British Transplantation Society

4th Annual Congress 2001

John Radcliffe Hospital Oxford

27-29th March 2001

OXFORD CITY MAP

Accommodation

- C — Christ Church
- O — Oriel College

Transport

- T — Taxi Ranks
- B — Bus Stop:
  13 & 13B to the John Radcliffe Hospital

Restaurants

1. Freud's
2. Al-Sharmi
3. Café Rouge
4. Pierre Victoire

British Transplantation Society, Triangle House, Broomhill Road, London SW18 4HX
Tel: 020 8875 2430 Fax: 020 8875 2422 email: secretariat@bts.org.uk

C15

1 AND 4 – YEAR PATIENT SURVIVAL AFTER ADULT FIRST LIVER TRANSPLANTATION IN THE UK, 1994 TO 2000

Mr S P Wijeyekoon[1], Miss L P Copley[1], Dr J van der Meulen[1,2], Mr G J Toogood[1,3] On behalf of the United Kingdom Liver Transplant Audit

1 The Royal College of Surgeons of England; 2 The London School of Hygiene and Tropical Medicine; 3 St James' University Hospital, Leeds.

Introduction: We report patient survival estimates by primary indication for transplantation for 3112 adult (age 15 years and above) first transplants performed between March 1994 and March 2000 in the UK & Ireland, using information derived from the UK Liver Transplant Audit.

Methods: The UK Liver Transplant Audit is a multi-centre prospective cohort study. Participation in Audit is mandatory for all 8 British and Irish transplant centres. The Kaplan-Meier estimate of the survivor function was used to describe patient survival as a function of the length of time after transplantation.

Results:

Indication for transplantation	n [percentage]	1 year patient survival estimate [95% CI]	4 year patient survival estimate [95% CI]
Acute disease	438[14%]	71%[67-76]	66%[61-71]
Cancer	270[9%]	75%[70-81]	45%[35-57]
Metabolic disease	141[5%]	80%[73-87]	65%[54-77]
Cholestatic disease	266[9%]	84%[79-89]	77%[72-83]
Cirrhosis	1843[59%]	84%[82-85]	74%[71-76]
primary biliary cirrhosis	541[17%]	85%[82-89]	80%[76-84]
alcoholic cirrhosis	504[16%]	83%[80-87]	71%[65-76]
viral cirrhosis	481[15%]	83%[80-87]	72%[67-78]
autoimmune cirrhosis	134[4%]	85%[79-92]	72%[62-82]
secondary biliary cirrhosis	27[1%]	84%[70-99]	54%[20-89]
cryptogenic cirrhosis	156[5%]	76%[69-85]	65%[56-76]
Others	154[5%]	72%[64-79]	61%[51-71]
Total	**3112[100%]**	**80%[79-82]**	**70%[68-72]**

Conclusion: Patients transplanted for cirrhosis (59%) form the largest indication group. Of all the indications, patients transplanted for primary biliary cirrhosis have the best survival estimates at both 1(85%) and 4 -years (80%). 16% of the cohort received a transplant for alcoholic cirrhosis and 15% were transplanted for viral cirrhosis. Both groups have comparable survival estimates at 1 and 4-years. The 4-year survival of patients in the acute group is an encouraging 66%. However, the long-term survival of patients transplanted for hepato-biliary cancer remains poor at 45%.

C101

AN EVALUATION OF ADULT SMALL BOWEL TRANSPLANTATION AT TWO CENTRES IN THE UNITED KINGDOM 1991-1999.

N V Jamieson[1], S J Middleton[1], S Pollard[2], P J Friend[1], P Lodge[2], E A B Cameron[1], C Watson[1], R Y Calne[1], M Davies[2], A E Gimson[1], J A Bradley[1], J. Shaffer[3], [1] Addenbrooke's Hospital, Cambridge, [2] St. James Hospital, Leeds, [3] Hope Hospital, Manchester

Small bowel transplantation (SBT) is a challenging field and it is only over the last decade that significant progress has been made in making this procedure a clinical reality. In 1996 the National Specialist Commissioning Advisory Group (NSCAG) commissioned two UK units to assess the value and place of SBT in adults. We report here our experience of adult small bowel transplantation over a ten-year period.

Patients referred for SBT were assessed using a protocol common to both centres. Patients were felt suitable for SBT if they had irreversible small bowel failure and could not be maintained on home parenteral nutrition (HPN) due to complications of HPN (eg. liver disease, failure of venous access). Multivisceral transplantation was considered where appropriate. Patients were assessed as to whether SBT was essential or whether other techniques might allow them to be safely treated without SBT. Outcome was assessed for all cases in terms of survival, quality of life estimates were made both pre and post operatively in three transplanted cases.

