total of 160 split liver transplants: 158 at transplant centres in the UK; 2 overseas. Of these 80 livers: 65 (81%) were each split and transplanted into one adult and one paediatric recipient; 11 (14%) were each split and transplanted into two paediatric recipients; 2 were each split and transplanted into two adult recipients; 2 were each split and transplanted into one adult recipient in the UK and one recipient overseas. 50 (32%) of the 158 split liver transplants in the UK used an imported lobe. Outcomes for 48 adult and 69 paediatric recipients were:

	Graft survival (95% CI)	Patient survival (95% CI)
Adult transplant recipients		
Imported lobe used	71% (48-95%)	71% (48-95%)
Local lobe used	81% (67-95%)	73% (58-88%)
Paediatric transplant recipients		
Imported lobe used	83% (68-98%)	-
Local lobe used	93% (86-100%)	-

- Insufficient events to provide reliable survival estimates

Conclusion: Split liver cadaveric first transplant adult recipients of imported liver lobes had poorer one year graft and patient survival rates than those who received local lobes. Although the differences were not statistically significant, the size of the difference for one year graft survival does have practical implications. As for adults, the survival rates for paediatric recipients were lower when imported lobes were used than when local lobes were used. Should centres be encouraged to use both lobes from their local donors and only transfer a lobe to another zone for transplant when it is not possible to achieve two local lobe transplants?

P7

THE URGENT HEART SCHEME IN THE UK: THE FIRST TWO YEARS

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Background: The UK operates urgent heart Schemes for adult and paediatric patients: the Adult Urgent Heart Allocation (AUHA) and the Urgent Paediatric Heart Allocation (UPHA) Schemes. These Schemes enable centres to register needy or rapidly deteriorating patients to receive priority over other more stable patients in the organ offering process. This study presents a summary of Scheme activity throughout their first two years of operation.

The Schemes: Both the Schemes were introduced on 1 April 1999 since when, donor hearts are offered preferentially to urgent heart patients. In any one year no more than 38 urgent adult patient registrations are permitted but there is no restriction on the number of paediatric registrations permitted. Centres are only allowed to register one adult and one paediatric patient as urgent at any one time. The AUHA Scheme incorporates a payback system that aims to make up for the loss of hearts to other centres. For every urgent heart transplant performed using an adult donor heart the transplanting centre must payback an adult donor heart into the national pool before they can register their next urgent adult patient.

Activity: Throughout the first two years of operation 77 patients were registered for an urgent heart transplant: 33 adult and 44 paediatric patients. All centres, except one, registered urgent patients. Only one centre used their full urgent adult quota for a year. 24 adult and 25 paediatric patients received an urgent heart transplant but 2 adult and 11 paediatric patients died on the urgent waiting list before receiving a transplant. A further 7 adult and 8 paediatric patients were returned to the routine waiting list, generally because they either had recovered sufficiently to no longer be considered urgent or were no longer considered suitable for an urgent heart transplant.

A total of 90 donor hearts were offered to urgent patients. Many of these hearts, 44%, were declined as the donor was unsuitable for reasons such as their age, medical reasons and their past history. A payback was required, and made, for 17 of the urgent heart transplants; 10 were offered to the centres that provided the urgent donor hearts.

Conclusions: Both Schemes appear to be working well with the majority of urgent heart patients either receiving an urgent heart transplant, 73% of adult and 57% of paediatric urgent patients, or recovering sufficiently to no longer require an urgent heart transplant, 21% of adult and 18% of paediatric urgent patients. The destination of payback hearts will continue to be monitored to ensure that centres are not disadvantaged by providing donor hearts for urgent patients at other centres.

P8

HLA-DR HOMOZYGOSITY AND THE NATIONAL KIDNEY ALLOCATION SCHEME

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On behalf of the UK Transplant Kidney and Pancreas Advisory Group

The fate of HLA-DR homozygous patients in the UK requiring a kidney transplant was investigated in 1999. It was found that these patients represented 22% of the active national transplant waiting list yet consistently only 14% of kidneys donors were HLA-DR homozygous. It was also apparent that many kidneys from homozygous donors were transplanted into prioritised groups of patients such as children and highly sensitised patients due to the large pool of patients who are well HLA matched for a homozygous donor. This resulted in long waiting times and poorly matched transplants for HLA-DR homozygous patients.

Evidence from simulations of revised allocation protocols showed that prioritising national homozygous patients over local heterozygous ones would increase access to well matched grafts for HLA-DR homozygous patients, while not disadvantaging children and highly sensitised patients. This proposed modification of the national Kidney Allocation Scheme was agreed by Renal Transplant Unit Directors and implemented in July 2000.

The effect of the modification to the Scheme has been investigated after its first year of operation. Results show that while HLA-DR homozygous patients still represent 22% of the active waiting list, the HLA matching of transplanted homozygous patients has improved significantly through increased access to kidneys from homozygous donors. In the two years to June 2000, only 25% of HLA-DR homozygous kidneys were transplanted in homozygous recipients. In the year that followed the modification of the Scheme, the corresponding proportion was significantly higher at 43% ($p<0.0001$). The effect of this is clearly seen in looking at HLA matching of transplants in HLA-DR homozygous patients in the two years prior to modification of the Scheme (July 1998 – June 2000) compared with the following year (July 2000 – June 2001):

	000		Favourable		Non-favourable	
	No.	%	No.	%	No.	%
July 98 – June 00	29	11	47	18	182	71
July 00 – June 01	22	14	52	33	84	53

The HLA matching results are still inferior to those for HLA-DR heterozygous patients, but represent a significant improvement over those for homozygous patients transplanted in the previous two years ($p<0.001$).

The process of identifying an inequality with regard to kidney allocation, using computer simulation of the Allocation Scheme to investigate potential modifications and then implementing agreed changes has been shown to be a successful strategy in the example of HLA-DR homozygous patients.

P9

REPEAT KIDNEY TRANSPLANTATION IN THE UK

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On behalf of the UK Transplant Kidney and Pancreas Advisory Group

Background: The factors affecting first graft outcome are well known and many of the same factors also affect repeat graft outcome. However, factors related to the first graft, such as duration of graft function, may also affect the outcome of repeat grafts. This analysis compares recipient characteristics of first and repeat kidney transplants in the UK and investigates three year transplant survival of second, and of third and subsequent, adult cadaveric kidney only transplants.

Method: The cohort analysed comprised transplants in 1991-2000 but for the three year outcome analysis, grafts in 1991-1997 were analysed. These included 988 second grafts and 118 third and subsequent grafts. Factors considered in the Cox regression analyses were: recipient age, waiting time, diabetes and gender; donor age, cause of death and gender; graft year, shipping, HLA match and kidney damage. In this analysis of regrafts, the following factors related to first graft were also investigated: transplant survival time, HLA matchgrade and donor type (cadaveric/living).

Results: Recipients of repeat grafts between 1991 and 2000 were significantly younger than first graft recipients: mean 39.1 years (s.e. 0.3) compared with mean 44.0 years (s.e. 0.1), p<0.0001. They also received significantly better HLA matched grafts: 41% of first grafts were 000 or favourably matched compared with 55% of repeat grafts, p=<0.0001. Finally, very few of the repeat transplant cohort were known to be diabetic: 2% compared with 6% of first grafts, p=<0.0001.

Three year transplant survival estimates for first and second grafts were the same – 76% (95% CI's 75-77% and 73-78% respectively). For third and subsequent grafts, survival was lower - 73% (95% CI 65-81%). The analysis of second graft outcome showed that survival time of first graft, donor age, recipient gender and kidney damage significantly influence three year transplant survival. Recipients whose first graft failed between 3 and 18 months have significantly inferior survival on regraft than recipients whose first graft survived three years or longer (relative risk of failure=1.93, p=0.001). As for first grafts, the risk of transplant failure significantly increases with donor age over 35 years. Female recipients appear to have a significantly increased risk of transplant failure than males (relative risk of failure=1.40, p=0.01), and the difference in transplant outcome on using damaged rather than undamaged kidneys is significant at the 10% level (relative risk of failure=1.33, p=0.07). For third and subsequent grafts the analysis of 118 grafts showed only donor age to be significant at the 10% level, with the greatest risk of transplant failure occurring within the 35 to 49 years age range.

Conclusions: Recipients of repeat grafts are significantly younger, are less likely to be diabetic and receive significantly better HLA matched grafts. Three year transplant survival of second grafts is significantly influenced by survival time of first graft, donor age, recipient gender and kidney damage. The analysis of a small number of third and subsequent grafts showed donor age as the only factor significantly influencing transplant outcome.

P10

URINARY N-ACETYL β-D GLUCOSAMINIDASE (NAG) EXCRETION IN RENAL TRANSPLANT RECIPIENTS WITH STABLE FUNCTION AND THOSE WITH CHRONIC ALLOGRAFT NEPHROPATHY (CAN)

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Background. There is interest in finding urine markers that are associated with renal allograft rejection. NAG is synthesised by proximal renal tubular epithelial cells and is therefore detectable in the urine if these cells are damaged. Low urinary NAG excretion in the immediate post-transplant period predicts stable early graft function, and a rise in NAG excretion typically precedes a rise in serum creatinine. Other work has suggested that NAG excretion is dependent on CsA dose. We have compared urinary NAG excretion in cyclosporin (CsA)-treated renal transplant recipients with CAN (group A, n=25, mean SeCr 225 umol/l) and those with stable long-term graft function (group B, n=59, mean SeCr 125 umol/l). Within A, the effect of immunosuppression conversion (CsA to FK506/ azathioprine to MMF with CsA dose reduction/ 'no change' controls) on NAG excretion was also studied.
Methods. Fresh morning urine samples were collected and stored at -20^{0}C. Repeat samples were collected after 6 months in A. A colorimetric assay was used to quantify urine NAG activity (PPR diagnostics, London, UK). Values were adjusted according to the urine creatinine concentration.
Results. No patients had microbiological evidence of urosepsis at the time of sample collection. There was large inter-patient variability in NAG excretion. Urine NAG values were not significantly different in A and B. There was no correlation between NAG excretion and glomerular filtration rate (GFR). In A, the change in NAG excretion over 6 months in the control and intervention groups was not significantly different, nor was there a significant difference between patients grouped by the rate of decline in their transplant function.
Discussion. These cross-sectional and longitudinal data suggest that urine NAG excretion is not a useful guide to GFR in renal allograft recipients, nor is it a specific marker for CAN.

P11

LONG-TERM OUTCOME OF LIVER RETRANSPLANTATION IN CHILDREN.

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Background
Retransplantation of the liver is the only means of prolonging survival in children whose initial graft has failed. Patient and graft survival after retransplantation has been reported to be inferior to first transplantation in most series. However the results are improving, especially after late retransplantation and patient survival is approaching that seen after first transplantation.

Patients and methods
Of 450 paediatric liver transplants performed between January1990 and March 2001, 50 underwent retransplantation once, 9 had a second retransplant, of whom one received a third retransplant. The overall retransplantation rate was 13.3%. The median age at retransplantation was 4 years (range, 54 days to 16 years) and the median weight was 15 kg (range, 3 kg to 80 kg). Twenty three (46%) children were retransplanted within a month of their first transplant and were classified as 'early retransplants'. The commonest indications for retransplantation were hepatic artery thrombosis (HAT) in 18 (36%) patients and chronic rejection in 13 (26%).

Results: The median post retransplantation follow up was 73 months (range, 6 months to 139 months). The Kaplan Meier patient survival for the group (n=50) was 71.7% at one year and 64.7% at 3,5 and 10 years. Graft survival was 65.6% at one year and 56.7% at 3, 5 and 10 years. This is significantly worse compared to that of children undergoing first liver transplant (one, 3, 5 and 10-year patient survival: 88%, 84%, 82.3% and 79.6% respectively; p=0.003). There have been 17 deaths, of which 9 occurred in the first month. Nine patients underwent a further retransplant, one of whom has had a fourth transplant. In the early retransplantation group, the Kaplan Meier patient survival figures are 69.6% at one, 3 and 5 years respectively; all deaths occurred within a year of retransplantation. The corresponding graft survival figures are 69.6% at one year and 64.6% at 3 and 5 years. In the late retransplant group, the patient survival figures are 73.4% at one year and 60.7% at both 3 and 5 years while the corresponding graft survival figures are 62.2% at one year and 49.4% at 3 and 5 years (p=0.8, not significant). Improved patient and graft survival was observed in the later phase of the retransplantation program, although the difference was not significant (p=0.4). Biliary complications occurred in 5 (10%) patients and vascular complications in 6 (12%). Increased preoperative serum creatinine (p=0.001) and serum bilirubin (p=0.02) were significantly associated with higher postoperative mortality.

Conclusion
Results of retransplantation in children are inferior to those seen with first transplantation. There is a trend towards improving results. Liver retransplantation makes an important contribution to overall survival in children.

P12

QUANTIFICATION OF EPSTEIN-BARR VIRAL LOAD IN PERIPHERAL BLOOD OF RENAL TRANSPLANT PATIENTS USING REAL-TIME PCR

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Introduction Increased risk of Epstein-Barr viral infection occurs with immunosuppression and uncontrolled proliferation of EBV-infected B cells has been strongly associated with Post transplant lymphoproliferative disease (PTLD). Conventional PCR has been used in the past to monitor EBV gene load for PTLD. However this is a time consuming approach and an alternative would be to use real time PCR.
Materials & Methods A longitudinal study was carried out on 30 renal transplant recipients and 7 haemodialysis patients on a weekly basis over a period of 4-5 months. 11 healthy control subjects, 3 patients with previous history of PTLD, and 7 patients with infectious mononucleosis were included. DNA was extracted from 2 ml of whole blood. TaqMan Methodology was used and samples were analysed using an ABI Prism 5700 Sequence Detection System. An EBV Polymerase gene probe was used with a Raji cell line for calibration.

Results None of the renal transplant recipients developed PTLD but all recipients displayed an increase in gene load. This generally peaked at two-week post transplant and returned to background levels within one month. Patients with infectious mononucleosis had higher levels of EBV gene copies/ µg amplified DNA than those seen after transplant. The three patients who had had PTLD in the past, demonstrated levels that were similar to post transplant patients. Healthy controls had very low levels of EBV and stable dialysis patients had slightly increased levels, which did not achieve levels seen in the transplant recipients.

	Healthy control (11)	Dialysis patients (7)	Transplant patients (30)	Infectious Mononucleosis (7)	Previous PTLD (3)
%individuals >35 gene copies/µg	0	28.6	40	85.7	33
Peak/copies/µg	2	64	211	582	56
Median/copies/µg	0.4	8	14	131	13
Mean/copies/µg	0.5	16	39	201	26

Conclusion Real time PCR can be successfully used to monitor the EBV viral load in transplant patients.

P13

CREG MATCHING AND KIDNEY TRANSPLANT FUNCTION: A SINGLE CENTRE STUDY.

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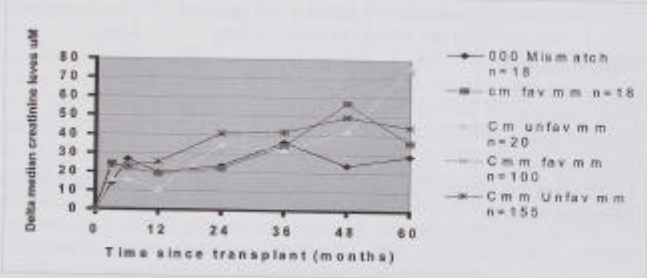
Introduction It is well documented that HLA antigen matching is beneficial to transplant survival. Ethnic minorities exhibit private HLA specificities that are rare in the donor population, and are therefore disadvantaged when allocation of organs is determined principally by HLA matching. Recent studies have suggested that it may be possible to address this problem through consideration of cross-reacting epitope groups (CREGs) in cases where private specificity matching cannot be achieved. These studies have implications within tier two of the UK allocation strategy. Accordingly, we have examined the association between CREG matching and long-term renal allograft function in patients who were transplanted in our unit between 1995 and 2000 (census December 2000).

Methods Follow-up data have thus far been obtained for 311 patients. CREG groups were assigned according to Rodey et al. Those who lost their grafts prior to census were accorded a serum creatinine score of 1000µmol/l for subsequent time intervals.

Results Data are presented as Δ median serum creatinine level at time intervals up to 60 months post transplant. Comparison of results for groups of CREG matched/HLA favourably mismatched patients and those that were CREG mismatched/HLA favourably mismatched shows that there was a marginal benefit associated with CREG matching. The same relationship was found in comparing CREG matched/HLA unfavourably mismatched with CREG mismatched/HLA unfavourably mismatched groups.

Conclusion These data confirm the influence of HLA matching on clinical outcome, but do not suggest an additional role for CREG matching in the allocation of donor kidneys.



P14

HOW OLD IS OLD FOR TRANSPLANTATION?

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AIM: Elderly patients with end stage renal disease are the fastest growing age group requiring transplantation. This study investigates whether transplantation is worthwhile in the elderly and whether there should be an age limit for transplantation.

METHODS: 1095 adult patients received a first transplant in Scotland between 1st of January 1989 and 31st of December 1999 and were followed up to 11 years. Sociodemographic data, comorbidity details and transplant outcome information were obtained from the national databases and patients' notes. Outcome variables (patient and graft survival, causes of death and failure, risk of graft failure and patient death adjusted for comorbidity and sociodemographic data) were compared in 4 age groups (18-49, 50-59, 60-64 and >65).