Thirty-five patients were evaluated from 1991-99. Sixteen patients were listed for SBT and 19 patients for non transplant management. The underlying diseases included mesenteric vein and artery thrombosis, volvulus, visceral neuropathy, Crohn's disease and Desmoid tumour. Of those listed for SBT 14 underwent transplantation. One patient died on the waiting list (listed for SB and liver) and one remains on the waiting list (listed for SB and liver). Six patients received small bowel alone (one living related donor), one SB and colon, one SB and liver and five multi-organ grafts. The indications for Tx were failure of venous access (n=3), PN related liver disease (n=3), Desmoid tumours (n=4), intra abdominal venous and arterial thrombotic disease (n=2), cholangiocarinoma (n=1) and neuroendocrine tumours (n=2). One patient had an identical living triplet donor and was transplanted as the absence of any risk of rejection of the graft.

Nineteen patients were considered unsuitable for transplantation because they could be managed safely with a lower risk of morbidity and mortality than that currently expected following SBT. Twelve of these patients remain alive on PN and 3 on enteral nutrition. One patient was felt to be depressed and suicidal and subsequently took his own life. A further patient died of pneumonia two years following assessment, one could not be transplanted because of ongoing intra abdominal sepsis, one patient awaits reassessment for SBT having active pulmonary sepsis at present.

Twelve patients survived transplantation surgery, 4 died within the first 6 months. All patients who survived for more than 6 months remained independent from PN for the majority of their remaining lives. In general transplanted patients spent on average 90% of post transplant life off PN and were able to take a normal diet.

Intestinal Transplantation offers patients with intestinal failure and complications of HPN a chance of restoration of normal enteral nutrition and good quality of life. Careful selection processes allowed a good outcome in 15/19 patients without the need for SBT to be undertaken.

C10

LATE CORTICOSTEROID WITHDRAWAL IN RENAL TRANSPLANT RECIPIENTS ON TRIPLE IMMUNOSUPPRESSIVE THERAPY – A RANDOMISED CONTROLLED TRIAL

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Corticosteroids have been the mainstay of immunosuppression in clinical transplantation for 30 years but cause significant morbidity including hypertension, obesity, diabetes mellitus, hyperlipidaemia and osteoporosis. Despite clinical trials showing the safety of late steroid withdrawal, over 70% of renal transplant recipients remain on long-term steroid therapy.

The aims of this study were to assess in a randomised controlled trial, the safety and benefits of late steroid withdrawal in stable renal transplant recipients with good graft function (Cr<200μmol/l) on triple immunosuppression (prednisolone, azathioprine and Neoral). Primary end-points were acute cellular rejection, graft and patient survival. Secondary end-points included serum creatinine, change in cystatin C, cholesterol, blood pressure, body weight, bone mineral density (BMD) and rate of change of creatinine.

In 1997 all patients at our centre were screened for their eligibility to enter the trial. Of 608 patients screened, 209 patients were deemed eligible. 92 patients were randomised (44 withdrawal, 48 control); all other patients were prospectively followed. 57 highly selected patients withdrew steroids outside the context of the trial and 60 patients declined entry. Prednisolone dose was reduced by 1 mg/month. A rise in serum creatinine of more than 15% above baseline resulted in a renal biopsy. BMD (DEXA) was measured at baseline and at 12 months.

	Non-randomised		Randomised	
	Declined	Withdrawn	Control	Withdrawal
N	60	57	48	44
Males n (%)	41 (68%)	25 (50%)	29 (59%)	30 (70%)
Mean age (SD)	40.8 (15.0)	44.2 (13.7)	44.6 (12.7)	41.5 (17.4)
Mean follow up/yrs (SD)	1.9 (0.3)	1.9 (0.8)	1.3 (0.3)	1.4 (0.3)
Acute rejection	2	0	1	1
Chronic Allograft Nephropathy	2	1	1	3
Start mean Cr μmol/l	125	112	131	131
End mean Cr μmol/l (p)	127 (ns)	113 (ns)	137 (ns)	142 (ns)
ΔCr μmol/l /year (p)	4.6	2.8 (ns)	2.0	7.9 (p=0.02)
Femoral neck BMD % change (p)	-	-	-0.1%	+3.9% (p<0.05)

The results of this study illustrate:

Low dose prednisolone (mean 6.2mg/day) has a detrimental effect on bone mineral density; prednisolone withdrawal improves bone mineral density.

One-year post-steroid withdrawal there is no significant change in mean serum creatinine in patients who have withdrawn steroids compared to controls.