RESULTS: The four age groups had a similar gender and social deprivation distribution and comparable incidence of diabetes as the primary renal disease or a comorbid condition. There was a significantly higher incidence of peripheral vascular disease, ischaemic heart disease, valvular disease, arrhythmias, heart failure, respiratory disease, and gastrointestinal disorders with increased age. The groups had comparable HLA matching, while the donor was younger and the cold ischaemic time was shorter in patients aged 18-49 compared with the other patients. Younger patients had a higher incidence of acute rejection episodes and a lower incidence of delayed graft function. There was no difference in the causes of death between the four groups. A higher incidence of death with functioning graft and a lower incidence of immunological failures were seen in older patients. The patient and graft half life (Log rank) as well as the adjusted risks of death and graft failure (Cox regression) are shown in the table.

	18-49 years	50-59 years	60-64 years	>65 years	p value
Patient half-life (years)	>11	10.98	6.39	6.86	0.0001
Graft half-life (years)	10.97	9.09	5.52	6.34	0.0001
Adjusted relative risk of death (95%CI)	1 (reference)	2.37 (1.28-4.39)	2.84 (1.12-7.18)	7.19 (3.54-4.59)	<0.0001
Adjusted relative risk of graft failure (uncensored) (95%CI)	1 (reference)	0.91 (0.59-1.38)	0.63 (0.28-1.42)	1.51 (0.86-2.64)	0.2012

Table 1. Estimated patient and graft half-life and relative risk of patient death and graft failure.

CONCLUSION: Despite a higher comorbidity index, transplantation in elderly patients is worthwhile and a careful patient selection rather than a set age limit should be used to ensure a sustained graft and patient survival.

P15

THE EFFECT OF HLA MATCHING ON THE OUTCOME OF LIVING-DONOR KIDNEY TRANSPLANTATION

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Introduction: There has been a steady increase in live kidney donor transplantation in the U.K. in an attempt to redress the shortage of cadaveric donor kidneys. Some studies have indicated inferior graft outcome associated with HLA-DR mismatching and have questioned the advisability of performing HLA-DR mismatched live kidney donor transplants. In two UK transplant units we have maximised the use of living donors for kidney transplantation, regardless of HLA mismatch grade.

Methods: Between 1995 and 2001, 117 living-donor kidney transplants were performed at the two centres. Clinical follow-up data was available for 89 genetically related and 22 unrelated transplants. Graft survival, one month and one year serum creatinine (Cr) levels, number and timing of rejection episodes were collated and their relationship with HLA mismatch (mm) grade was examined. Statistical analysis was performed using the Epi-Info statistical package (World Health Organisation).

Results: Three (3.2%) of 94 live-related and one of 23 (4.3%) live-unrelated donor transplants failed within one year. In addition, one recipient of a live-unrelated transplant died with a functioning graft. Acute rejection occurred in 10% of favourably HLA matched transplants (N=29) compared with 60% of non-favourably matched transplants (N=82), p<0.001. There was a strong influence of HLA-B and HLA-DR mismatches on the incidence of acute rejection (Table 1). Acute rejection occurred in 10% (N=29) of 0 HLA-DR mismatched (0-DRmm) transplants, compared with 56% (N=66) with 1-DRmm and 75% of 2-DRmm transplants (p<0.001).

HLA locus	0 mismatch	1 mismatch	2 mismatches	p-value
HLA-A	34% (N=29)	49% (N=75)	71% (N=7)	NS
HLA-B	20% (N=30)	50% (N=64)	82% (N=17)	<0.001
HLA-DR	10% (N=29)	56% (N=66)	75% (N=16)	<0.001

Of the surviving transplants there was a correlation between the presence of acute rejection and one month Cr levels (p=0.05). Transplants with 2 HLA-B mismatches had higher one month Cr (median 159μmol/l) compared with 0 HLA-B mismatches (median 130μmol/l), p=0.03. Despite the strong effect of HLA mismatches on acute rejection, there was no correlation between HLA-A, -B or -DR mismatch grade and patient serum creatinine levels at one year.

Conclusion: Living-donor kidney transplants which are poorly HLA matched have a high incidence of early rejection and increased one-month serum creatinine levels. However, graft function at one-year is comparable to well-matched grafts, and further evaluation of HLA mismatched living-donor kidney transplantation is justified.

P16

COMBINED LIVER AND KIDNEY TRANSPLANTATION FOR PRIMARY HYPEROXALURIA (PH1)

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Introduction: PH1 is an autosomal recessive deficiency of peroxisomal alanine: glyoxylate aminotransferase which may be partial or complete. PH1 occurs at a rate of approx 1per million per year. In the absence of the normally located functional enzyme in the peroxisomes of hepatocytes to convert glyoxylate to glycine the glyoxylate is metabolised in the cytoplasm by LDH to form oxalate. The only route of oxalate excretion is via the kidney and here formation of calcium oxalate crystals leads to nephrocalcinosis, stone formation, obstructive uropathy and eventually to renal failure. Systemic oxalate deposition then rapidly ensues with progressive clinical deterioration and death. The first liver transplant for PH1 was carried out in Cambridge in 1984 and confirmed that this could reverse the metabolic defect and led to the adoption of combined liver and kidney transplantation as a treatment for PH1. A registry is maintained in Cambridge of patients undergoing liver transplantation for PH1 at European centres.

Results: Between 1984 and 2001 thirty-two European Centres reported 103 liver transplants (usually combined with a renal transplant) in 98 patients with a diagnosis of PH1: 76 of these 98 patients are currently alive. Mean age of onset of first symptoms was 5.2 ± 7.5 years with a delay to diagnosis of 4.1 ± 5.4 years. Mean age at transplantation was 18.3 ± 12.9 years and the duration of dialysis was 3.3 ± 3.1 years. There was a family history in 40% of cases including 29 siblings with PH1, 4 cousins, one parent and 13 cases not otherwise specified. One, two and five year patient survival rates were 85%, 82% and 79% and graft survival rates were 80%, 73% and 71% at the same time intervals. Patients who had been on dialysis for <2 years at the time of transplant were more likely to be assessed as being in good general condition at the time of transplantation. These patients had better survival than those who had been on dialysis for longer and came to transplantation with evidence of marked systemic oxalosis. Combined liver/kidney transplantation appears to give excellent results in patients with PH1 although the results are poor when transplantation is delayed until advanced sytemic oxalosis has developed.

P17

ATTITUDES TO ORGAN TRANSPLANTATION IN A MUSLIM INDO-ASIAN COMMUNITY IN THE UK

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The lack of organs available for transplantation is a major problem in the UK, but the immigrant Muslim Indo-Asian community, with its higher incidence of end stage renal failure, suffers more acutely. Since donation is low from within that community, we sought to understand the attitudes to organ donation in this particular ethnic group, using an exploratory qualitative study. This approach also assessed awareness of organ donation for transplantation within this community in West London.

The fieldwork was divided into three stages using participants' observations, focus group discussion, and in-depth individual interviews. A sample of forty Muslim Indo-Asians participated in this study (13 female, 27 male), 18 years of age or older, with a variety of socio-demographic backgrounds. Most of the respondents were aware of both live and cadaveric organ donations. This information had been acquired from the national media and through the received experience of people undergoing transplants within the same community. However, most were opposed to it, for a mixture of religious and cultural reasons. This was true, despite the fact that Islamic religious authorities have pronounced the practice acceptable under religious law. However, the respondents believed that organ donation is inconsistent with Islamic beliefs, such as the principle that the body should be buried intact and complete. Those who would consider donation required that the recipient be from within their own community. The only four that had at one time held donors cards had subsequently canceled them. There was remarkably few differences between different participants' answers. Young participants were more willing to learn about the process of organ donation than were older people. In conclusion, there is a dissonance between the facts of Islamic law and public perceptions.

P18

LIVE KIDNEY DONATION IN THE ETHNIC MINORITY COMMUNITIES

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For many people with chronic renal failure, renal transplantation is the best treatment option, potentially improving their life expectancy and quality of life.
In general there are over four people waiting for each cadaveric kidney that is donated. However, the situation is worse for the members of the non-Caucasoid ethnic minority groups. In the United Kingdom, only 11% of patients receiving a kidney transplant were from these communities, although they currently represent 35% of the patients on the transplant waiting list.

At the end of 2000 there were 6,284 people waiting for a kidney but only 1,487 cadaveric and 336 live transplant occurred in that year. Over 95% cadaveric kidneys donated come from the Caucasians. In the Hammersmith Hospitals NHS Trust, West London, there are currently 257 patients on the kidney transplant list of whom 52% are from the Indo-Asian and African-Caribbean communities.

In 1998 two posts were established to help increase knowledge and awareness of transplantation in these minority communities with the hope that this might increase cadaveric and live donation. The awareness campaign uses the local media, which targets the Indo-Asian and African-Caribbean communities (newspapers, magazines, radio, and press organisations) and their communal institutions (religious, such as temples (Hindu and Sikh), mosques, churches and Secular, such as social organisation and associations.

116 Asian patients have been reviewed and 37 (32%) potential donors identified. Of these, 14 (12%) were blood group compatible. After undergoing further investigations, 10 (9%) potential donors were found to be suitable. To date, two live-related transplants have occurred and others are planned for the next few months.

77 African-Caribbean patients have been seen and 22 (29%) potential donors identified. Due to incompatible blood grouping and patients refusing to accept donation from their families, as well as potential donors changing their minds, there are now 3 (4%) potential recipient in the latter stages of workup. To date, one live-related transplant has occurred.

In conclusion, an intensive personalised programme to encourage donation from these ethnic groups has identified appropriate donors for only 12% Asian chronic renal failure patients and 4% African-Caribbean patients at this institute. The impact on the cadaveric donation rate is not yet known.

P19

PROGNOSTIC SIGNIFICANCE OF REVERSAL OF DIASTOLIC FLOW AT COLOUR DUPLEX SCANNING IN RENAL ALLOGRAFTS

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Background: Reversal of flow during diastole (RFD) demonstrated by duplex scanning has been reported to carry a dismal prognosis. This study was carried out to determine long term graft outcome in a large series of patients with RFD.
Methods: A database consisted of 6489 (serial) duplex scans performed in 669 patients (9.7 scan per patient) in the period 1992 to 2001. Of these, 63 grafts(9.4%) in 62 patients had RFD.
Results: The following were the underlying pathology: acute rejection in 33, acute tubular necrosis (ATN) in 17, ATN with ureteric obstruction in 1, renal vein thrombosis in 8, pyelonephritis in 1, contrast induced ATN in 1, post-infective glomerulonephritis in 1 and cyclosporine toxicity in 1. Median follow up was 72 months. 29/63(46%) patients lost their graft, 17 (27%) of which necessitated nephrectomy. The mean serum creatinine in surviving grafts at 1 year and 5 year respectively, was 175 and 202 µmol/l. The overall graft survival was 66.7% and 50% respectively, at 1 year and 5 years, respectively.
Conclusion: RFD is a bad prognostic sign and is associated with higher incidence of severe resistant rejections. RFD with RI of >1.2 is associated with high incidence of graft loss.

P20

OUTCOME OF CADAVERIC RENAL ALLOGRAFTS CONTAMINATED BEFORE TRANSPLANTATION

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Background: This analysis was performed to define the frequency of pre-transplant contamination and resultant morbidity including infections requiring therapy, and its impact on outcome including graft loss.
Methods: Case records of 638 renal allograft recipients patients transplanted in our centre during the period 1990 to 2000 were studied. 775 reports of perfusion fluid swabs, kidney swabs and ureteric tissue were retrieved. A single dose of intra-venous antibiotic (most commonly Co-amoxyclav) is used in this unit for all the recipients at the time of induction of anaethesia.
Results: Of 638 patients, 58 received renal grafts with evidence of pre-operative contamination. Twenty two out of 58 patients were treated with antibiotics. Thirty patients out of 32 who had contaminant from skin flora had a benign course (recipients who neither had positive blood culture nor graft infection). By contrast, 7 out of 9 recipients with positive culture of perfusion fluid due to lactose fermenting coliform (LFC) required antibiotics and three of them (33%) suffered graft loss as a result. Two of these patients had bacteraemia due to LFC, and one died. Three out of 5 patients with positive cultures due to yeast were treated with antibiotics. At one year 46/54 (85.2%) of these patients survived with functioning graft. One year patient survival was 50/54 (92.6%).
Conclusion: This data suggest that the contamination of renal allografts by LFCs or yeast needs to be treated aggressively. By contrast, the contamination by gram positve organisms does not pose risk to the graft; the single dose of prophylactic antibiotics appears enough to prevent serious risk.

P21

AUDIT OF LABORATORY ACTIVITY SUPPORTING A LIVING DONOR TRANSPLANT PROGRAMME

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Introduction: With the increasing shortfall in cadaveric kidney donors a number of options for increasing the donation rate have been explored. One of these is to promote living donation. We have audited activity in our laboratory in order to assess the impact of promoting living donation.
Method: A database was established in 1997 to record all potential living donors who were referred to the laboratory. Data from April 1997 to March 2001 was analysed. Factors included in the analysis were HLA mismatch, relationship, donor suitability and outcome data. The endpoints for the work up of a potential donor were living donation, cadaveric transplant of the recipient or establishing either donor or recipient as unsuitable.
Results: 422 potential donors were HLA typed for 255 potential recipients over the 4 year period from 5 dialysis centres. Only 56 (21.9%) recipients received a living donor transplant. 34 (13.3%) recipients received a cadaveric transplant during the course of live donor work-up and 2 potential recipients died before transplant. When this data was broken down by financial year there was an increase in the number of potential donors HLA typed in 1998/99, after which the level of activity was maintained. This is illustrated below:

Referral Date	1997/98	1998/99	1999/2000	2000/01
Potential Donors	67	126	129	100
Potential Recipients	44	81	80	66
Live related transplants	17	13	17	9
Cadaveric transplant	5	8	16	5

Whilst the number of potential donors investigated has increased, the number donating has not increased proportionally. 238 potential donors for 167 recipients were considered unsuitable for the following reasons;

ABO incompatible	11 donors (4.6%)
Did not fulfil local HLA matching criteria	67 donors (28.1%)
Donor specific HLA sensitisation	76 donors (31.9%)
Social / personal reasons for withdrawal	39 donors (16.3%)
Medically unsuitable	45 donors (18.9%)

47 donors are still being considered for 32 recipients. For these recipients the time from initial donor specimen receipt to March 2001, the endpoint for this audit, ranged from 1 to 33 months.

Conclusion: This audit has shown that despite the increase in laboratory activity we have seen no increase in transplant rate over the study period. This illustrates that the cost implications of promoting a living donor transplant programme are considerable.

P22

THE ROLE OF N-ACETYL CYSTEINE ADMINISTERED DURING THE DONOR OPERATION ON SUBSEQUENT LIVER TRANSPLANTATION: A RANDOMISED PILOT STUDY

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Background and Aims: Anti oxidant and a glutathione precursor, NAC has been used for several years in treating acute liver failure from poisoning such as paracetamol over dose. There is experimental evidence that N-acetyl cysteine (NAC) reduces liver preservation damage. This study was undertaken to study the effect of NAC administered in the donor operation on transplantation (OLT).
Methods: 22 patients were randomised to receive NAC (Group A) or placebo (Group B) during donor operation performed by the RFH team. IV infusion of NAC (150mg/kg, ~ 10 grams) was started 15 minutes before cardiac arrest and also in the perfusion solutions for portal vein (75mg/kg,~5grams) in the cold phase of dissection and bench perfusion before bagging.
Results: Four patients were excluded from analysis (re-exploration within 48 hours and >30 units peri-operative blood transfusion x 3, OLT for acute x 1). The two groups were matched for recipient and donor ages and sex. Viral hepatitis accounted for cirrhosis in 2 patients in group A and 6 patients in group B while ALD was more frequent in group A (4:1). The mean cold ischaemia time and warm ischaemia time were similar in both groups as was the use of blood and blood products. The changes in liver function tests (Serum bilirubin, ALP, AST, ALT, Prothrombin time) did not show any significant differences in the first week post transplant. Baseline post-reperfusion biopsy showed minimal reperfusion injury in 6/9 in group A and in 6/8 in group B. The incidence of acute cellular rejection was also not effected by the use of NAC.
Conclusion: NAC administered during donor operation did not show a protective effect on IR injury or acute rejection.

P23

USING THE JOINT BRITISH SOCIETIES CORONARY RISK PREDICTION CHARTS TO CALCULATE CORONARY HEART DISEASE RISK AFTER LIVER TRANSPLANTATION

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Background
Hypertension, hypercholesterolaemia and weight gain are common after liver transplantation. It is not known whether development of these complications alters the cardiovascular risk profile after liver transplant.

Methods
The case notes of 110 consecutive adult liver transplant recipients surviving beyond one year were reviewed.

Results
Median follow-up was 52 months (range 6-90 months). 74 % of patients developed hypertension compared with 3 % being hypertensive before transplant ($P<0.001$). Hypercholesterolaemia was present in 16 % before and 60 % after transplant ($P<0.001$). 29 % had a body mass index > 25kg/m^2 at the time of transplant compared with 58 % after transplant ($P<0.001$). Diabetes mellitus developed in 4 % after transplant, resulting in a post-transplant prevalence of 12 %. There were two non-fatal cardiac events: one myocardial infarct and one heart failure. One patient sustained a non-fatal cerebellar infarct. The Joint British Societies Coronary Risk Prediction Charts categorise 10-year coronary risk (on the basis of total cholesterol:high density lipoprotein cholesterol ratio, systolic blood pressure, smoking, diabetes mellitus, age and gender) as < 15 %, 15-30 % and > 30 %. Using these charts we categorised coronary heart disease risk before and after transplant.