Rate of change of serum creatinine (ΔCr) appears to be worst in those patients who withdrew steroids (in RCT); longer follow up may demonstrate a difference in mean serum creatinine in this group

4. The highly selected patients who withdrew steroids outside the trial experienced no detrimental effect on serum creatinine and rate of change of creatinine was near zero.

C22

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

Johnson RJ, Armstrong SA, Belger MA, Briggs JD, Fuggle SV, Morris PJ on behalf of the UK Transplant Kidney and Pancreas Advisory Group, Bristol, UK

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 children and local patients receive priority and 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 two years have been compared with those of the last 18 months of the previous scheme. There have been significant improvements in HLA matching for adult and paediatric transplants ($p<0.0001$ and $p<0.003$, respectively), achieved through greater exchange of organs between centres. The proportion of 000 mismatched grafts has increased from 7% to 13% for adults and from 5% to 13% for children. There has also been a threefold increase in the number of 000 mismatched grafts for highly sensitised patients (HSP). For those kidneys allocated to adults through the national Scheme there have been some changes with regard to the points scoring factors. Firstly, transplanted recipients were significantly younger than previously ($p<0.01$). This was not an objective of the new Scheme and is a trend that will have to be carefully monitored. Secondly, the mean donor-recipient age difference has decreased by 2 years suggesting an effect over and above the trend of increased mean donor age. Also, patients who are moderately difficult to HLA match have received proportionally more transplants at the expense of those who are easiest to match ($p<0.03$). The median waiting time of adults receiving nationally allocated kidneys has continued to increase, namely from 336 days (IQ range, 135-669) to 439 days (IQ range, 173-910), ($p<0.0001$). It is not clear whether patients who have had to wait a long time have benefited through points scoring.

In conclusion, the new UK Kidney Allocation Scheme has been associated with improved HLA matching for adults and children and for both first graft and regraft recipients; a threefold increase in the number of HSP 000 mismatched grafts; younger adults receiving kidneys allocated through the national Scheme and a decrease in donor-recipient age differences. Finally, matchability points scoring may have helped to achieve an increase in the number of transplants for patients who are moderately difficult to HLA match.

C96

Long-term renal function after transplantation from NHBD kidneys

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Introduction

As marginal kidneys such as NHBD provide an increase in the donor pool, its recruitment is limited by the poor results of transplantation. With suspicions of poor quality kidneys from NHBD donors, we looked at the long-term function of NHBD kidney transplants as to compared to HBD kidney transplants. A case control study was carried out to look at the renal function of NHBD & HBD kidney transplants at The Freeman Hospital from 1998 till current times.

Materials & methods

Renal function was assessed by time-course serum creatinine / urea values, from the time of hospital discharge. Creatinine clearances were calculated using Cockcroft-Gault formula. Survival statistics were calculated using Kaplen Meier curves.

Results

A control group of 37 HBD kidneys were selected by taking the next consecutive HBD after a NHBD. This group was found to be matched for:- tissue HLA mis-match, donor & recipient factors. However, cold ischaemia time was significantly less for the HBD kidneys (Mann Whitney U, p = 00006).

	NHBD	HBD control	Mann Whitney U (p value)
DFG (%)	97.3	56.8	0.002
1st year survival rate			(Log rank)
patient	90.9	88.7	p = 0.754
kidney	88.7	96.4	p = 0.153
Hospital stay (days)	24.9 ± 2.0	21.6 ± 2.6	p = 0.014
Biopsy proven acute rejection (Y : N)	17 : 20	15 :20	0.688
Creatinine clearance (mls/min)			
Discharge	22.9 ± 2.3	44.4 ± 3.0	< 0.0001
3 months	40.9 ± 3.2	46.0 ± 3.3	0.119
6 months	42.3 ± 3.3	47.9 ± 4.2	0.203
12 months	47.2 ± 4.1	48.5 ± 4.5	0.874
18 months	56.6 ± 5.4	54.7 ± 5.4	0.878

Conclusion

NHBD kidneys have a high incidence of delayed graft function (DGF), which is reflected in reduced creatinine clearance at discharge. However, from 3 months onwards the creatinine clearance is no different to HBD kidneys.