10-year risk	Before transplant	After transplant
> 30 %	0	5 %
15-30 %	18 %	42 %
< 15 %	82 %	53 %

If we assume patients require therapeutic intervention aimed at reducing risk when risk is 15 – 30 % or greater, 20 patients (18 %) would require treatment before transplant compared with 52 (47 %) after transplant.

Conclusions
Coronary heart disease risk increases after liver transplant. The number of observed cardiovascular events is low. We would expect more events than we have seen.

P24

A NEW, FAST AND SAFE TECHNIQUE OF BENCHWORK PREPARATION OF THE PANCREATIC GRAFT

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Introduction: The meticulous benchwork preparation of the pancreatic graft is vital to perform the transplant with minimal difficulties and no complications. The ETS-FLEX (articulating) Endoscopic Linear Vascular Cutter has been used extensively for dividing vessels in endoscopic surgery. When positioned on a vessel, it applies three staple lines proximally and three distally and it divides the vessel between them. We describe a new simplified technique for the benchwork preparation of the pancreatic graft using these staples.
Methods: There are three steps in the benchwork preparation where the vascular staples are applied: a) the splenic hilar vessels are stapled and divided by applying the vascular staples and performing a splenectomy, b) the mesenteric root which has already been divided with the TA 90 stapler during the procurement is reinforced proximally by applying the vascular staples and c) any peripancreatic lymphatic tissue or small vessels that have not been ligated during the procurement are identified and stapled with the same stapler.
Results: We have used this technique in ten pancreatic grafts. The time for the benchwork preparation including the Y-graft anastomosis (mean $\pm$ SD) was 45 $\pm$ 5 minutes. Following revascularisation, there was excellent reperfusion of all grafts with minimal and easily controllable bleeding, not requiring blood transfusion.
Conclusions: The use of the vascular staples simplifies the benchwork preparation of the pancreatic graft resulting in: a) significantly shorter benchwork preparation time, b) minimal manipulation of the pancreatic graft and c) minimal bleeding following revascularisation.

P25

A FAST AND SAFE LIVING DONOR NEPHRECTOMY TECHNIQUE

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Introduction: The living donor nephrectomy needs to be done in a way that exposes the donor to the minimum possible risks, with excellent graft function. We describe the use in the living donor nephrectomy of ETS-FLEX (articulating) Endoscopic Linear Vascular Cutter which allows a quick division of the renal vessels and ureter and perfect haemostasis, which are of vital importance in achieving the above mentioned goals.
Methods: When positioned on a vessel, the Vascular Cutter applies three staple lines proximally and three distally and divides the vessel in between them. The distal end of the Vascular Cutter that has the staples can be rotated in a way that allows it to fit perfectly on a vessel regardless of the angle this is lying in the operating field. The kidney is approached through a 9±1cm Loin incision. An Omnitract retractor is used. After dissection-free of the vessels, the ureter is stapled and divided and then the vascular staplers are applied initially to the renal arteries, first on the main renal artery and subsequently in smaller arteries if present. Then the staplers are applied on the main renal vein followed by smaller renal veins if present. The kidney is then flushed after removal of the staple lines.
Results: Between October 2000 and November 2001 we have performed 16 living donor nephrectomies using this technique. Four kidneys had a second renal artery, two kidneys had two renal veins and one kidney had three renal veins. The warm ischaemia time (mean ± SD) was 60 ± 5 seconds. The operative time was (mean ± SD) 60 ± 10 minutes. In all cases, there was no need for further haemostasis after the removal of the kidney and there were no operative complications. All grafts were successfully revascularised with 100% patient and graft survival (range of follow up 1-11 months).
Conclusions: The use of the vascular staples in living donor nephrectomies reduces significantly the warm ischaemia and total operative time and allows a very safe removal of the kidney via a very small incision, especially when there is more than one renal arteries or veins.

P26

MARKERS OF RENAL TUBULAR INJURY IN TRANSPLANT PATIENTS WITH CHRONIC ALLOGRAFT DYSFUNCTION: EFFECTS OF IMMUNOSUPPRESSION AND PROTEINURIA

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Renal transplant (T/P) patients have tubular dysfunction, which is multifactorial in origin. Nephrotoxic drugs, such as Cyclosporin and Tacrolimus contribute by several mechanisms. Also, tubular markers of injury can be difficult to interpret in view of the additional and variable tubular damage from proteinuria. In this study we measure urinary N-acetyl β,D-glucosaminidase (NAG) & its more tubulo-specific A2 isoenzyme as a marker of tubular dysfunction and injury. We also use regression analysis of urinary NAG (UNAG) vs urinary protein (Upr) to identify drug nephrotoxicity separately from the effects of proteinuria.

UNAG & its A2 isoenzyme were measured in 106 urines from 68 T/P patients, 9.7 ± 6.2 (1 -18) years after T/P. Creatinine clearance (CrCl) was 32 ± 22 (7-111) ml/min, Upr 2.0 ± 2.0 (0 –10.9) g/24h. 32 patients were taking Cyclosporin but not Tacrolimus, 20 were on Tacrolimus but not Cyclosporin, and 16 patients were taking neither Cyclosporin nor Tacrolimus. Other agents included prednisolone, azathioprine, and mycophenolate mofetil.
A group of 120 non-T/P patients with native nephropathies (CrCl 41 ± 30, 4 -152 ml/min, Upr 3.1 ± 2.3, 0.1-11.3 g/24h) were also studied.

In 12 T/P, A2 isoenzymes predominated in urine (83.5 ± 9.5% of total), indicating a tubular origin for UNAG. In both T/P & non-T/P patients, UNAG and Upr were correlated ($r=0.57$, & 0.65 respectively, $p<0.001$). In T/P on Cyclosporin, the slope of the regression UNAG/Upr (161 ± 35.8 mmol/g) was similar to non-T/P (161 ± 17.7 mmol/g). However, the intercept for UNAG, when Upr = 0, was higher (690 ± 106 mmol in T/P vs 387± 68 mmol in non-T/P, $p < 0.001$). In T/P on Tacrolimus, both the slope and the intercept of the regression of UNAG/Upr were similar to T/P on Cyclosporin (116 ± 43.6 and 659 ± 149.7 mmol respectively).

By contrast, in T/P on neither Cyclosporin nor Tacrolimus, UNAG in 14 of the 16 patients fell below the UNAG/Upr trend line for T/P on Cyclosporin at any level of Upr. The intercept of the regression gave a value of UNAG of 261 ± 72mmol, when Upr = 0,which was less than in patients on Cyclosporin or on Tacrolimus ($p<0.001$).

These data suggest that suprisingly Tacrolimus is as toxic to the tubules as Cyclosporin. UNAG can be used to identify such toxicity in T/P patients even in the presence of chronic graft dysfunction and proteinuria.

P27

DONOR GLOMERULAR SCLEROSIS HAS A SIGNIFICANT IMPACT ON FUNCTION AND SURVIVAL OF CADAVERIC RENAL TRANSPLANTS.

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In the quest to expand transplantation, an increasing number of kidneys are retrieved from "marginal" donors. This calls for the need to develop algorithms to assess the quality of grafts prior to allocation.

Aim: To determine if the percentage of donor glomerular sclerosis accurately reflects the "quality" of the organ and has an impact on graft function and survival.

Material & Methods: All cadaveric renal transplants with an adequate renal wedge biopsy at the time of transplant were included. Glomeruli were assessed on PAS stained sections and a percentage of sclerosed glomeruli (PSG) was calculated. Clinical data was obtained from the WTRG. **Results**: 210 patients satisfied inclusion criteria. There were 129 grafts without sclerosed glomeruli (PSG = 0% - Group1) and 81 kidneys (39%) showed glomerular sclerosis ranging from 1% to 60%. Patients with PGS>0% were divided into 3 groups based on 50th and 75th percentile values. Group 2 represented PGS of up to 10% (n=42), Group 3 PGS 10%-20% (n=22) and Group 4 PGS>20% (n=17). The median observation period was 3.8 years (range 1.0 - 6.3 years). Grafts with sclerosed glomeruli demonstrated inferior renal function as early as 3rd post-operative day (median GFR 17 ml/min vs. 24 ml/min; p<0.001, Mann Whitney) and the significant difference persisted throughout the observation period (median GFR at 1 and 4 years was 49ml/min vs. 62 ml/min; p<0.001 and 51ml/min vs. 31 ml/min; p=0.01 respectively). The correlation between PGS and Cockroft-Gault GFR was highly significant (p<0.001, p<0.001 and p=0.003 at 3 days, 1 year and 4 years respectively -Pearson Correlation). Based on median GFR between year 1 and 4 linear trends were extended to predict function at 10 years. Patients without glomerular sclerosis had the highest GFR at 1 year (66.5 ml/min) and the slowest deterioration (-3.8 ml/min/year), in contrast, patients with PGS>20% had the lowest GFR at 1 year (46.0 ml/min) and the steepest rate of annual decline (-9.0 m/min/year). A multivariate linear regression analysis of a dependable variable "GFR at 4 years" constructed of independent variables: PGS, donor age (DA), number of acute rejection episodes (AR), donor GFR (DGFR) and tacrolimus or cyclosporin based therapy (DRUG) showed that PGS and AR were significant with p=0.008 and p=0.02 respectively, whereas DA (p=0.31), DGFR (p=0.06) and DRUG (p=0.14) were not statistically significant in this model. This analysis demonstrated that every increment of glomerulosclerosis by 1% in biopsy at time zero was responsible for 0.8 ml/min drop in the GFR at 4 years. A corresponding value of the coefficient (B) for each acute rejection episode was 7.0 ml/min thus, PGS of 10% has an equal detrimental effect on the GFR at 4 years as a single acute rejection episode. To compare it with an effect of the age of donor, an increase of 30 years in DA would have a similar effect. Older donors represent a considerable proportion of the donor pool and in this series 33% of donors were over the age of 55. Although, there was a natural correlation between PGS and donor age (p<0.001; Pearson's Correlation), 43% of older donors (DA ≥ 55 years old) had no glomerular sclerosis and further 26% showed PGS<10%. Furthermore, a considerable proportion of younger donors (29%) were found to have sclerosed glomeruli with 13% showing PGS>10%. A 5-year Kaplan Meier graft survival for the four groups was 80%, 88%, 59% and 35% respectively (p=0.04, Log Rank). A Cox Regresion constructed of categorical donor factors (age over 55, total ischaemic time over 24 hours, Cockroft-Gault donor GFR< 70 ml/min and PGS>10%), demonstrated that a significant overall score (Chi-Square 11.2, p=0.02) was achieved with PSG>10% being the significant covariate (p=0.01, Exp (B)=2.7 and 95% CI 1.2 - 5.6). The corresponding values for donor age over 55 were: p=0.88, Exp (B)=1.1, 95% CI 0.5-2.3, for ischaemic time: p=0.11, Exp(B)=0.4, 95% CI 0.1-1.2 and for donor GFR: p=0.47, Exp(B)=1.3, 95% CI 0.6-2.8. **Conclusions:** The degree of donor glomerular sclerosis has a major impact on graft function and survival and appears to be the best predictor of outcome of all other donor factors. The findings of this study indicate that a routine biopsy should be performed at the time of procurement as it may be relevant to the subsequent allocation of a cadaveric kidney.

P28

UPREGULATION OF HEME OXYGENASE-1 IS ASSOCIATED WITH PROTECTION OF THE RENAL ALLOGRAFT FROM ISCHEMIA REPERFUSION INJURY.

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Ischemia reperfusion injury (I/RI) leads to early graft dysfunction characterised by a reduction in GFR. Ischemia upregulates Hypoxia inducible factor 1 α (HIF-1) which in turn increases endolethin 1 (ET-1) mRNA and heme oxygenase-1 (HOX-1) expression. ET-1 is a potent vasoconstrictor which causes a reduction in GFR. In contrast HOX-1 overexpression protects organs from ischemic injury in animal models of IR/I. The aim of this study was to determine whether HOX-1 protects the renal allograft from (IR/I). We have used quantification of HIF-1 and ET-1 mRNA as a sensitive measure of ischemic injury. Fifty two renal allograft recipients underwent protocol renal biopsies following allograft reperfusion. Renal allograft expression of HIF-1, ET-1 and HOX-1 mRNA were quantified using real time RT-PCR. Two groups were identified on the basis of graft function at 7 days. Results are expressed as mean±SEM; statistical significance (*p<0.05) between groups was assessed by t test.

		Primary Function	Delayed Function
N		26	26
7 day Creat.	μ mols/l	135±5	427±54*
Cold Ischaemia Time	hrs	17±2	23±2
Donor Age	yrs	42±2	47±3
HIF-1 mRNA	Log Co No.	3.4±.2	3.0±.2
ET-1 mRNA	Log Co No.	2.7±.2	2.2±.2
HOX-1 mRNA	Log Co No.	3.4±.1	2.7±.2*

Both groups suffered similar ischemic injury as demonstrated by cold ischemia times, HIF-1 and ET-1 mRNA expression. Donor age could not account for differences in renal function between the two groups. However, HOX-1 expression within the renal allograft was significantly increased following reperfusion in those patients with good primary graft function.

This study supports the hypothesis that HOX-1 protects the renal allograft from ischemic injury.

P29

HEALTH AUTHORITY RATES FOR PREVALENT TRANSPLANT PATIENTS, COMPARED WITH THEIR DIALYSIS POPULATION.

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Identifying patients by their transplant unit has always caused difficulty in calculating incidence and prevalence rates as many transplant centres additionally transplant for several dialysis units. This is also complicated by the fact that some dialysis units send patients to more than one transplant centre. After transplantation, some transplant centres transfer patients back to their local renal unit while other centres continue to follow patients up indefinitely.

In the year 2000, the Renal Registry covered 33 million of the UK population and collected quarterly follow up on all patients in end stage renal failure, including transplant patients. Using a post-coding package (QAS systems), the post codes were first 'cleaned' by validation and correction against their full address. The postcodes are mapped to health authorities and regions, permitting analysis against these defined populations. The prevalent data collected by the Registry was analysed by these populations.

The transplant prevalence rate by health authority varies significantly from 120 to 400 per million population. These variation may be caused by several factors. Low prevalence rates may be as a result of fewer patients starting renal replacement therapy. Comparing these rates against the dialysis population provides a basis for comparison.

The median Registry transplant and dialysis prevalence rate was 251 and 278 pmp respectively. Morecambe Bay has a low prevalent transplant rate of 126 pmp with a low dialysis population of 203 pmp patients compared with the adjacent North Cumbria with figures similar to the UK median of 254 pmp. and 251 pmp respectively. In contrast Wolverhampton has a low transplant prevalence rate of 161 pmp and from a large dialysis population of 520 pmp. These figures may also be influenced by lower rates of listing for transplantation and / or lower rates of transplantation (in some regions caused by lack of donors with the tissue types found in the ethnic minority population). Central Nottingham has transplant rate of 238 pmp, close to the UK median although the dialysis prevalence rate very high at 415 pmp. This can be compared with adjacent North Nottinghamshire rate of 216 and 334 respectively.

Further work on equity of access will be in collaboration with UK Transplant, comparing these data with the incidence transplant and waiting list rates by health authority .

P30

FUNDING DECISIONS FOR ANTI-REJECTION THERAPIES: THE USE OF REAL COSTS AND ECONOMIC MODELS TO PREDICT COST-EFFECTIVENESS

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The National Institute for Clinical Excellence (NICE) has indicated that immunosuppressive agents will be evaluated in a forthcoming wave of appraisals. This will include assessments of efficacy and cost effectiveness. Traditionally, there has been considerable interest in the funding of these agents, particularly since their increasing availability in the mid 1990's. Indeed, the difficulties encountered securing funding from local agencies has led to a substantial variation in usage across the UK. These difficulties exist despite often compelling statistical advantages in favour of the new agents both in terms of efficacy and tolerability. There has been no large, UK-based, economic evaluation of these agents and as such we have analysed our clinical and economic outcomes over a 1 and 3 year period.

The case notes of 111 patients receiving renal transplants during 1997 were examined and recorded clinical events and investigations costed and collated. All patients received Neoral based triple therapy. A Markov 'economic modelling' technique was used to predict how these patients would fare in terms of clinical events and costs if Mycophenolate Mofetil (MMF) had been substituted for azathiaprine in their regimen. Predictions for MMF were based on published multi-centre studies up to one year and extended on the basis of UNOS three year data.

The acute rejection rate for the azathioprine and MMF groups was 31% and 17% respectively. The one year acquisition cost of MMF was £3,200/year against £545/year for azathioprine. However, the additional cost of MMF treatment was reduced to £1,200 at one-year when the reduction in acute rejection events and their associated costs were taken into account. Hence, at one year the incremental cost effectiveness ratio is moving to favour MMF. Moreover, when the outcomes and costs are extended beyond 1 year, MMF usage becomes cost neutral at approximately 2.5 years, after which cost savings would be anticipated.

Decisions concerning the funding of immunosuppressive agents should not be based on short-term data, particularly isolated drug acquisition costs. The results of this study reinforce the concept that a longer view, based on the real costs of the whole transplant process, is required if an assessment of cost-effectiveness is to be made. The use of economic models, based on such costs may, by providing an early prediction of cost-effectiveness, assist funding bodies to make early decisions on the funding of agents, so bringing a more unified approach to UK practice.