L28

DONOR-SPECIFIC DENDRITIC CELLS MODULATE CARDIAC ALLOGRAFT REJECTION

J.S.Billing, J.W.Fry, P.R.Wheeler, P.J.Morris and K.J.Wood. Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU

Objective: Antigen-presenting cells are central to the adaptive immune response. T cell activation requires both T cell receptor ligation (signal 1) and interaction with a number of costimulatory molecules expressed by professional antigen-presenting cells (signal 2). Delivery of signal 1 in the absence of costimulation can produce a state of T cell anergy. Immature dendritic cells (DC) express MHC class II but are deficient in costimulatory molecules. We investigated the effect of mature and immature donor-specific DC on rejection of a cardiac allograft, including syngeneic DC transduced with an adenoviral vector for the donor MHC class I antigen H-2K^b.

Methods: Dendritic cells were generated from bone marrow (BM) by culture in medium containing GM-CSF. Culture of BM cells in GM-CSF for 7 days produced immature DC and very few mature DC, as shown by expression levels of MHC class II and costimulatory molecules. Extending the culture period to 12 days resulted in a mixed population of mature and immature DC. The functional phenotype of 7-day and 12-day DC was assessed by their ability to act as stimulators in mixed lymphocyte culture (MLC). CBK (H-2^k + K^b as a transgene) dendritic cells were administered to CBA (H-2^k) recipient mice 27 days pre-transplant, in conjunction with two doses of anti-CD4 mAb on days –28 and -27. This was followed by transplantation of a fully allogeneic C57BL/10 (H-2^b) heart on day 0. Additionally, 7-day autologous CBA DC were transduced with an adenoviral vector encoding the MHC class I gene H-2K^b (AdSV40K^b) and were given one month pretransplant with anti-CD4 mAb.

Results: In an MLC assay, 7-day DC were far less potent stimulators of allogeneic lymphocytes than 12-day DC. A dose of 10^5 12-day CBK DC, given with 2 doses of anti-CD4, accelerated cardiac allograft rejection relative to antibody controls (MST 10 days, p=0.004). However, 7-day CBK DC at doses of 10^5 and 10^6 cells induced long-term survival of 75% of cardiac allografts in each group (MST >100 days, p<0.05). Transduction of 7-day DC with AdSV40K^b at a multiplicity of infection of 100 resulted in surface expression of K^b by 41% of cells and these transduced DC also prolonged the survival of a fully allogeneic cardiac graft when given with anti-CD4.

Conclusions: Donor-specific DC had a profound influence on allograft rejection, with mature DC accelerating the process and immature DC inducing long-term survival. Adenoviral transfer of a donor MHC class I gene rendered syngeneic DC able to prolong allograft survival. These findings suggest that BM-derived immature DC may provide a useful vehicle for the delivery of donor alloantigen in tolerance induction strategies.

Parallel Sessions

Tuesday 27 March

16.00-17.30

Session A

Scientific Session

L59

CONTRIBUTION OF DONOR HYPERTENSION TO RECIPIENT BLOOD PRESSURE AFTER RENAL TRANSPLANTATION

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Essential hypertension is a polygenic, multifactorial disease affecting 20% of the Western population. Although, hypertension is also a common complication following kidney transplantation due to the use of calcineurin inhibitors there is also evidence to suggest that causative genes could be acting via a renal-based mechanism. We have investigated the origins of such genetic hypertension using the Spontaneously Hypertensive Rat (SHR) strain and the normotensive Wistar-Kyoto (WKY) rat strain. From these strains we constructed a unique congenic strain called WKY.SHR-Sa in which we transferred a small region (around 34 cM) of rat chromosome 1 from SHR into the WKY thought to be important in the renal control of blood pressure. Addition of this gene region into the WKY strain resulted in approximately a 20% increase in blood pressure in the new congenic strain WKY.SHR-Sa.

As the hypertensive WKY.SHR-Sa congenic strain is essentially >99% genetically equivalent to WKY, we transplanted a kidney from a hypertensive rat into a normotensive rat. The use of congenic strains was designed to overcome any problems of immune rejection and by transplanting a kidney, the origins of any changes in blood pressure in the recipient would be isolated to the donor kidney genotype alone. A contralateral nephrectomy was performed at day 7. Post transplantation, indirect blood pressures were measured at 12 weeks and 20 weeks using tail plethysmography. The experiment was terminated at 28 weeks and tissues taken for histology that showed no immune mediated damage. The controls used were syngeneic WKY to WKY transplants or uninephrectomised WKY rats and WKY.SHR-Sa rats.

Transplantation of a congenic kidney, but not a WKY kidney in to a WKY recipient, increased blood pressure significantly in WKY recipients at all ages.

	WKY.SHR-Sa to WKY Tx Blood Pressure	WKY to WKY Tx Blood Pressure	significance
12wk BP	115.8 ± 5.6	108.5 ± 3.3	<0.001
20wk BP	134.9 ± 4.1	124.1 ± 3.5	<0.001
28wk BP	139.7 ± 12.7	129.7 ± 12.0	0.049

The data show that transplantation of a kidney from a hypertensive donor was responsible for blood pressure elevation in the recipient strain. The genetic mechanisms within rat chromosome 1 and the specific genes responsible for this effect are currently being investigated. The results also demonstrate the transfer of a donor phenotype to the recipient that could form a risk factor for post transplant hypertension in clinical transplantation that is known to adversely affect long-term outcome.

L21

INHIBITION OF ARTERIAL ALLOGRAFT VASCULOPATHY WITH CONCURRENT BLOCKADE OF THE CD28 AND CD40 PATHWAYS IN THE NON-HUMAN PRIMATE MODEL.

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The use of cadaveric human arterial allografts represents a potential source of physiologically ideal vascular conduits to treat cardiovascular disease. Application of this therapy is limited by the failure of conventional immunosuppressive agents to prevent rejection of these allografts characterized by transplant vasculopathy. The aim of this study was to test the ability of costimulation blockade to protect allogeneic arterial grafts from immunologic injury and improve graft patency in a non-human primate model.

Aortic replacement was performed by the exchange of infrarenal aorta between MHC disparate unrelated Rhesus Macaques. All pairs had a negative flow cytometry crossmatch prior to transplantation. Human CTLA4-Ig (20 mg/kg) and H106 (anti-human CD40 ligand monoclonal antibody, 20 mg/kg) were given intravenously on days 0, 4, 7, 14,28,42,56 and 70. Control recipients were treated with saline on the same days. The development of anti-donor antibodies was monitored in the post-operational period. To assess graft patency and structure of the graft wall, angiography and intravascular ultrasonography (IVUS) was performed on days 0, 28, 70 and 150. Graft histology was analyzed at the time of sacrifice on days 28,70 and 150.

In control recipients, the angiogram showed marked narrowing of the graft lumen on day 75. One out of 3 recipients had a complete occlusion of the graft lumen on day 75. IVUS image also showed not only significant increase in thickness of the graft wall and intima ($p<0.0001$) after day 28, but also significant decrease in graft wall compliance after day 75 ($p<0.001$). Graft histology showed intimal hyperplasia, medial thickening, leukocyte infiltration (detected by CD45 immunohistochemistry) and cellular proliferation (detected by BrdU immunohistochemistry). Anti-donor antibody responses generated in all control recipients after day 75. Treatment with CTLA4-Ig and H106 resulted in significant reduction in wall and intimal thickening of the grafts ($p<0.01$) and a marked decrease in the degree of vasculopathy by histology. Anti-donor antibody responses were also inhibited by the treatment.

These data show that concurrent blockade of CD28/B7 and CD40/CD40L pathway markedly inhibits the alloimmune response to arterial grafts and improves graft patency in this non-human primate model.

L30

DO INTRATUBULAR CD103 T CELLS PLAY A ROLE DURING RENAL ALLOGRAFT REJECTION?

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Inflammation of renal tubules, or tubulitis, is a defining feature of acute renal allograft rejection. Renal tubules, a basic functioning unit of the kidney, are epithelial in origin. Several groups have observed that a subset of graft infiltrating cells in murine transplant models express CD103, which defines the α subunit of the $\alpha^E\beta_7$ integrin. This molecule is expressed by over 95% of intestinal intraepithelial cells but by fewer than 2% of peripheral blood lymphocytes. The only known ligand for this integrin is E-cadherin, a molecule restricted almost exclusively to epithelial cells. We propose that the expression of CD103 by kidney graft-infiltrating T cells may promote preferential adherence of cytotoxic effector cells to renal tubular epithelial cells (RTEC) during rejection.

This hypothesis was tested initially by a series of immunohistochemical analyses to determine the localisation of CD103 cells following renal transplantation. Examination of 34 frozen biopsy specimens taken during episodes of clinical dysfunction after kidney transplantation revealed that the mean number of CD8 and CD103 cells per renal tubular cross-section correlated significantly with the severity of rejection ($p<0.01$). Furthermore, the proportion of CD103 expressing cells increased proportionately more than CD8 cells alone ($p<0.03$). In all specimens, CD103 T cells were only found juxtaposed to RTECs and not within the interstitial infiltrate. Transforming growth factor-β (TGFβ), a known regulator of CD103 expression, was also found to be expressed on RTECs at an increased level during rejection ($p<0.03$).