P31

MRA IN THE EVALUATION OF LIVE RENAL DONORS – THE VALUE OF CLINICAL AUDIT & SOURCES OF REPORTING ERROR

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Introduction: Magnetic resonance angiography (MRA) is a non-invasive tool for evaluation of potential live donors. The aim of this retrospective study was to assess the accuracy of MRA reports when compared with operative findings, look for the sources of any errors and determine how reporting accuracy can be improved.

Methods: The reports on the findings of patients who underwent gadolinium contrast enhanced MRA as pre-operative imaging were compared with the subsequent surgical findings. Where there was discrepancy, studies were retrieved to the MRI workstation and subjected to detailed joint clinical and radiological review. Scan quality was assessed and sources of error were classified as either due to radiological/interpretation error, communication error, technical limitations, patient related factors or a combination of multiple factors.

Results: There were 32 patients, 17 men and 15 women with a mean age of 43 years. The MRA findings were fully confirmed in 27 of the 32 patients, these scans were not further analysed (4 patients known to have accessory vessels were operated upon with this knowledge and the MRA findings were confirmed). In 5 patients, the reported MRA findings were discrepant. In four instances, an accessory artery was found where none had been reported. In one instance, with good scan quality this was purely a radiological error, in the other four a combination of factors were present. In two instances, we found what was thought to be an accessory vessel but on review was a single artery with early division. In another instance, 2 accessory vessels were encountered at surgery where the MRA report indicated only one, at review this further vessel was visualised only faintly on MRA, which was again an early division of the upper hilar artery. An accessory vessel was found at surgery in one of the patients, which was not detected on MRA retrospectively. A duplex collecting system found at surgery was also not reported at MRA, however, the MRA protocol had at that time not been designed to assess for this specific information. The feedback from surgery has been used to inform the radiologists interpreting the MRA studies and our work up protocol has been made more comprehensive to include MR Urography.

Conclusion: The prospective accuracy of MRA in the evaluation of renal donors is related to multiple factors and we believe this can be improved through careful audit with interdisciplinary co-operation.

P32

INTERIM REPORT OF THE USE OF STREPTOKINASE IN THE NON HEART BEATING DONOR.

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Introduction

The discard rate of non heart beating donor kidneys is high (9 – 45%) for a number of reasons. One of the reasons being, poor perfusion due to intravascular thrombosis. Recent animal work has suggested that the use of thrombolysis is beneficial in the perfusion of organs from such donors. We have conducted a randomised blinded study using streptokinase (1.5MU) or placebo (10mls normal saline) as a warm preflush in addition to the standard heparinised preservation solution. The code on the study had to be broken recently after a recipient had a significant haemorrhage in the post transplant period. The results to date are summarised below:

Results

Group	Non Streptokinase	Streptokinase	Fisher's test, *p* value
Initial NHB donors	12	12	
Kidneys harvested & perfused	22	14	
Paleness at retrieval Easy back table flush	27.3 % (6/22) 36.4 % (8/22)	85.7 % (12/14) 85.7 % (12/14)	< 0.005 < 0.01
Recipient requiring transfusion < 1/52	44.4 %	28.6 %1	ns
Total graft failure & discard rate	63.6 % (14/22)	50 % (7/14)	ns

Conclusion

There was no significant risk of haemorrhage in the recipients of kidneys from donors treated with streptokinase. In addition the quality of the kidneys obtained on gross appearances were significantly better with thrombolysis. As yet the discard rate in the streptokinase group though lower has not yet reached significance.

P33

WHY CARDIOTHORACIC ORGANS FROM NOMINAL CARDIOTHORACIC DONORS ARE UNUSABLE

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Background: It is not always feasible to retrieve cardiothoracic organs from donors who donated other solid organs. This study examined the reasons given for cardiothoracic organs not progressing through the various stages of the offering process. This may help identify a cohort of potential donors whose cardiothoracic organs are not fully utilised for reasons that could be addressed and overcome.

Data: Data on 787 adult cadaveric nominal cardiothoracic donors in the UK and Republic of Ireland during 2000 were obtained from the UK National Transplant Database. The reasons cited by transplant centres for the non-availability, non-retrieval and non-use of the cardiothoracic organs from these nominal donors were also obtained. A cadaveric nominal cardiothoracic donor is defined as an individual aged 60 years old or under who did not die from a heart attack and for whom at least one solid organ was made available for transplant. Heart valve only donors, asystolic donors and donors whose cardiothoracic organs were only donated for research were excluded.

Nominal heart donors: During 2000 there were 725 adult cadaveric nominal heart donors from whom 600 (83%) hearts were made available for transplant. Hearts were primarily unavailable as permission for donation was not given, 64 (51%), the donors were unsuitable for medical reasons, 24 (19%), or the donors were unsuitable ages, 20 (16%). 377 (63%) of the available hearts were not retrieved, mainly because the donors were unsuitable for medical reasons, 231 (61%). Of the retrieved hearts, 218 were used but 5 were unusable, 4 because the donors were unsuitable for medical reasons.

Nominal lung donations: In 2000 there were 1446 adult cadaveric lungs nominally available for transplant and 1135 (78%) of these became available. Lungs were mostly unavailable as no permission was given for donation, 125 (40%), or the donors were unsuitable for either medical reasons, 102 (33%), or their age, 42 (14%). Of the available lungs, 896 (79%) were not retrieved, mostly because the donors were unsuitable for medical reasons, 571 (64%), or the lungs had poor function, 135 (15%). 207 (87%) of the 239 retrieved lungs were used in 35 single and 86 double lung transplants. The remaining 32 lungs were unusable, most frequently because the donor was unsuitable for medical reasons, 15 (47%).

Conclusions: At least three-quarters of the hearts and lungs from adult cardiothoracic nominal donors were made available for transplant. Cardiothoracic organs from nominal donors were unavailable for transplant because no permission for donation was granted or the donors were unsuitable ages. Permission was generally refused by the family; understanding why they refuse may identify a training or publicity need that could help change the attitudes of other potential donor families. It was not feasible to retrieve many of the available cardiothoracic organs, as the donors were unsuitable for medical reasons. Retrieved organs were generally transplanted although some proved to be unusable, as the donors were unsuitable for medical reasons.

P34

PRE-EMPTIVE TREATMENT OF CYTOMEGALOVIRUS DISEASE IN HIGH-RISK RENAL TRANSPLANT PATIENTS

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Introduction:
Prevention of cytomegalovirus (CMV) disease in the high-risk renal transplant patients (seropositive donor to seronegative recipient) can take the form of prophylaxis with antiviral medication for 3 months or pre-emptive therapy, which targets treatment only to those patients who have evidence of viremia before the onset of symptoms. It has been shown that the qualitative polymerase chain reaction (PCR) gives the earliest indication of CMV transmission and this has been used as the basis for guiding pre-emptive therapy. In this study, we have evaluated this policy since its introduction in 1997.
Methods: 51 consecutive high-risk patients were monitored by qualitative PCR test, done twice weekly for the first three months. There were 31 cadaveric and 20 living related transplants. The immunosuppression protocol was based on a calcineurin inhibitor with or without an adjunctive agent. No patient received OKT3 or a polyclonal antibody. Patients with 2 consecutive positive tests were treated with intravenous gancyclovir for a minimum of 2 weeks, 30%(n=8) of whom were treated on an outpatient basis.
Results: 20(39.%) patients had 2 consecutive positive PCR test. 3 patients developed all features of the CMV syndrome. The mean length of treatment was 16.25 days (range 10-21days), only 2 patients requiring a further course of treatment. No patient developed CMV associated organ dysfunction or died of CMV disease over a mean length of follow-up of 18.7 months (range 4 – 46 months). The actuarial patient survival is 95.5% and the graft survival 88.8%.
Conclusions: Only 40% patients in this high-risk group showed evidence of virus transmission and therefore 60% of the cohort did not require antiviral therapy. Routine prophylaxis would have resulted in considerable over-treatment. Our policy of pre-emptive management of cytomegalovirus infection with gancyclovir, based on a screening protocol using qualitative PCR appears to be safe and effective.

P35

DIFFERENCES IN TACROLIMUS DOSAGE REQUIREMENTS BETWEEN UK ETHNIC GROUPS

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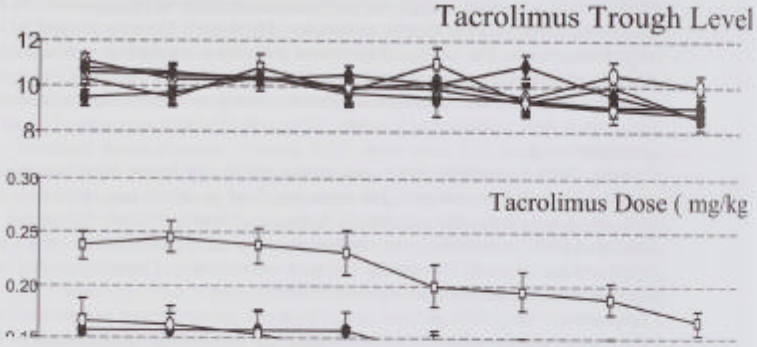
Differences in the pharmacokinetics of Tacrolimus have been described between white and African-American transplant recipients. We retrospectively examined 3,448 Tacrolimus trough levels (paired with patients dosing and weight) in 184 renal transplant recipients from five ethnic groups (119 Caucasian, 29 Indo-Asian, 17 Afro-Caribbean, 10 Middle-Eastern, and 9 Oriental). Immunosuppressive regimes included prednisolone and azathioprine or MMF, with Tacrolimus in twice daily dosing.



Results: There were no significant differences in trough levels between the groups. Afro-Caribbean patients required significantly higher doses than Caucasian patients with the difference being greater than previously reported from US studies (mean dose difference vs Caucasian 0.114 mg/kg, $p<0.001$). Indo-Asian patients dose requirements were closely similar to the Caucasian group. The Middle-Eastern and Oriental patient groups required significantly higher doses than the Caucasian patients (Mid-East vs Caucasian mean dose difference 0.038 mg/kg, $p<0.001$: Oriental vs Caucasaian mean dose difference 0.039 mg/kg, $p<0.001$), and significantly less than the Afro-Caribbean group (Mid-East vs Afro-Caribbean mean dose difference 0.074 mg/kg, $p<0.001$: Oriental vs Afro-Caribbean mean dose difference 0.075 mg/kg, $p<0.001$).

Summary: Our Afro-Caribbean patients require more Tacrolimus than comparable US group. In addition we have shown that Middle-Eastern and Oriental patients require more Tacrolimus than our Caucasian and Indo-Asian graft recipients.

P36

TACROLIMUS DOSE REDUCTION IN STABLE RENAL ALLOGRAFT RECIPIENTS: A RANDOMISED CONTROLLED TRIAL

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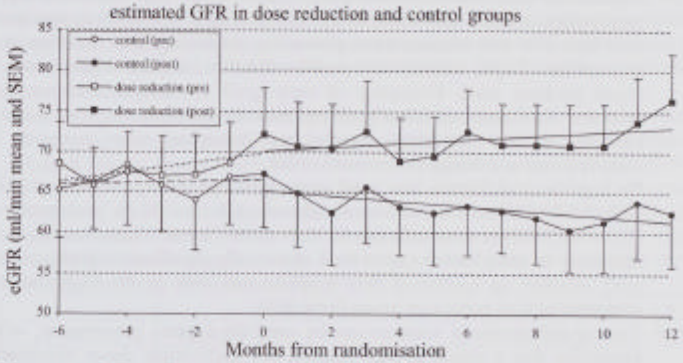
We undertook a randomised, controlled trial to assess the benefits and risks of reduction in Tacrolimus dose in stable renal allograft recipients. 19 patients were randomised to reduction in target trough levels from 10-15 ng/ml to 5-8ng/ml at a mean 23 months post transplantation (range 15 – 39). 10 patients were randomised to remain at target levels of 10-15 ng/ml at a mean 22 months (range 15-33 months). Patients were followed for 12 months post randomisation with measurement of creatinine, weight, estimated and isotope GFR, HbA1, and with oral Glucose tolerance tests at the time of acceptance into the study and after 12 months.



Mean trough Tacrolimus levels fell from 13.4 to 8.3 ng/ml in the dose reduction group over the 12 months from randomisation. Trough levels did not change significantly in the control group (13.1 to 12.7 ng/ml). Isotope GFR improved significantly after dose reduction (from 64 to 72 ml/min $p=0.002$ 95% CI for change 2.5 to 11.4) There was no significant change in isotope GFR in the control group (95% CI for change –1.95 to 9.25 ml/min) Although the incidence of *de novo* diabetes during the study period was less in the dose reduction than in the control group (3/19 vs 3/10) this was not significant, and there was no significant change in HbA1 in either group. There were no rejection episodes in either group.

Summary: Reduction in target Tacrolimus trough levels to a target range of 5-8ng/ml after 1-2 years of stable graft function produces a significant improvement in graft function without any identifiable risk of rejection.

P37

LONG AND SHORT TERM RESULTS OF PROSPECTIVE RANDOMISED TRIAL OF LIVER PRESERVATION USING HIGH SODIUM VERSUS HIGH POTASSIUM LACTOBIONATE/ RAFFINOSE SOLUTION

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High sodium as opposed to the commonly used high potassium lactobionate /raffinose preservation solution (UW solution) offers potential theoretical advantage in improving the quality of storage of livers for transplantation.

In this study we evaluated in a prospective randomised trial the efficacy of a high sodium (n= 32) versus high potassium solution (n=29) for organ preservation in patients undergoing cadaver donor liver retrievals and subsequent transplants. Livers were harvested from heart beating cadavers and perfused in vivo through canulae in the portal vein and abdominal aorta with ice cold Baxter's kidney perfusion solution. After removal of the liver, the portal vein, hepatic artery and the bile duct were flushed respectively with 1000ml, 500ml and 250 ml of either high sodium or high potassium preservation solution and stored in ice. The quality of preservation was assessed in the post operative and intraoperative phases by measuring liver function tests, namely transaminase levels, bilirubin and prothrombin time, and the requirement of blood and blood products used. Frequency of early graft dysfunction and nonfunction were recorded. Incidences of acute graft rejection as confirmed by liver biopsy requiring treatment in the first three months post transplant were recorded. Long term complications and deaths were also recorded.

No significant difference was seen in the post operative peak AST (1274.9 vs 879), peak ALT (976 vs 835), minimum albumin (36.1 vs 37.3), peak prothrombin time (25.06 vs 37.07) or peak bilirubin (132.3 vs 160) levels. There was also no significant difference in complications apart from statistically significant higher incidence of early graft rejection as confirmed liver biopsies was seen in the high sodium group as compared to high potassium group ($p< 0.005$).

Thus a sodium based solution can be used for hepatic preservation, advancing the possibility that a single storage solution for multi-organ donor operations could be developed.

P38

HEAT SHOCK PROTEIN, INDUCIBLE NITRIC OXIDE SYNTHASE AND APOPTOTIC MARKERS IN THE ACUTE PHASE OF HUMAN CARDIAC TRANSPLANTATION

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Objective: Solid organ transplantation may be associated with activation of apoptotic pathways and other stress markers. It is however not clear to what extent these pathways are active in clinical cardiac transplantation, where, following brain stem death acute heart failure frequently affects the right ventricle. We aimed to describe the pattern of expression of Bax and Bcl-2, two critical apoptotic markers, and of iNOs and Hsp70 together with their relationship with donor organ failure, if any.

Methods: 12 patients undergoing heart or heart-lung transplantation (including 2 domino cases) were studied with serial trucut biopsies from the right (RV) and the left ventricles (LV) at the following time points: after donor optimisation; at the end of ischaemic time; and after 10 minutes of reperfusion. The 1-week endo-myocardial RV biopsy was included too. Five donor hearts turned down purely on functional grounds were also analysed. Bcl-2, Bax, iNOs and Hsp70 were examined with immunohistology.

Results: There was no difference between the RV and the LV for any of the markers at intraoperative assessment. The pattern of expression was however not predictive of allograft failure. Donor hearts have a strong pro-apoptotic phenotype, which is largely unopposed by the protective factors Bcl-2 and Hsp70 (see table). Furthermore, the intensity of myocyte staining increases over time for Bax ($p<0.001$) and iNOs ($p=0.02$). Domino hearts (free of brain stem death) showed a similar pattern of expression. Compared to usable organs, poorly functioning donor hearts have stronger myocardial staining for Bax ($p=0.002$) and iNOs ($p=0.01$).

	Intraoperative		Postoperative, 1 week	
Marker	Endothelium	Myocytes	Endothelium	Myocytes
Bcl-2	-	-	-	-
Bax	+/-	+	+	+
Hsp-70	-	-	-	+
iNOs	+	+	+	+

Conclusions: Clinical cardiac transplantation is associated with activation of the Bax and iNOs pathways in both ventricles. The myocardium is affected in time-dependent fashion but this is compatible, to a certain extent, with satisfactory allograft function. Our findings may assist future organ preservation efforts and may be relevant in preventing future allograft vasculopathy.

P39

STRESS ASSOCIATED WITH ORGAN RETRIEVAL

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Introduction: There has been recognition by members of our Transplant Unit that multi-organ retrieval is associated with unique stressors that may be both practical, with donor surgery often taking place during the night in unfamiliar surroundings and emotional, in dealing with bereaved relatives. There has been little research in this area and any published work has focused on the effect of organ retrieval on donor hospital theatre staff, highlighting several stressors.

Aims:

1) To determine whether members of the team found multi-organ retrieval to be a stressful part of their work
2) To identify stressors, as practical, emotional or both
3) To determine current mechanisms of stress management adopted by staff
4) To determine whether staff felt that a more formal support system was indicated.

Methods: Thirty-two questionnaires were sent to members of a multi-organ retrieval team. Anonymity of the respondents was assured.

Results: Twenty-six questionnaires were returned. The grade and time with retrieval team, and number of retrievals (mean) attended in 2000 are summarised in the table below:

Grade	Consultant Surgeon	SPR/SHO	Theatre Nurse	Perfusionist	Transplant Coordinator
Number of respondents	5	7	6	5	3
Time with team	>5 years	0.5 - > 5 yrs	2 - > 5 yrs	0.5 – 4 yrs	0.5 - > 5yrs
Retrievals	7	9	13	16	9

Respondents found some (11/26) or all (15/26) retrievals stressful. On a linear scale of 1-10, the most stressful retrieval scored a mean of 5.4 (range 2.2-7.8). Aspects of retrieval were divided into practical, emotional or both as shown below:

Grade	Consultant Surgeon	SPR/SHO	Theatre Nurse	Perfusionist	Transplant Coordinator
Practical	3	3	1	1	1
Emotional	2	2	4	3	0
Both	0	1	1	1	2

Sixteen respondents made additional comments. Of these 8/16 mentioned additional stress associated with organ retrieval from children/young people. The majority of coping mechanisms were talking to family/friends (22) and talking to contemporaries (18). Seventeen of the respondents would like some type of support mechanism, with 9/17 wishing access to individual support.

Conclusion: This pilot study demonstrated that staff involved in the process of multi-organ retrieval acknowledged the stress associated with it. The majority of the group felt that a more formal support mechanism would be beneficial.

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ESTIMATION OF INDIVIDUAL RESPONSE TO ALLO-STIMULATION AND IMMUNOSUPPRESSION.

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Long term survival of renal allografts is limited by complications of potent immunosuppressive drugs (ISD). In the clinical forum individuals differ in their response to ISD. An allo-specific model of sensitivity to ISD at a cellular level may enable variations in individual response to be predicted.

Peripheral blood mononuclear cells (PBMC) from 12 children ('recipients') awaiting renal transplantation and 10 healthy age-matched controls were incubated for 1 hour in the presence of CsA 0-250ng/ml. PBMCs from a parent (prospective live donor) were irradiated (30Gy) and cultured with those of the child in the presence of interleukin 2. In some cases, the assay was repeated using stimulator cells from individuals with a two-haplotype mismatch with the child. Non-irradiated PHA-stimulated target cells from the parent were labelled with non-radioactive europium and added to the culture after 7 days incubation. Target cell lysis was quantitated by time-resolved fluorometry. CsA-mediated inhibition of target cell lysis was calculated and used to compare individual responses to the drug. Two-colour flow cytometry was performed after 7 days to identify activated subsets of lymphocytes at varying concentrations of CsA (0-250 ng/ml).

There was a wide variation between individuals in percent lysis following allo-stimulation and a wide variation in inhibition of cell lysis by CsA that was independent of degree of lysis. CsA-mediated inhibition of cell lysis was also independent of the level of HLA mismatch between donor and recipient, indicating that response to CsA may be a recipient phenomenon.

Maximal inhibition of target cell lysis in patients and controls ranged from 18% to 98%. Seven out of twelve patients and 5/10 controls were 'sensitive' to CsA in-vitro, in that they achieved maximal inhibition of cell lysis at low concentrations of the drug (<50ng/ml). Eleven of the twelve patients have subsequently received a renal transplant. Interestingly, five out of seven patients who were 'sensitive' to CsA in our assay have had problems in the post-transplant period (severe viral infections, nephrotoxicity and graft vasculopathy), possibly reflecting over-immunosuppression.

Immunophenotyping indicated expansion of $CD8^+$ and $CD25^+$ subsets after allo-stimulation that was partially or completely inhibited by CsA. This correlated with CsA-mediated percent inhibition of target cell lysis.

These data imply a potential role for this model in prediction of individual response to immunosuppressive therapy following allo-stimulation in the pre-transplant setting.

P41

A PROSPECTIVE RANDOMISED TRIAL OF LAPAROSCOPIC AND OPEN LIVE DONOR NEPHRECTOMY: EFFECTS ON POST-OPERATIVE RESPIRATORY FUNCTION.

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Introduction
Laparoscopic live donor nephrectomy is a new minimally invasive technique devised to avoid the effects of a large loin incision. A prospective randomised trial has been established to compare donor recovery following transperitoneal laparoscopic donor nephrectomy (LDN) and retroperitoneal open donor nephrectomy (ODN) without rib resection. The aim of this analysis was to compare post-operative respiratory function and analgesic requirements after these two operations.

Patients and Methods
A consecutive series of 22 patients were randomly allocated in a 1:1 ratio to undergo live donor nephrectomy either by LDN or ODN. Post operatively all donors were managed using a patient controlled analgesia system (PCAS). Respiratory function was assessed by pre- and post-operative spirometry and overnight pulse oximetry recordings. De-saturation was defined as a reduction in overnight arterial oxygen saturation of greater than 4% for greater than 10 seconds.

Results
In-patient stay (mean ± sem) was significantly shorter in patients undergoing LDN (4.6 ± 0.5 vs 5.5 ± 0.5 days: $P<0.05$). Patients undergoing the laparoscopic procedure recorded less pain on the first post-operative day (linear analogue score 71 ± 26 vs 42 ± 24; $P<0.05$). On the third post-operative day patients in the ODN group had lower mean (95%CI) arterial oxygen saturations (94.4 ± 0.61 vs 96.7 ± 0.43; $P<0.01$) and a higher de-saturation index (5.86 ± 2.04 vs 1.83 ± 0.53; $P<0.05$).

Conclusions
These preliminary results suggest that laparoscopic live donor nephrectomy is associated with less post-operative pain and an improvement in respiratory function measured by overnight pulse oximetry. Further studies are required to define the potential benefits of the laparoscopic approach.

P42

A RANDOMISED TRIAL OF MYCOPHENOLATE MOFETIL VERSUS AZATHIOPRINE AS CALCINEURIN INHIBITOR SPARING AGENTS IN THE TREATMENT OF CHRONIC ALLOGRAFT NEPHROPATHY

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Introduction Two of the factors implicated in the pathogenesis of chronic allograft nephropathy (CAN) are the nephrotoxicity of the calcineurin immunosuppressive drugs, and immune response against the allograft. Therefore calcineurin inhibitor dose reduction is a theraputic strategy that may be employed to treat CAN, with cover by an alternative, non-nephrotoxic immunosuppressive agent. Azathioprine (aza) has been used for this purpose in the past, however mycophenolate mofetil (mmf) has been shown to be a more potent immunosuppressive agent in the prevention of acute rejection, and to have antifibrotic properties. The aim of this prospective randomised trial was to compare the relative efficacy of aza and mmf used with calcineurin inhibitor dose reduction as a treatment for CAN.
Methods Patients on cyclosporin or tacrolimus based immunosuppression underwent a 40% dose reduction of their calcineurin inhibitor and were randomised to receive either aza (1 mg/kg/day) or mmf (1g b.d.). Subjects underwent transplant biopsy and isotope GFR measurement at entry and 6 months later. Inclusion criteria were a functioning graft at least 6 months post transplant with biopsy proven CAN and informed consent. Exclusion criteria were recurrence of primary disease, acute rejection, pregnancy or a GFR <15ml/min. The rate of change of GFR over time was calculated in the six months prior to, and the 6 months during the study. The changes in slopes between treatment groups were compared by the Mann-Whitney U test. Significance was taken at the $p=0.05$ level.
Results 21 patients receiving cyclosporin (10 mmf; 11 aza) and 22 receiving tacrolimus (12 mmf; 10 aza) were recruited into the study. All groups were well matched for the potentially confounding variables of donor and recipient ages, cold ischaemic times, donor source (HBD or NHBD), previous acute rejection episodes and incidence of delayed graft function. Rate of change of GFR is presented in the table below, values are median (range).

	Pre-trial GFR slope (Δ(ml/min)/month)	Post-trial GFR slope (Δ(ml/min)/month)	P value
CyA and MMF	-0.37 (2.5)	0.85 (3.2)	0.01
CyA and AZA	-0.31 (3.6)	-0.45 (2.9)	NS
FK506 and MMF	-0.28 (1.5)	0.92 (5.8)	0.02
FK506 and AZA	-0.23 (2.4)	1.25 (4.3)	0.05

Discussion Both mmf and aza allowed tacrolimus reduction with a significant improvement in GFR over the trial period. For patients on cyclosporin, dose reduction covered by mmf also improved GFR, however azathioprine did not. This may be due to the relative immunosuppressive, nephrotoxic and pro-fibrotic potencies of the drugs involved. Longer follow-up is required to assess whether those treatment groups showing improved GFR confer long-term benefit.

P43

CHRONIC RENAL ALLOGRAFT SALVAGE: MYCOPHENOLATE MOFETIL CONVERSION WITH REDUCTION OR ELIMINATION OF CALCINEURIN INHIBITORS.

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BACKGROUND: Long-term maintenance therapy with calcineurin inhibitors (CIN) such as cyclosporin A (Cs A) and tacrolimus (FK 506) presents a considerable incidence of chronic renal toxicity. In this prospective study we demonstrate the safety and efficacy of rescue therapy with mycophenolate mofetil (MMF) in patients with chronic allograft nephropathy (CAN) in kidney transplantation.

METHODS: 25 renal transplant recipients with chronic deteriorating graft function were converted to MMF. Patients were maintained on MMF and Prednisolone with or without low dose CIN. The mean period from transplantation to conversion was 47.5 months (range 2-173). The mean follow-up was 12.2 months (range 3-40). 12 recipients (48%) had CIN monotherapy as primary immunosuppression.
Patients were also divided into CIN dose elimination (group A = 17) and dose reduction (group B = 8). The mean daily dose of MMF, 1.54g and of Prednisolone 8.4 mg. In group B the dose of CIN was reduced in order to achieve 1/2-1/3 reduction of target through levels. Chronic allograft nephropathy and/or chronic drug injury was present on biopsy of 21 recipients (84%). The other 4 were converted without biopsy.

RESULTS: During the follow up the mean serum creatinine concentration (S Cr) decreased from 336.2 to 242.44 mg/dl ($p<0.0001$). Mean glomerular filtration rate (GFR) values increased from 26.37 to 38.7 ($p<0.0001$). Initial weight loss was observed in 9 patients however, they all regained their original body weight during the follow up period. 10 patients required treatment for anaemia. Leucopoenia was experienced in 3 and gastrointestinal disturbances in 10 patients and all were managed by MMF dose adjustment. No patient experienced acute rejection and none have returned to dialysis. There were marginal but not statistically significant difference between the mean S Cr and GFR values in the two groups (mean S Cr decreased from 322.3 to 221.18 mg/dl and mean GFR values increased from 25.65 to 38.22 in group A, whereas, mean S Cr decreased from 366 to 287.6 mg/dl and mean GFR values increased from 27.9 to 39.67 in group B).

CONCLUSIONS: Chronic exposure to CINs is one of the main factors of chronic allograft nephropathy leading to graft dysfunction. This study shows that conversion from CIN to MMF + Prednisolone, with or without elimination of CINs, is safe and allows a significant improvement in graft function.

P44

AUDIT OF A NORTH WEST ACCIDENT AND EMERGENCY DEPARTMENT

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Introduction
There are many new initiatives being introduced by UK Transplant to promote organ and tissue donation. However, many of these initiatives are focused on intensive care units, with less emphasis on Accident and Emergency Departments. We therefore completed an eight month audit of a North West Accident and Emergency Department to ascertain the level of activity of donation requests.

Method
A random sample of 50 deaths from the period March to October within a North West A&E department was taken. An audit form was completed for each death to ascertain whether a request for donation had been made and the outcome of this request.

Result
Out of 50 deaths, there were no cornea or tissue donors and no family was approached re donation. 5 out of 50 patients (10%) had known contraindications to cornea or tissue donation. 7 out of 50 patients (14%) were aged less than 60 and could therefore have been considered for both cornea and tissue donation and of this group, all could have been considered for non heart beating kidney donation. The remaining 38 patients (76%) were potential corneas donors.

Conclusion
The A&E department may well hold an important key to increasing donor numbers. It was apparent from this study that 90% of patients could have been considered for donation and that 7 patients may well have been suitable asystolic donors had a NHBD programme been operational, thus yielding a potential of 14 kidneys and 90 corneas in addition to skin and bone for transplantation. This highlights an urgent need for protocols and training within the A&E department.

The audit did not reveal any patients who may have proceeded to brain stem death and therefore have gone on to donate organs for transplantation and is therefore an area worthy for investigation.

We believe that A&E should become a target for further research.

P45

C4d DEPOSITION IN EARLY RENAL ALLOGRAFT PROTOCOL BIOPSIES

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Background
Acute renal allograft rejection may be classified as either T cell mediated (tubulitis and endothelialitis) or antibody mediated. Although identification of cellular rejection by routine histopathology is straightforward, detection of antibody-mediated rejection can prove difficult. The complement protein C4d may be an effective marker of antibody-mediated damage as it binds covalently and stably to endothelial cells following antibody-mediated activation by the classical pathway. Recent evidence suggests that C4d is a useful marker of acute humoral rejection in biopsies performed for graft dysfunction. The aim of this study was to determine the frequency of C4d deposition in consecutive early protocol biopsies taken irrespective of graft dysfunction and to correlate these findings with routine histopathology, presence of circulating alloantibodies and clinical outcome.

Materials and Methods
Indirect immunoperoxidase staining for C4d was performed on snap frozen tissue obtained from 53 consecutive cadaver (n=44) and living related (n=9) donor renal allografts at two time points: (i) pre-transplantation (after cold storage, before implantation) and (ii) day 7 post-transplantation (irrespective of clinical function). C4d positivity in the peritubular capillaries (PTC) was graded semi-quantitatively by two independent observers blinded to the clinical information. The presence of circulating alloantibodies was determined using flow cytometry and cytotoxic antibody screening of post-transplant serum samples.

Results
No peritubular capillary (PTC) C4d deposition was detected in any of the pre-transplantation biopsies (0/53). In marked contrast, there was extensive C4d positivity within the PTC in 6 of 53 post-transplant day 7 protocol biopsies. Acute rejection was diagnosed histologically in 16 of 53 biopsies, 12 with acute cellular rejection and 4 vascular rejection. There was a significant association between C4d deposition and acute rejection, 5 of 16 biopsies showing acute rejection were C4d positive, whereas only one of the 37 non-rejecting kidneys was positive (p=0.002). Circulating alloantibodies were evident in 4 of 6 recipients with C4d positive transplant biopsies. Deposition of C4d was not significantly associated with the incidence of delayed graft function, elevated serum creatinine at 3 months or 1 year, or graft survival at one year in this patient cohort.

Conclusions
We conclude that C4d is absent in donor kidneys before implantation but present in 11% of routine protocol biopsies one week post-transplant. Our results suggest an association between C4d deposition in the peritubular capillaries, acute rejection and the presence of circulating alloantibodies.

P46

MONITORING SYSTEMIC DONOR LYMPHOCYTE CHIMAERISM FOR THE DIFFERENTIAL DIAGNOSIS OF GRAFT VERSUS HOST DISEASE AFTER LIVER TRANSPLANTATION

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Background:. The diagnosis of graft versus host disease (GVHD) after liver transplantation can be difficult as early symptoms are often non-specific and there is no diagnostic test available. In this study we examined whether the presence of donor lymphocytic macrochimaerism was a useful diagnostic marker for GVHD.

Materials and methods: Between 1996 and 2001, 34 liver transplant patients with a clinical suspicion of GVHD were investigated for peripheral blood donor chimaerism. The possibility of GVHD was considered in the presence of the following clinical features:- rash, diarrhoea, pyrexia, bone marrow suppression, isolated anaemia and normal LFTs. The relationship between the presence of donor chimaerism, clinical suspicion and the final diagnoses based on clinical and histological criteria was examined.

Evidence of donor chimaerism was detected by PCR, using HLA sequence specific oligo-nucleotide primers, of genomic DNA extracted from recipient peripheral blood. In cases where donor HLA alleles were detected, quantitative measurements of peripheral blood CD3+ T-cells were carried out by two-colour flow cytometric (FC) analysis using donor and recipient HLA specific alloantisera. Dot-blots were generated to identify dual cell populations corresponding to donor or recipient HLA phenotype and electronic gates were set to calculate the percentage of donor and recipient T-cells.

Results: Six of the 34 patients had evidence of chimaerism by PCR and flow cytometric confirmation of donor T-cells, ranging from 4% to 40% of circulating lymphocytes. All had normal LFTs, a characteristic skin rash and the diagnosis confirmed by skin or gut biopsies showing histological features consistent with GVHD. Two patients are alive and four died of sepsis and multi organ failure. Twenty eight patients had no evidence of chimaerism and of these 22 eventually had a definitive diagnosis other than GVHD. These consisted of CMV disease (n=9), bacterial sepsis (n=7), Clostridium difficile infection (n=2), GI Bleed-(n=2) and adverse drug reaction (n=2). The remaining 6 patients recovered spontaneously, no evidence of GVHD having been discovered.