Following demonstration of CD103 positive T cells *in situ*, a series of *in vitro* assays was performed to examine the regulation of expression of the $\alpha^E\beta_7$ integrin on T cells. Activation of allospecific T cells was achieved using the mixed lymphocyte reaction and the resultant cell lines were used to characterise the effect of TGFβ stimulation. Following TGFβ treatment, CD103 was found to be preferentially expressed on CD8 cytotoxic T-cells; >60% of CD8 cells were CD103 positive by day 7 in culture. Proliferating T cells were identified by CFSE staining and flow cytometry; secondary immunofluorescence identification of CD103 showed that TGFβ only induced expression of the $\alpha^E\beta_7$ integrin after at least one cycle of cell division. Intracellular immunofluorescence demonstrated co-expression of perforin and CD103.

Collectively, this evidence suggests strongly that the CD103 T cells found within renal tubules during acute rejection are induced to express the $\alpha^E\beta_7$ integrin by tubule-associated TGFβ, and are of an allospecific cytotoxic phenotype with an adhesive preference for RTECs. Clearly, these cells have the potential to play an active role in tubular damage during acute rejection.

L31

UNDERSTANDING THE ACCEPTANCE OF HUMAN RENAL ALLOGRAFTS: REGULATORY MECHANISMS IN DONOR-SPECIFIC HYPORESPONSIVENESS

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We have studied 22 long standing recipients of live-related renal allografts, 13 with have good graft function and 9 with chronic allograft nephropathy (CAN), to detect the presence and mechanisms of donor-specific "tolerance".
(i) The direct pathway of allorecognition was investigated using limiting dilution analysis (LDA); all 22 patients exhibited significant donor-specific hyporesponsiveness for IL-2 secretion. (ii) Based on the hypothesis that the hyporesponsiveness was induced by encounter with graft parenchymal cells this analysis was further refined by the study of naïve (CD45RA) versus memory (CD45RO) recipients CD4+ T cells. Given that only memory T cells cross the vascular endothelium and traffic through peripheral tissues, we predicted that donor-specific hyporesponsiveness would be more pronounced in the CD45RO subset. 9 patients were analysed before and 4 months after transplantation and donor-specific hyporesponsiveness was indeed more pronounced in the CD45RO population (Wilcoxon $p<0.05$). (iii) To address the possible contribution of anergy to the observed hyporesponsiveness, recipient CD4+ T cells were either tested immediately or were cultured for 3 days in recombinant IL-2 prior to LDA to recover antigen reactivity in the responding cells. In 5 out of 8 hyporesponsive patients culture with IL-2 led to full restoration of anti-donor frequencies. (iv) Given our previous finding that anergic T cells act as regulatory cells, we analysed our LDA for evidence of regulatory CD4+ T cells and deviation from single hit kinetics. Significantly more regulatory cells were detected in response to donor, compared to third party stimulators ($p<0.05$). In 8/22 patients the frequency of donor specific regulatory cells was substantially elevated. No evidence of Th1/Th2 polarisation was obtained. (v) To measure indirect anti-donor alloreactivity, the LDA protocol was modified by the addition of recipient antigen presenting cells and the use of whole cell protein extracts as the source of donor antigen. Significantly elevated frequencies of T cells with indirect anti-donor specificity were observed in the patients with CAN.

This data suggests that the direct pathway of anti-donor alloreactivity diminishes with time in most, if not all, renal allograft recipients. This induced hyporesponsiveness may be due to the induction of anergy as a result of encounter with graft parenchymal cells. In contrast the indirect anti-donor alloresponse appears to be the major immunological driver of CAN. These observations are of clear relevance to the design of assays of clinical transplantation tolerance.

LABORATORY

L58

CHEMOATTRACTION OF T CELLS EXPRESSING CCR5 AND CXCR3 BY PROXIMAL TUBULAR EPITHELIAL CELL CHEMOKINES

Dr P Cockwell[1,2], Mr CJ Brooks[1], and Professor COS Savage[1,2]. [1]Division of Medical Sciences, University of Birmingham and [2]Department of Nephrology and Renal Transplantation, Queen Elizabeth Medical Centre, Birmingham

Chemotactic factors produced by resident renal cells in acute renal allograft rejection promote the differential infiltration of activated T cell subsets. We have analysed the chemotactic responses of resting and activated CD3+ peripheral blood lymphocytes (PBLs) to factors secreted by proximal tubular epithelial cell (PTEC) supernatant, assessing the role of chemokines and chemokine receptors in this process.

By FACS we analysed expression of the chemokine receptors CCR5, CXCR3, CCR2, CXCR1 and CXCR2 on both freshly isolated and activated PBLs. Using Boyden chambers we studied the chemotactic activity of supernatant from resting and cytokine (TNF-α and IFN-γ) stimulated PTEC towards PBLs. Blocking antibodies were used to study the role of chemokine receptors. Using ELISA we analysed the levels of the chemokines RANTES (for CCR5), IP-10 (for CXCR3), MCP-1 (for CCR2), and IL-8 (for CXCR1 and CXCR2) in PTEC supernatant.