Conclusion: Flow cytometric analysis to detect the presence of donor lymphocytes in the peripheral blood of patients following liver transplantation is a helpful diagnostic tool for GVHD.

P47

CARDIOVASCULAR INSTABILITY IN DONOR IS NOT A MAJOR DETERMINANT OF OUTCOME IN LIVER TRANSPLANTATION

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Abstract

Background: Cardiovascular instability and prior cardiac arrest were determinants of primary liver allograft non-function (PNF). In this era of severe organ shortage, improved donor management and organ procurement, we re examine these factors, which traditionally render potential donors marginal.

Aim: To determine the effects of donor cardiac instability (cardiac arrest or severe hypotension) on patient and graft outcome in OLT.

Methods: Prospectively collected data of 556 primary adult OLTs performed between Jan 1995 and Dec 1999 were analysed. The OLT recipients were divided into three groups according to the severity of the donor's cardiovascular instability.

Group 1 (n=41) received livers from donors who had suffered a cardiac arrest with a median of 30 (6-90) minutes. Group 2 (n=194) recipients' donors suffered a prolonged period of hypotension (mean BP <80 mm Hg for at least 1hour). This group was further subjected to a sub-analysis according to the duration of hypotension (1, 2-4, >4 hrs). Group 3 (n=321) donors were haemodynamically stable.

Results: Donor and recipient demographics (age, sex, and cause of disease) did not differ significantly between the groups. Cardiac arrest resulted in a significant rise in mean donor AST levels ($p<0.001$) and reduction in mean serum sodium ($p=0.04$). There was a trend for an increased incidence of PNF in G1 ($p=0.1$). Patient ($p=0.3$) and graft survival ($p=0.6$) at 1 year were lower in G1 but did not differ significantly. Postoperatively, a significantly higher INR and lower platelets count was noted in G1. There was no significan difference between the incidence of hepatic artery thrombosis, biliary complications, the incidence and severity of acute or chronic rejection between the study groups. Sub-analysis of G2 revealed significantly lower posttransplant AST levels using livers from donor suffering prolonged (>4 hrs) hypotension which may indicate ischaemic preconditioning.

Conclusions: Although donor cardiac arrest history was associated with a trend towards an slightly higher incidence of PNF. Donor circulatory instability without cardiac arrest is not a significant determinant of allograft outcome.

Laboratory Posters

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CAN *IN VITRO* TESTING OF T CELL FUNCTION IDENTIFY LIVER ALLOGRAFT RECIPIENTS WHO ARE SUITABLE FOR IMMUNOSUPPRESSION WITHDRAWAL?

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Background: The current 2-year survival rate after liver transplantation is 80%. As patient survival increases, the side effects of lifetime immunosuppression, namely opportunistic infection, malignancy and renal failure are becoming apparent. A re-evaluation of liver allograft tolerance without immunosuppression may be required, yet in the absence of prospective monitoring, withdrawal will inevitably expose patients to increased risk of graft loss due to late rejection. In order to detect and monitor recipients suitable for immunosuppression withdrawal an *in vitro* assay of T-cell function was developed to identify donor specific non-responsiveness and used to monitor 65 human liver allograft recipients of which 21 were studied serially.
Methods: IL-2 secretion by peripheral blood mononuclear cells in response to soluble recall antigen (SRA), donor alloantigen (DAA), 3rd party alloantigen (3rdAA), PHA and in the absence of antigen was quantified by highly sensitive ELISA. All recipients were tested alongside healthy controls.
Results: 44 recipients were studied at a single time point at least 1 month after transplantation. On the basis of the functional testing, recipients were classified as 1) immunosuppressed (IS, n=4, 9%), reduced or absent response to all antigens; 2). Graft reactive (GR, n=34, 77%), response to all antigens including DAA; 3). Donor non-responsive (DNR, n=6, 14%), absent or markedly reduced response to DAA with preserved 3rdAA and SRA response. IL-2 secretion in response to PHA (2400pg/ml), SRA (250pg/ml) and 3rdAA (450pg/ml) was similar in DNR recipients compared with GR recipients (PHA 1800pg/ml; SRA 225pg/ml; 3rdAA 450pg/ml) and healthy controls (PHA 2550pg/ml; SRA 450pg/ml; 3rdAA 750pg/ml) despite reduced responses to DAA (DNR 85pg/ml; GR 525pg/ml; controls 675pg/ml; P=0.001). Four of the six DNR recipients were retested 6 months later when 3 patients had developed strong responses to DAA. The fourth patient subsequently responded to DAA after a further 6 months. Serial measurements of the same responses were carried out pre transplantation and up to 2 years post transplant in a further 21 liver allograft recipients. 16/21 (76%) patients remained GR throughout and 1 patient who was initially IS subsequently became GR. Donor non-responsiveness was seen in two patients who were initially IS (0-2 months post transplant) then GR for 6-7 months and DNR from approximately 9 months post transplant. Three patients were initially GR for 6 months following transplantation then IS throughout the remainder of the study.
Conclusions: Although initial findings suggested 14% of liver allograft recipients were hyporesponsive to donor alloantigen whilst maintaining recall and 3rd party responses this hyporesponsiveness was unstable since reactivity re-emerged over time. Serial monitoring confirmed the transient nature of such hyporesponsiveness and suggests that most recipients remain immunologically reactive to graft alloantigen. Thus *in vitro* assays based on IL-2 secretion in response to DAA do not identify functional tolerance although they can reveal over-immunosuppression and as such, may help to optimise clinical immunosuppression.

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A NEW MODEL OF CHRONIC CYCLOSPORINE NEPHROTOXICITY FOR COMPARATIVE MORPHOLOGICAL AND HISTOLOGICAL STUDIES

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Since the introduction of cyclosporine A (CsA) in 1976, the survival of solid organ transplantation has improved markedly, and has also found utility in the treatment of autoimmune diseases. However, the benefits of CsA therapy have been potentially offset by the occurrence of its nephrotoxicity. The aim of this study was to introduce a model of cyclosporine nephrotoxicity that mimics the clinical situation for comparative histological and morphological studies, without using high doses given over a long period of time,
Male Sprague-Dawley rats (6 per group) were anaesthetised with halothane and the left renal pedicle clamped for 30 minutes through a flank incision. Control animals were sham operated. The animals were daily injected intraperitoneally either with CsA 15 mg/kg or the drug vehicle for 30 days. The experiment was terminated after 30 days. Kidney sections were stained with Masson's trichrome for fibrosis scoring (0-3) , haematoxylin and eosin for histological scoring (tubular dilatation, and inflammatory infiltrate) and PAS for arteriolar hylinosis. Immunohistochemical staining was performed for TGFβ1, CD68 (macrophage marker), collagen III and collagen IV. Results are expressed as mean ± sem and differences considered significant when $P<0.05$.
There was a significant increase in the fibrosis score when renal warm ischaemia was induced in CsA-treated rats (1.96 ± 0.49) compared to either warm ischaemia alone (0.31 ± 0.14) or CsA alone (0.31 ± 0.07). Both the tubular dilatation score and inflammatory infiltrate score were significantly higher in CsA-treated ischaemic kidneys (1.09 ± 0.33 and 2.2 ± 0.43 respectively) compared to either CsA alone (0.27 ± 0.09 and 0.4 ± 0.15 respectively) or ischaemia alone (0 and 0.5 ± 0.14 respectively). All the CsA-treated animals showed a significant increase in arteriolar hylinosis (47 ± 5%) compared to ischaemia alone (0%) or vehicle treated sham operated animals (0%). Both TGFβ1 immunostain and the number of CD68 positive cells per field were significantly higher in CsA-treated ischaemic kidneys (39.5 ± 7.3% and 64.9 ± 15.4 per field respectively) compared to either ischaemia alone (5.9 ± 0.8% and 4.9 ± 2.6 per field respectively) or CsA alone (10.5 ± 3.7% and 1.8 ± 0.6 per field respectively). Collagen type III and type IV immunostain were significantly increased in CsA-treated ischaemic kidneys (37.9 ± 12.1% and 58.1 ± 3.1% respectively) compared to either ischaemia alone (8.46 ± 3% and 39 ± 4.9% respectively) or CsA alone (10 ± 2.5% and 36.3 ± 4% respectively). There was no difference in CsA blood concentration between groups treated with this drug.
This study documents an animal model of CsA nephrotoxicity with arteriopathy and striped interstitial fibrosis induced by a 30 minute period of renal warm ischaemia and CsA 15 mg/kg for 30 days. This model offers the opportunity to test therapeutic interventions that might ameliorate the nephrotoxic effect of this drug.

P50

THE ROLE OF INDIRECT ALLORECOGNITION IN PATHOGENESIS OF OBLITERATIVE BRONCHIOLITIS FOLLOWING LUNG TRANSPLANTATION.

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Introduction: Chronic rejection is the main obstacle preventing successful long-term transplantation of solid organ allografts. The prevalence and severity of chronic rejection is greatest in lung recipients, where it presents as Obliterative Bronchiolitis (OB) in 50% of patients at 2 years. We hypothesise that lungs are more immunogenic than other organs and OB is the result of sustained T cell activation by donor MHC antigens. It is established that recipient T cells recognise antigens via two pathways known as direct and indirect recognition. There is evidence from heart and kidney transplant recipients that long-term patients become hyporesponsive in the direct pathway, but that patients who develop chronic rejection become hyperresponsive to donor antigens. In contrast, little work has been done looking at pathways of antigen presentation after lung transplantation. Here we have investigated indirect recognition in patients with and without OB following lung transplantation.
Methods: Limiting dilution analysis was carried out to determine the frequency of CD4+ T cells with indirect reactivity to donor antigens in 5 patients with OB (2-5 years post-transplant) and 4 patients without OB (3-7 years post-transplant). Purified recipient CD4+ T cells were incubated with self-APC and with donor or third party antigens(frozen/thawed splenocytes from donor or third party). After 3 days, supernatant was assessed for Interleukin 2 using a bioassay (the IL-2 dependent cell line, CTLL-2).
Results: 4/5 patients with OB showed significantly higher frequencies(f) to donor(mean f 1/100,694, range 1/13,513-1/260,085) compared to third party (mean f 1/234,108, range 1/210,484-1/257,732). In contrast none of the 4 patients without OB were hyperresponsive to donor antigens (mean f 1/376,184, range 1/252,324-1/528,722).
Conclusions: The results support the hypothesis of sustained T cell activation, via the indirect pathway, in patients with OB. Whether T cells are also driven by direct recognition is currently being investigated.

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INHIBITION OF ANTIBODY-MEDIATED ACTIVATION OF HUMAN ENDOTHELIAL CELLS BY FLUVASTATIN.

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We have previously described the presence of antibodies directed against donor MHC class I proteins after allotransplantation. Ligation of MHC class I with purified anti-donor class I antibodies derived from patients, or with anti-MHC class I Mabs, leads to endothelial cell (EC) activation, including translocation of NF-κB to the nucleus, increased cell proliferation and growth factor expression.
In this study we have ligated MHC class I with the MAb W6/32, on the surface of EaHy.926, a human EC line. TNF was used as a positive control. We examined cell culture supernatants for IL-1β and IL-6 by ELISA. There was very little secretion of either cytokine from resting EaHy.926. After ligation of MHC class I for 24h, IL-6 secretion increased 6-fold ($p<0.05$), and increased 60 fold after TNF treatment ($p<0.05$). There was no increase in IL-1β secretion after MHC class I ligation with W6/32.
We examined the effect of the lipid lowering drug fluvastatin, an HMG-CoA reductase inhibitor, on IL-6 secretion. Antibody-mediated but not TNF-mediated IL-6 secretion was inhibited after pre-treatment of cells with 5μM fluvastatin. Pretreatment with other statins (pravastatin or simvastatin) or inhibition of PKA or PKC signalling pathways had no effect on either W6/32-induced or TNF-induced IL-6 secretion. All Trans retinoic acid inhibited IL-6 secretion with either stimulus.
We postulate that in vivo, anti-donor antibodies capable of EC activation could be partly responsible for increased IL-6 in the serum of transplant patients with accelerated atherosclerosis. As well as lipid-lowering effects, statin therapy in transplant recipients may reduce antibody-mediated cell activation.

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DONOR LIVER PRESERVATION AND STIMULATION OF ADHESION MOLECULES : RELEASE OF STIMULATORY FACTORS DURING COLD ISCHAEMIA

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Hepatic cold preservation leads to varying degrees of ischaemia / reperfusion injury, which can affect both early and late outcomes. One aspect of I/R is the expression of adhesion molecules which can initiate the inflammatory cascade in the grafted organ. An unresolved question is the dependence of such pro-inflammatory stimulation on factors present during cold hypoxia and before revascularisation. It is known that several cytokines can be detected in the effluent preservation fluid flushed from the liver at the end of storage (1), and adhesion molecule up-regulation can be detected in animal models of liver preservation (2). The present study was undertaken to investigate whether the mixture of cytokines released into the effluent during clinical hepatic transplantation can stimulate adhesion molecules (ICAM-1 & E-Selectin) expression using cultured endothelial cells as a defined model system.

Cultures of human endothelial cell line (ECV304) were established. The effluent albumin solutions were collected from 10 adult donor livers at the recipient operation, with local institutional approval. The mean cold ischaemic time was 11.2±2.4 h. The solutions were centrifuged to remove cellular components and stored at –40°C before use. Upon thawing, the effluents (4 ml aliquots) were dialysed overnight against 2.5 l of saline solution at 4°C to remove the residual high potassium content from the UW solution. The final dialysis was performed against 50 ml of DMEM culture medium. Cells were cultured in serum-free DMEM for 24 h before addition of the dialysed effluent (1 ml) to 6 well dishes of established cells. The cultures were then maintained for 5h at 37°C, before termination of the experiment and extraction of the mRNA content using a Qiagen Rneasy Mini Kit. Levels of mRNA for ICAM-1, E-Selectin, and house-keeping genes β-actin and GAPDH were measured by rt-PCR using appropriate probes. Results showed that ratios of β-actin and GAPDH were constant (1.03±0.01), whilst those for E-Selectin / β-actin (1.21±0.05) and ICAM-1 / β-actin (1.14±0.03) were significantly elevated ($P<0.01$ & $P<0.05$, ANOVA plus Dunnett's test). Control cells exposed only to fresh albumin solution showed no changes. These results confirm the presence of pro-inflammatory agents in the stored livers before re-oxygenation, presumably resulting from the agonal phase in the donor and/or subsequent cold hypoxia. Specific targeting of this early-phase inflammatory response may be one way to reduce overall I/R damage.

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P53

REEVALUATION OF THE SENSITISATION STATUS OF A COHORT OF PATIENTS PREVIOUSLY CLASSIFIED AS HIGHLY SENSITISED AND AWAITING TRANSPLANTATION.

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Under the current UK strategy for the allocation of cadaveric kidneys, consideration is given primarily to the degree of HLA match between donor and recipient. Thereafter a series of criteria, linked to a points system are applied to permit allocation in situations where recipients with equal degrees of HLA mismatch are identified. Amongst the criteria considered is the HLA sensitisation status of the recipient and the extent to which this has been defined. Precedence for available organs is given to patients with the most comprehensive definition of their HLA specific antibodies.

Following publication of the UK kidney allocation scheme in 1998 an audit of this laboratory's serum screening capability concluded that we lacked the ability to match the standards required to ensure maximum eligibility of our patients for available cadaveric kidneys. Of particular concern was the large proportion of patients classified as highly sensitised (HSP) who constituted 18% (52/288) of our patient group compared with the UK average of 10%. Moreover the mean sensitisation points score for patients within this group was just 0.76 out of a maximum points score of 3. Of potential relevance to this was the heavy bias (99:1) in the proportion HLA class I to class II antibody specificities detected, which indicated an inability to define class II in the presence of, class I specificities. The average number of HLA specificities detected/sample was 3.

The concerns raised by these findings provided the impetus for a revision of our serum screening protocol. The approach taken was to employ a tiered screening strategy linking together flow cytometry methods permitting rapid identification of positive samples and subsequent definition of antibody specificity. Full details of our protocol will be provided.

A measure of the efficacy of our approach is provided by the revised statistics relating to the originally defined HSP cohort. Following reanalysis only 9/52 patients (equivalent to 3% of those listed) within this group were found to have serum PRA values relating to the presence of HLA antibody at levels supporting their classification as HSP. Mean sensitisation points score for patients within the group increased to 1.9 and the proportion of HLA class I to class II antibody specificities detected was 61:39. The average number of specificities detected/sample was 8

The extent to which a laboratory is able to provide a comprehensive definition of serum specificity impacts directly on the opportunity for patient call-up. Variation between laboratories in terms of this capability therefore raises important issues of equality of access to treatment.

Based on our experience we recommend that all centres review their serum screening strategies to be satisfied that they are capable of meeting the necessary standards to ensure maximum eligibility of their patients for available cadaveric organs.

P54

APOPTOSIS AND EXPRESSION OF Bcl 2 AND BAX IN CYCLOSPORINE INDUCED EXPERIMENTAL RENAL FIBROSIS

AIM: The introduction of cyclosporine has had a considerable impact on renal allograft survival and has revolutionized the field of organ transplantation. However it is associated with cyclosporine toxicity, which is characterized by interstitial fibrosis. We evaluated the role of apoptosis in cyclosporine induced renal fibrosis and expression of death promoter gene Bax and survival promoter gene Bcl 2.