Only a small proportion of freshly isolated cells expressed the chemokine receptors analysed and there was low grade chemotaxis of these cells to cytokine-stimulated PTEC supernatant when compared to unstimulated PTEC supernatant. After activation 84% of PBLs expressed CCR5 and 90% expressed CXCR3. There were low expression levels of CXCR1 (<15%), CXCR2 (<5%) and CCR2 (<7%). Activated PBLs showed strong chemotactic responses to supernatant from cytokine- stimulated PTEC compared to unstimulated PTEC ($p<0.0001$). Chemotaxis of these cells was completely blocked by pertussis toxin and inhibited by blocking antibodies to CCR5 and CXCR3 by 66% and 59% respectively. Cytokines stimulated high levels of RANTES (up to 63.3 ± 1.0 ng/ml), IP-10 (up to 477 ± 19 ng/ml), MCP-1 (up to 104 ± 15.6 ng/ml) and IL-8 (up to 6.6 ± 2.2 ng/ml). There was little chemokine production by unstimulated PTEC.

In conclusion, chemokines produced by cytokine activated PTEC promote the selective recruitment of activated T cells via the receptors CXCR3 and CCR5. These may be key processes in promoting acute renal allograft rejection. Thus these receptors and their targeting chemokines, including RANTES and IP-10, may be potentially important therapeutic targets.

LABORATORY

L70

HLA-DP MISMATCHING IN "000" CADAVER DONOR KIDNEY TRANSPLANTS.

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Manchester Institute of Nephrology and Transplantation

HLA-DP is a Class II molecule, encoded centromeric to HLA-DR and -DQ. HLA-DP-specific antibodies have been implicated in allograft rejection, suggesting that matching for this molecule may influence transplant outcome. HLA-DP has restricted expression on CD4+ T cells, B lymphocytes and antigen presenting cells. Until very recently, there was no suitable typing system available for -DP alleles, which meant that it was not possible to assess the importance of matching for this locus in renal allografts.

We have adapted an HLA-DP DNA based typing system, to define 93 -DP alleles, including the 18 most common -DPB1 alleles, and 8 -DPA1 alleles. Using this system, we have defined -DPA1 and DPB1 alleles present in 60 kidney transplant donor / recipient pairs who were not mismatched ("000") for HLA-A, -B and -DR. Transplant outcome and incidence of acute rejection were available for all patients included in the study.

For HLA-DPB1, graft survival was increased in recipients who were not mismatched for any -DPB1 alleles with their donor, over those who were mismatched for 1 or 2 -DPB1 alleles (93.3% vs 79.7%, not significant). -DPA1 mismatching did not adversely influence survival. When there was no mismatch at DPB1, one year survivals were high (93.3%) and there were no further losses at five years. In addition, -DPB1 mismatching was significantly associated with the occurrence of acute rejection in "000" mismatched transplants; when there was no -DPB1 mismatch, only 1/13 (7.7%) recipients had a single acute rejection episode. In contrast, when there was -DPB1 mismatching, 12/31 patients (38.7%) had one or more acute rejections ($p=0.04$).

Our data show that -DPB1 mismatching in "000" mismatched cadaver kidney transplants identifies those patients

- whose grafts are more likely to fail after one year
- who will experience acute rejection episodes.

L16

THE EFFECT OF ACUTE REJECTION ON INTRAGRAFT TGFβ EXPRESSION AND THE SUBSEQUENT DEVLOPMENT OF CHRONIC RENAL ALLOGRAFT INJURY.

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Acute rejection (AR) is an important determinant of chronic allograft injury. Previous studies have demonstrated that chronic allograft injury is mediated by enhanced allograft TGFβ expression. In this study we sought to determine the effect of severity of AR on renal allograft TGFβ expression and the subsequent development of chronic renal injury. Thirty two patients underwent protocol biopsies at the time of engraftment (0 months) and at 3, 6, and 12 months following transplantation. Unscheduled biopsies were taken in response to a significant increase in creatinine. Severe rejection was defined as greater than Banff 4 grade II using the Banff 97 criteria. Two groups were identified: Group 1 (n=21): No rejection; Group 2 (n=11): Severe rejection. All patients were on cyclosporine based triple immunosuppression. Renal allograft TGFβ expression was measured using quantitative RT-PCR. Structural injury was quantified using morphometric analysis. Results were expressed as mean ±SEM. Statistical significance between groups was calculated using Mann Whitney U significance was taken as * $p<0.05$. Paired t test analysed temporal changes in TGFβ and chronic renal injury compared to baseline. Significance was taken as $^{\phi}$ $p<0.05$.