METHODS: Adult male Sprague – Dawley rats were used. They were administered intraperitoneal injection of cyclosporine (25mg/kg/day) or vehicle (olive oil) and were sacrificed in groups at 1, 2, and 4 weeks numbers per group, N=6 experimental; N=4 control. Rats were weighed regularly and blood was taken for determination of serum creatinine and cyclosporine level. 24hr urine was also collected for estimation of creatinine clearance rates. Kidney sections were stained with Haematoxilin and Eosin (H & E), Periodic Acid Schiff (PAS), and Masson's trichrome. Further we stained the nuclear chromatin by the acridine orange fluorescent method and detected signs of DNA cleavage by endonucleases via the principle of TUNEL staining (ApopTag). Immunohistological staining was performed for expression of Bcl 2 and Bax.

RESULTS: Cyclosporine treated rats gained weight (g) more slowly than vehicle treated controls (268 ± 5.2 Vs 333 ± 4.3) P<0.005. There was a reduction in creatinine clearance (ml/min) in experimental rats (0.8 ± 0.2 Vs 1.68 ± 0.23) P<0.05. Significant increase in fibrosis score was found in cyclosporine treated rats (2.265 Vs 0.7675) P<0.002. Experimental cyclosporine treated rats showed a marked and progressive increase in number of apoptotic cells. Week 1(0.478 Vs 0.167), week 2 (0.894 Vs 0.175: P<0.001), week 4 (1.725 Vs 0.15: P<0.001). Cyclosporine induced the expression of Bax (P<0.01) and decreased that of Bcl2 (P<0.05). The changes occurred as early as one week and remained statistically significant at four weeks.

DISCUSSION AND CONCLUSION: We conclude that cyclosporine nephrotoxicity is associated with marked increase in apoptosis and cyclosporine induced apoptosis correlates with interstitial fibrosis. The expression of Bax and Bcl2 remained diametrically opposite; cyclosporine treatment favored Bax expression. Future work can lead to better understanding of the mechanisms of apoptosis and may provide safer and more specific therapeutic intervention for cyclosporine induced nephrotoxicity.

P55

ADDITION OF GLUCONATE IN A PRESERVATION SOLUTION INDUCES CELL SWELLING THAT MAY BE DETRIMENTAL. COMPARISON WITH LACTOBIONATE AND SUCROSE

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Most transplant preservation solutions contain an impermeant to prevent cell swelling during cold and warm ischaemia. Lactobionate (MW of sodium lactobionate = 380) is an important component of University of Wisconsin (UW) solution. Its main role is as an impermeant to prevent cell swelling. Gluconate is a similar anion (MW of sodium gluconate = 218) but much cheaper than lactobionate. This is used in the modified UW solution for machine perfusion. We tested the efficacy of gluconate and lactobionate as impermeant in a cell model which allows measurement of cell volume in real time. The results were compared with that of sucrose, the impermeant used in PBS140.

Phosphate buffered sucrose contain 140mM of sucrose and 69mM of phosphate buffer. The pH is 7.0 and osmolality 300. The test solutions were prepared by replacing 140mM of sucrose with either lactobionate (PBL) or gluconate (PBG). The kidneys of anaesthetized NZW rabbits (1.4-2.2kg) were flushed with and stored in one of the three test solutions (PBS140, PBL140, or PBG140) and stored at 0-4°C. Isolated proximal convoluted tubules were then set up unperfused on an inverted microscope and bathed in oxygenated physiological saline at 37°C for 15 minutes to equilibrate cell volume. The bathing fluid was then exchanged for the test solution (used to flush that kidney) for 35 minutes. The bathing fluid was finally replaced with physiological saline for a further 20 minutes. These two periods in saline acted as controls. Outside tubule diameter (μm) was measured every 5 minutes and are shown as mean ± SEM (n=6) for the end of each of the three periods after 0-4 hour hypothermic storage..

Storage solution	Initial diameter in saline	Tubule diameter in the test solution	Tubule diameter in saline
PBS140	34.2 ± 0.9	31.8 ± 1.0	34.7 ± 1.1
PBL140	35.0 ± 0.6	34.2 ± 0.6	37.0 ± 0.8
PBG140	43.1 ± 1.7	42.9 ± 1.9	48.8± 2.1

Whilst, both sucrose and lactobionate prevented cell swelling, kidneys flushed with gluconate containing solution showed marked increase in cell volume even after short period of preservation. The cell volume was further increased in the second control period suggesting that the cell volume regulation mechanisms are affected when kidneys are flushed and stored in gluconate containing solution. It is suggested that the addition of lactobionate to a preservation solution or a machine perfusion solution may be detrimental.

P56

EURO-COLLINS DOES NOT PROTECT AGAINST WARM ISCHAEMIA OF RAT KIDNEY: DEMONSTRATION IN AN IN-SITU WARM ISCHAEMIA MODEL.

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Euro-Collins (EC) is an established solution for kidney preservation. We evaluated the effect of clinically proven preservation solution upon in-situ warm ischaemia of rat kidney of 45 minutes preceded by a normothermic flush with either EC, hyperosmolar citrate (HOC), phosphate buffered sucrose (PBS140) or University of Wisconsin (UW) preservation solutions.

Male Wistar rats (300-350g)* were anaesthetized by intraperitoneal injection of inactin (120 $mg.kg^{-1}$). An Intravenous saline infusion was set up at $6ml.hr^{-1}$ also containing $37MBq.l^{-1}$ of ^3H inulin (for inulin clearance). Both ureters were cannulated for serial urine collection. An equilibration period of 1 hour was allowed following surgery after which urine was collected from each kidney for one hour (control). The left kidney was then flushed with 0.5 ml of EC, HOC, PBS140, UW at 37°C. A clamp was then applied to the left renal pedicle. After 45 minutes the clamp was released to allow reperfusion and a right nephrectomy was performed.

One rat in the EC group died during the 3rd hour without producing any urine. Of the remaining five, one had no urine output and 3 had isosthenuria or near-isosthenuria (urine osmolality similar to or only slightly higher than plasma). This made comparison of post-ischaemic renal function of the other groups irrelevant all of which had progressively concentrated urine with good inulin clearances.

Groups	Period	Urine flow rate ($\mu L.min^{-1}.100g$ body wt^{-1})	Inulin clearance ($\mu L.min^{-1}.100g$ body wt^{-1})	Urine osmolality ($mOsm.kg^{-1}$)
HOC	-1 (6)	2.71 ± 1.05	171 ± 27	1051 ± 236
	2 (5)	15.54 ± 4.63	80 ± 34	399 ± 23
	4 (4)	5.06 ± 2.50	39 ± 19	443 ± 40
PBS140	-1 (7)	2.26 ± 0.36	205 ± 25	1024 ± 98
	2 (7)	10.44 ± 3.14	105 ± 21	464 ± 34
	4 (6)	3.76 ± 0.78	50 ± 7	552 ± 54
UW	-1 (6)	1.67 ± 0.13	180 ± 26	1094 ± 127
	2 (6)	18.88 ± 5.74	111 ± 26	432 ± 27
	4 (6)	5.03 ± 0.95	59 ± 13	625 ± 71

Numbers in parenthesis indicate the numbers of completed observations for the given period

It is suggested that EC does not provide protection against in-situ warm ischaemia in rat kidney. This may have implication in transplant preservation.

P57

ADDITION OF PHARMACOLOGICAL AGENTS TO PHOSPHATE BUFFERED SUCROSE (PBS140) CONFER IMPROVED PROTECTION AGAINST WARM ISCHAEMIA IN RAT KIDNEY

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The role of addition of pharmacological agents to a preservation solution has been debatable. University of Wisconsin solution contain a number of these agents. We have evaluated the effect of the addition of selected pharmacological agents to phosphate buffered sucrose (PBS140) upon in-situ warm ischaemia of rat kidney. Allopurinol, diltiazem, aspirin and glutathione were added to PBS140. Small quantities of potassium (5 mM) and glucose (10 mM) were also added. The results were compared with that of PBS140 and UW.

Male Wistar rats (300-350g)* were anaesthetized by intraperitoneal injection of inactin (120 $mg.kg^{-1}$). An Intravenous saline infusion was set up at $6ml.hr^{-1}$ also containing $37MBq.l^{-1}$ of ^3H inulin (for inulin clearance). Both ureters were cannulated for serial urine collection. An equilibration period of 1 hour was allowed following surgery after which urine was collected from each kidney for one hour (control). The left kidney was then flushed with 0.5 ml of PBS140, UW or Solution I (PBS140 and pharmacological additives) at 37°C. A clamp was then applied to the left renal pedicle. After 45 minutes the clamp was released to allow reperfusion and a right nephrectomy was performed. Urine flow rate, inulin clearance and urine osmolality for each group is presented below for pre-ischaemic period (control) and one and four hour post-ischaemia (mean ± SEM, n=6).

Groups	Period	Urine flow rate ($\mu L.min^{-1}.100g$ body wt^{-1})	Inulin clearance ($\mu L.min^{-1}.100g$ body wt^{-1})	Urine osmolality ($mOsm.kg^{-1}$)
PBS140	Pre-ischaemia	2.3 ± 0.36	205 ± 25	1024 ± 98
	1 hr	18.1 ± 6.23	144 ± 24	426 ± 35
	4 hr	3.8 ± 0.78	50 ± 7	552 ± 54
UW	Pre-ischaemia	1.7 ± 0.13	180 ± 26	1094 ± 127
	1 hr	26.9 ± 7.68	104 ± 25	365 ± 16
	4 hr	5.0 ± 0.95	59 ± 13	625 ± 71
Sol I	Pre-ischaemia	2.5 ± 0.7	190 ± 14	902 ± 156
	1 hr	22.1 ± 3.7	*196 ± 51	*480 ± 53
	4 hr	4.2 ± 0.8	*106 ± 21	*822 ± 61

*p <0.05 (ANOVA)

Whilst post ischaemic inulin clearance (1st hr) fell significantly in both PBS140 and UW groups (70% and 57% of pre-ischaemic control respectively), this was higher (103%) than pre-ischaemic control in solution I. Inulin clearance gradually declined over the 4 hour post-ischaemic period in all groups. Whilst this fell to 24% and 33% of pre-ischaemic control in PBS140 and UW, solution I maintained inulin clearance of 55% of pre-ischaemic control. Similarly, the new solution showed increased urinary concentrating ability. The first and fourth hour urine osmolalities for solution I were recorded as 53% and 91% of pre-ischaemic control Four rats in this groups achieved a 4th hour urine osmolality higher than pre-ischaemic control. These were of 41% and 54% for PBS140 and 33% and 57% for UW respectively. The addition of the pharmacological agents therefore yielded significant advantage in the protection against in-situ warm ischaemia in our model.

P58

GLUTATHIONE S-TRANSFERASE AS AN INDICATOR OF TUBULAR DAMAGE IN A WARM ISCHAEMIA MODEL

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The enzyme glutathione s-transferase (GST) is found in rat kidney in two distinct forms: α-GST, in the proximal tubules and, μ-GST in the distal tubules. Elevated levels of these enzymes in serum and urine may indicate damage to the respective parts of the kidney. In the present study the excretion of these enzymes were measured following 45 minutes of in-situ ischaemia of rat kidney preceded by a normothermic flush with either hyperosmolar citrate (HOC), phosphate buffered sucrose (PBS140) or University of Wisconsin (UW) preservation solutions.

Male Wistar rats (300-350g)* were anaesthetized by intraperitoneal injection of inactin (120 $mg.kg^{-1}$). An Intravenous saline infusion was set up at $6ml.hr^{-1}$ also containing $37MBq.l^{-1}$ of ^3H inulin (for inulin clearance). Both ureters were cannulated for serial urine collection. An equilibration period of 1 hour was allowed following surgery after which urine was collected from each kidney for one hour (control). The left kidney was then flushed with 0.5 ml of HOC, PBS140 or UW at 37°C. A clamp was then applied to the left renal pedicle. After 45 minutes the clamp was released to allow reperfusion and a right nephrectomy was performed. GST was measured in the collected urine by enzyme linked immunoassay (ELISA, Biotrin).

Inulin clearance at 2 hour post-ischaemia were similar in all three groups. GST excretion for these groups are given in the table below for pre-ischaemic control and 2 hour post-ischaemia.

Groups	Period	Inulin clearance μL/min/100g body wt	α-GST excretion ng/min/100g body wt	μ-GST excretion $ng.min^{-1}.100g$ body wt^{-1}	% sodium absorption	Free water clearance μL/min/100g body wt
HOC	-1 (6)	171± 27	0.411 ± 0.253	0.377 ± 0.228	99 ± 0.5	-3.77 ± 1.1
	2 (5)	80 ± 34	16.888 ± 4.360	11.380 ± 5.528	62 ± 7.3	-5.55 ± 2.3
PBS140	-1 (6)	205 ± 25	0.312 ± 0.128	0.139 ± 0.041	99 ± 0.3	-4.81 ± 0.8
	2 (6)	105 ± 21	*9.591 ± 2.702	8.315 ± 3.630	*87 ± 2.8	-4.49 ± 1.3
UW	-1 (6)	180 ± 26	0.161 ± 0.043	0.067 ± 0.024	99 ± 0.1	-3.69 ± 0.5
	2 (6)	111 ± 26	28.791 ± 7.062	30.812 ± 11.66	72 ± 7.5	-7.88 ± 2.3

Numbers in parenthesis indicate the numbers of completed observations. * p≤0.05
Period: -1=pre-ischemia, 2=2hr post-ischaemia. Numbers in parenthesis indicate the numbers of completed observations. *P<0.05)

Excretion of both α and μ-GST were raised in all three groups in the post-ischaemic period indicating ischaemic tubular damage. PBS140 was associated with a significantly lower rate of excretion of α-GST at 2 hr post-ischaemia when compared to HOC and UW and a significantly lower μ-GST excretion compared to UW. Whilst, the former reflected well in a significantly lower percentage sodium reabsorption in UW, the excretion of μ-GST did not correlate well with the distal tubular function, namely, free water clearance. Measurement of GST can serve as a marker of tubular damage and to assess the effectiveness of warm ischaemic protection provided by the preservation solutions.

P59

EFFECT OF INSPIRED OXYGEN CONCENTRATION ON HEPATIC OXYGENATION AND BLOOD FLOW IN CONTROL AND DIABETIC RATS.

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We have previously shown that in diabetic rats breathing 100% oxygen for 48 hours following intraportal transplantation of syngeneic islets, the number of islets that survive is increased whilst the number of islets required to restore euglycemia is reduced. In order to investigate the mechanism by which hyperoxia may alter oxygenation at the graft site, we have studied hepatic oxygenation and portal blood flow in control and diabetic rats breathing various inspired oxygen concentrations. Diabetes was induced in male Lewis rats by i.p. injection of streptozotocin (55 mg/kg) and confirmed after 3-4 days by plasma glucose determination (33.9±1.8 vs 9.5 mmol/l in controls). On day 7, isofluorane anaesthesia was induced, laser Doppler probes placed on hepatic tissue & around the portal vein and the inspired oxygen content varied (13, 21, 50 or 100%). In control and diabetic rats breathing 21% oxygen, the portal flow rates were 7.7±0.8 and 6.1±0.7 ml/min respectively. Maximum rates of portal blood flow were observed when the inspired oxygen content was 100% in both control (12.3±0.5 ml/min) and diabetic (8.4±0.5 ml/min) rats and were significantly lower ($p<0.05$) in the diabetic animals. When the animals were breathing 13% oxygen the rates were 33 and 35% of maximum values in control and diabetic animals respectively. In diabetic animals, increasing the inspired oxygen concentration from 21 to 100% increased the oxy-haemoglobin content (by 126.8±17.4 vs 146.4±17.8 μM in controls) and reduced the deoxy-haemoglobin content by 109.2±12.6 vs 101±15.9 μM in controls. The incremental changes in oxy-haemoglobin and deoxy-haemoglobin content were not significantly different between control and diabetic animals. Reducing the inspired oxygen concentration from 21 to 13% reduced oxy-haemoglobin and increased deoxy-haemoglobin content in livers of both control and diabetic animals. The incremental changes were not significantly different between control and diabetic animals. These data suggest that increasing the inspired oxygen of diabetic rats (after intraportal islet transplantation) may increase hepatic oxygenation by increasing splanchnic blood flow which in turn may help to increase oxygen delivery by haemoglobin.

P60

UP REGULATION AND CELLULAR RELEASE OF TRANSGLUTAMINASE IN A NOVEL MODEL OF CHRONIC ACIDOSIS IN PORCINE LLC-PK1 CELLS

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Background. Acidosis occurs commonly in transplanted patients and is multifactorial especially with chronic allograft nephropathy (CR) and declining graft function. Acidosis itself may be pathogenic at the level of the proximal renal tubule (PTC). We have previously shown that acidosis can alter the PTC oxidative metabolic rate. This can directly affect free radical generation and disturb cellular defense mechanisms, which may include the tissue transglutaminase (tTg) enzyme system. tTg up regulation in tubule cells and its cellular release has been linked with the progression of renal scarring including that associated with CR. Transglutaminases (Tg) are able to crosslink glutamine and lysine amino acids on adjacent peptides to form an irreversible ε(γ-glutamyl) lysine di peptide bond. The formation of this bond within the extracellular matrix is thought to accelerate the rate of ECM deposition, while making qualitative changes to the ECM that blunt the action of proteolytic enzymes such as the matrix metalloproteinases. Alternatively, activation of intracellular Tg results in crosslinking of cytosolic proteins causing a novel type of cell death that is thought to be a significant factor in tubular atrophy. However, it remains to be determined what factors are able to both up regulate and cause the release of Tg from the cell. In this study we use a model of chronic acidosis in PTC to determine if acidosis may influence Tg expression and its cellular release.

Methods. Porcine LLC-PK1 cells were grown under standard conditions until 30% confluent. Bis-Tris buffer was thereafter used and the pH of the media modified to recreate increasing levels of chronic acidosis between pH 7.4 (control) and pH 6.7. After three days (i.e. 100% confluent) including the last 24 hours in serum-free media, media was collected and concentrated by freeze-drying and the cells harvested. These were then assayed for transglutaminase levels using a ^{14}C-putrescine incorporation activity assay. Cell growth, tissue viability (dead-live stain method, scanning electron microscopy) and LDH release were measured to assess cell status.

Results. There were no adverse effects of changes in pH of the culture media on the rate of growth, cell morphology, tissue viability or permeability (extracellular LDH). At pH 7.4, intracellular tTg activity was 0.015 ± 0.004 units/ mg protein. With reducing pH this increased by 168% ($p<0.01$) by pH 7.0 and gave a 3 fold increase at pH 6.7 ($p<0.01$). This increase in intracellular Tg resulted in increased levels of Tg in the extracellular environment with extracellular levels increasing 2.5 fold from 0.027 ± 0.004 units /mg protein at pH 7.4 to 0.067 ± 0.009 units/ mg protein at pH 6.7.

Conclusion. We have used a novel model of chronic acidosis in porcine cells to closely mimic that associated with progressive renal disease & CR. This caused an up regulation of intracellular Tg activity that resulted in increased extracellular Tg activity. Extracellular Tg was not associated with increased LDH release indicating the release in Tg was not a consequence of cell leakage. Acidosis may therefore be a regulator of Tg while the action of Tg may be one of the factors behind the cellular injury resulting from acidosis.

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NITRIC OXIDE AND RENAL WARM ISCHAEMIC INJURY: AN INVESTIGATION USING PULSATILE MACHINE PERFUSION

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Non-heart-beating donor kidneys (NHBDs) are subject to a period of warm ischaemia, often of unknown duration and severity, which is responsible for the high rates of delayed graft function seen with this donor source. Nitric oxide is believed to be involved in the pathogenesis of renal ischaemia. The aim of this study was to determine the relationship between the warm ischaemic time (WIT), nitric oxide production, and intrarenal resistance (IRR) during hypothermic machine perfusion.

Porcine kidneys (n=36) were subjected to varying periods of in-situ warm ischaemia (10-90 minutes). The kidneys were retrieved, flushed with hyperosmolar citrate and stored on ice for 2 hours. Hypothermic preservation on a pulsatile perfusion machine was undertaken for 6 hours at a systolic pressure of 60 mmHg. IRR (mean pressure/flow) was measured throughout perfusion. Perfusate and tissue samples were analysed for nitrate and nitrite concentrations and endothelial nitric oxide synthase (eNOS) production.

A strong correlation was found between the initial IRR during MP and prolonged WIT (correlation coefficient $r=0.962$, $r^2=0.9278$, $p=0.002$). No such correlation was found at the end of perfusion ($r=0.6139$, $r^2=0.3769$, $p=0.2$). MP produced a significant fall in IRR for all treatment groups. Nitrate concentrations increased during WIT and MP ($p<0.01$). The levels of eNOS correlated with nitrate production during warm ischaemia ($p=0.01$) but not with that produced during MP ($p>0.05$). Neither the nitrate concentration nor the eNOS levels correlated with the fall in IRR.

In conclusion, early IRR accurately reflected the WIT. This could prove useful in pre-transplant viability assessment. MP reduced IRR, possibly via increased NO production, but this does not appear to be mediated by eNOS. MP may partially ameliorate the deleterious effects of warm ischaemia on non-heart-beating donor kidneys.

P62

THE EFFECT OF PULSATILE MACHINE PERFUSION ON ISLET ISOLATION FROM THE PORCINE PANCREAS

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Objectives

Pulsatile machine perfusion has been used successfully for renal preservation. The aim of this study was to compare pancreas preservation by cold pulsatile machine perfusion with simple cold storage and the two-layer method (UW/PFC) prior to islet isolation.

Materials and Methods

24 Porcine pancreata were retrieved from landrace pigs and randomised to 4 groups (n=6 each group). Group A underwent islet isolation immediately (controls). Group B underwent cold storage in UW at 4°C (4 hours), Group C underwent preservation by the two-layer method (UW/Perfluorocarbon) with continuous oxygenation (4 hours) and Group D underwent cold pulsatile perfusion (4 hours). In all cases the period of warm ischaemia was minimal (mean=7 mins). The modified automated method for islet isolation was employed with porcine Liberase® used for pancreas digestion. Islet yield was calculated as islet equivalents (IEQ) per gram of pancreatic tissue. The digest was purified on continuous minigradients (n=5 each group) and each 1ml fraction was assayed for insulin and amylase. Purity was calculated as the percentage exocrine contamination (EC) at 60% islet yield.

Results

All experimental groups had a comparable period of warm and cold ischaemia. The mean pancreatic weight was 137g. The mean percentage weight gain during preservation period was 13% for the cold storage group (B), 3.9% for the two-layer method (C), and 92% for the pulsatile perfusion group (D) (P=0.01). The IEQ per gram of pancreas was 2607 for group A (controls) and 3854, 2274, and 1378 for groups B, C and D respectively (P= 0.28). There was no statistical difference in islet diameter between groups. The exocrine contamination rate at 60% islet yield was 47% for group A (controls) and 25%, 34%, and 36% for groups B, C and D respectively (P=0.21).

Conclusions

Four hours of pulsatile machine perfusion produces comparable islet yields and gradient purity when compared to the two-layer method, cold storage method and a control group. 4 hours of pulsatile perfusion resulted in significant pancreatic oedema. However, although this does not appear to affect islet yields, further detailed studies are required to establish islet viability.

P63

A PAIRED STUDY COMPARING THE EFFICACY OF RENAL PRESERVATION BY NORMOTHERMIC AUTOLOGOUS BLOOD PERFUSION AND HYPOTHERMIC PULSATILE PERFUSION

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Introduction The reduction in metabolic rate affected by hypothermia constitutes the central tenet of traditional organ preservation. The antithesis of this approach is to provide for metabolic demand during normotheric preservation. This latter strategy has the potential benefit that if metabolic requirements are met during preservation, then longer preservation with better post-transplant function may be achieved than for an organ suffering prolonged cold ischaemia. The aim of this study was to compare the efficacy of preservation of porcine kidneys by normothermic autologous blood perfusion and hypothermic machine perfusion.

Methods 6 large white pigs weighing 80-100Kg were killed by exsanguination, collecting the blood into 25,000 units of heparin. The kidneys were retrieved and one of each pair was perfused on the Portable Organ Preservation System (POPS®) with autologous blood at 37°C and the other on the Waters RM3® system with Belzer's II solution at 4-8°C. The organs were perfused for 16 hours, at the end of which their functions were assessed and compared. The measurement of renal function was performed ex-vivo on the POPS® system. Results are median (range) and statistical comparison was performed using the Mann-Whitney U test. Significance is taken at the p=0.05 level.

Results The ratio of urine to perfusate creatinine and sodium concentrations were calculated as indices of glomerular and tubular function. Values are median (range).
* denotes significance.

	creatinine concentration	Fractional sodium excretion (%)	Proteinuria (g/l)	Glycosuria (mmol/l)	Vascular resistance (ml/min/mmHg)
POPS	21.2 (15.1)	0.10 (0.16)	0.10 (0.18)	0.8 (1.4)	0.07 (0.06)
RM3	3.0 (4.2)	0.70 (0.55)	0.25 (0.41)	1.3 (2.6)	0.10 (0.08)
P value	0.03	0.03	0.2	0.3	0.3

Discussion For each parameter measured, the POPS preserved kidneys performed better than the RM3 preserved organs. In the fields of creatinine concentration ratios and fractional sodium excretion these differences were significant. Transplant experiments are required to confirm that the ex-vivo measurements of function correlate with post-transplant in vivo function.

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THE ROLE OF HEME OXYGENASE-1 (HO-1) DURING COLD STORAGE AND RENAL AUTOGRAFT TRANSPLANTATION IN RABBITS

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Ischemia reperfusion (I/R) injury represents an inevitable pathophysiological event during organ transplantation. Heme oxygenase-1 (HO-1), the inducible isoform of heme oxygenase, is known to protect tissues against oxidative stress originating from various disease states. At present, it is not known whether HO-1 overexpression is capable of alleviating I/R injury caused by cold storage and renal transplantation. In this study, we investigated the effect of HO-1 induction on protection of renal tissue during cold storage and maintenance of post-ischemic renal function. New Zealand White (NZW) rabbits were administered with either saline or hemin (50 mg/kg), an inducer of HO-1. Kidneys were then removed at various time points after treatment and analyzed for heme oxygenase activity, HO-1 mRNA and protein expression. For the transplant study, NZW rabbits were treated with hemin 24 hr prior to renal nephrectomy, followed by hypothermic storage for 24 hr in cold University of Wisconsin solution before autotransplantation. Rabbits were then sacrificed at the end of 24 days and the kidneys were harvested for light microscopic analysis. Hemin, substrate and potent inducer of the heme oxygenase pathway, increased renal HO-1 protein expression and enzymatic activity. The autotransplanted hemin-treated group showed increased survival compared with controls (untreated). Less than 17% of the control animals survived to term post-transplantation, while the hemin-treated group had 83% of the animals survive to term (83 % *vs.* 17 %, $P < 0.05$) (see Figure 1).

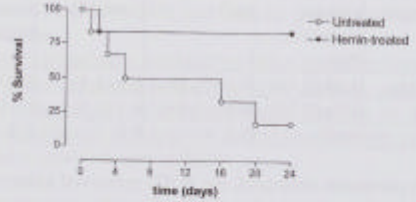

Figure 1

We conclude that hemin promotes increased expression and enzymatic activity of HO-1 in rabbit kidney. In addition, overexpression of HO-1 prior to renal transplantation protects kidneys subjected to hypothermic storage leading to an increased survival rate. These findings suggest the activation of the heme oxygenase pathway may alleviate the effects of reperfusion injury on kidneys which have been subjected to cold storage prior to transplantation.

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ANALYSIS OF IL-6 CYTOKINE LEVELS IN RENAL PRESERVATION SOLUTIONS AFTER STATIC STORAGE.

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The quality of stored organs is likely to be affected by both warm ischaemia (WI) and cold ischaemia (CI) time, which is associated with hypoxia and hypothermic stress to the graft. Prolonged ischaemic times are likely to influence early graft function as damaged organs are less likely to function. Recent data suggests IL-6 may be important in ischaemic injury. We have collected preservation solutions from 12 kidneys about to undergo cadaveric transplantation at Derriford Hospital, Plymouth. Solutions were collected at the time of operation, filtered and stored at -80^0C until use. Total protein concentrations were measured in each solution and used to standardise levels. Commercial ELISA kits were used to measure IL-6 and the anti-inflammatory cytokine IL-4. Kidneys were separated into groups according to early graft function (primary or delayed), WI time (less than or greater than 30 minutes) and CI time (less than or greater than 24 hours). CI times ranged from 13 hours to 37 hours (mean 22 hours). WI times ranged from 20 minutes to 65 minutes (mean 35 minutes). IL-6 levels ranged from 98.75pg/ml to 476.5 pg/ml (mean 264.24 pg/ml). IL-4 levels ranged from 1.29 pg/ml to 46.89 pg/ml (mean 16.32 pg/ml). There was no correlation between increased CI or WI time and IL-6 or IL-4 levels in the solutions. In kidneys with greater than 24 hours CI time the average IL-6 level was slightly higher than in those with less than 24 hours CI time (308.2 $\pm$ 40.1pg/ml vs 249.5 $\pm$ 47.1 pg/ml p=NS). Similarly in solutions with greater than 30 minutes WI, IL-6 levels were higher (312.5 $\pm$ 54.7 pg/ml vs 215.9 $\pm$ 44.4 pg/ml p=NS) and in grafts which had delayed function, IL-6 levels were found to be higher (275.8 $\pm$ 54.1 pg/ml vs 248.1 $\pm$ 53.7 pg/ml). In contrast, IL-4 levels were higher in solutions with less than 24 hours CI time (16.6 $\pm$ 5.7 pg/ml vs 15.3 $\pm$ 4.3 pg/ml p=NS), in solutions with less than 30 minutes WI (16.8 $\pm$ 6.8 pg/ml vs 14.12 $\pm$ 6.3 pg/ml p=NS) and in grafts which had good primary function (17.6 $\pm$ 6.2 pg/ml vs 14.9 $\pm$ 6.5 pg/ml p=NS). In conclusion, cytokines are measurable in preservation solutions obtained from static stored kidneys used for transplantation. This preliminary study found a trend in higher IL-6 cytokine levels in kidneys with longer CI and WI times and delayed function. Higher anti-inflammatory IL-4 levels were found in kidneys with shorter CI and WI times and good primary function. The role of IL-6 in the donor kidney still remains unclear, however analysis of a larger number of kidneys may determine its use as a marker of graft injury.

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DEVELOPMENT OF NON-VIRAL TECHNIQUES FOR REGIONAL GENE DELIVERY TO THE LIVER VIA BRANCHES OF THE BILE DUCT

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Gene delivery to the liver has many potential clinical applications, especially in transplantation. Although adenoviruses are excellent DNA vectors for hepatocytes, they have many disadvantages. A particular problem in transplantation is immunogenicity of viral proteins, which could trigger unwanted allogeneic responses.

We have developed a small, bifunctional, synthetic peptide as an integrin-targeted DNA vector. It consists of a $(lys)_{16}$ chain at the amino terminus for electrostatic binding of DNA and the 15 amino acid integrin-binding domain of the venom of an American pit viper, Crotalus molossus molossus. The *in vitro* characteristics of this vector (polylysine-molossin) have been studied extensively as a preliminary to *in vivo* application. Exit from endocytic vesicles after endocytosis of vector/DNA complexes is a critical step for effective gene delivery. Here we report an analysis of gene delivery to the isolated lobes of the rat liver, via local perfusion through a branch of the bile duct, using chloroquine as the agent to promote endocytic exit.

Dose-response studies were performed for local, oral and systemic chloroquine administration, singly and in combination, for gene delivery via the bile duct. Gene expression in the liver was directly related to serum chloroquine levels at the time of gene transfer, there being no detectable gene expression at serum chloroquine concentrations of 0.6 mg/litre or lower.

Optimal DNA concentrations in the vector/DNA complexes was 50μg/ml. Higher DNA concentrations did not increase gene delivery, possibly because the size of vector/DNA particles increased at the higher DNA concentrations, from 80 nm at 50μg/ml of DNA to 250 nm at 200 μg/ml of DNA.

Chloroquine administration was limited by systemic toxicity. At maximal chloroquine dosage and optimal DNA concentrations in vector/DNA complexes, ~1% of hepatocytes in normal, adult rats were shown to express the β galactosidase reporter gene.

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SENESCENCE AS A CONTRIBUTORY FACTOR IN CHRONIC ALLOGRAFT NEPHROPATHY (CAN).

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CAN is a major cause of kidney transplant failure, with the associated economic and human sequelae. The pathogenesis of CAN remains poorly understood and non-immunological processes are implicated. Halloran has proposed that accelerated senescence, through which the accumulated burden of injury and age, exhausts the ability of key cells to repair and maintain tissue integrity, is key to the process of CAN . We have proposed that injury at a molecular level, resulting from oxidative damage as a consequence of ischaemia and hypoxia, manifests as cellular senescence in grafts, and may result in the features of CAN. Several observations support this hypothesis, including the association of CAN with increased donor age, ischaemic damage prior to organ retrieval, and similarities in the histological features of CAN and aged kidneys, including the expression of senescent-associated extra cellular matrix proteins.

We have measured the expression of several senescence-associated genes (SAGs) in kidney allografts in pre-implantation biopsies from both living and cadaveric donors and from transplant nephrectomy specimens with histologically confirmed CAN. In a split-plot analysis of variance a significant difference was observed in expression of several SAGs (overall ANOVA; $p< 0.01$). Gene differences were assessed by comparison with the residual variation within samples and were significant at $p< 0.001$. Overall differences between kidney types with genes were assessed by comparison with a combination of between and within sample variation, and were significant at $p< 0.05$. The observed differences in expression data did not appear to be correlated with the age of the donor organ) inclusive of time post transplant in CAN samples, but rather a specific reflection of senescence associated processes. A significant difference was also observed in the expression of one senescence associated gene in T_0 Cadaveric samples when compared to T_0 Living, despite no difference being observed between T_0 Living and CAN samples ($p<0.05$).

These results indicate potential early markers for the development of CAN and a possible method for the screening of "marginal" donor kidneys prior to transplantation. They provide clues to the molecular mechanism by which oxidative damage promotes cellular senescence through the action of individual SAGs and thus may permit changes in therapeutic strategy, to prevent or retard the development of CAN. The level of SAG expression may also prove beneficial in pre-transplant screening of cadaveric kidneys. We are now expanding this work through a cDNA macro-array screening strategy and investigations into telomere length dynamics in kidneys undergoing CAN.