Time	Log Co TGFβmRNA		IF %		GFR ml/min	
	Group 1	Group2	Group 1	Group2	Group 1	Group2
0	1.9±0.2	2.3±0.4	12±1	13±1		
Unsched	2.9±0.3	3.6±0.2*				
3	2.6±0.3	4.2±0.6*	18±1	27±3*	55±3	31±3*
6	3.2±0.3$^{\phi}$	3.3±0.6$^{\phi}$	31±3	42±5$^{\phi}$	58±3	40±4*
12	2.9±0.3$^{\phi}$	3.6±0.4$^{\phi}$	39±3	49±7$^{\phi}$	59±4	40±4*
24					58±3	40±5*

In agreement with our previous studies we have demonstrated a progressive increase in TGFβ expression and interstitial fibrosis in renal allografts of patients who have not had AR. We have hypothesised that this represents a response of the allograft to cyclosporine. Group 2 patients also demonstrated a progressive increase in TGFβ expression and interstitial fibrosis in protocol biopsies. The rate of rise of interstitial fibrosis in protocol biopsies was similar between groups 1 and 2. However group 2 patients had significantly higher TGFβ expression at the time of rejection and at 3 months compared to group 1. Correspondingly interstitial fibrosis at 3 months was greater in group 2 patients. We propose that this enhanced expression of TGFβ and interstitial fibrosis at 3 months together with the progressive structural damage contribute to the lower GFR in group 2. Our study suggests that the severity of the AR is of primary importance in contributing to the development of progressive chronic allograft injury.

L63

THE INDIRECT PATHWAY OF ANTIGEN RECOGNITION IS INSENSITIVE TO CYCLOSPORIN A: IMPLICATIONS FOR CHRONIC REJECTION

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Cell-mediated rejection of allografts is primarily orchestrated by T cell responses via two distinct pathways, the direct and indirect presentation pathways for alloantigens. Direct alloantigen recognition involves direct interaction with intact donor MHC molecules on the surface of donor antigen-presenting cells. This response is highly sensitive to immunosuppressive drugs such as cyclosporin A (CsA). Indirect recognition of donor antigens is identical to the presentation of nominal antigens such as tetanus toxoid, in that T cells recognize processed donor antigens presented by recipient antigen-presenting cells. However, the effects of immunosuppression in the indirect pathway, which has been implicated to play a role in chronic rejection, has been shown to be inconclusive with regards to sensitivity. In order to clarify this discrepancy, we have carried out both direct (allogeneic MLR) and indirect (tetanus toxoid recall response) T cell proliferation assays in the presence or absence of CsA.

Using purified APC-depleted T cells, a striking difference in CsA sensitivity was observed between the direct and indirect response. The effect of CsA in the T cell response to allogeneic PBMC resulted in an ID_{50} value of 42.1 ± 30.1ng/ml (n=7). However, in the indirect T cell response to T cell-depleted autologous APC presenting tetanus toxoid, the ID_{50} value for CsA inhibition was significantly higher at 115 ± 33.2ng/ml (n=5) indicating that the indirect response is relatively insensitive to CsA inhibition. In an attempt to ascertain the mechanisms that may be involved, the role of the cytokine IL-15 was investigated. Recent reports have indicated that IL-15-driven T cell responses show resistance to CsA inhibition, unlike IL-2-triggered immune responses. Primary results have indicated that indirect T cell responses to tetanus toxoid are inhibited in a dose-specific manner following the addition of anti-IL-15 monoclonal antibody (range from 1.25 – 20 μg/ml). In contrast, there was no reduction in the allogeneic T cell response using the same range of anti-IL-15 concentrations. This suggests that IL-15 contributes to the indirect T cell response and the difference in CsA sensitivity between direct and indirect T cell responses could be due to the differential cytokine requirements.

In conclusion, these preliminary results suggest that the indirect T cell response is insensitive to CsA inhibition and that this resistance may in part be attributed to IL-15.

Session B

Kidney/Immunosuppression Session

C86

MONOTHERAPY IN LIVING DONOR KIDNEY TRANSPLANTS. A RETROSPECTIVE STUDY OF A LARGE COHORT.

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AIMS: Immunosuppression in transplant recipients has to be finely balanced between achieving maximum possible graft survival and avoiding the undesirable and debilitating side effects of immunosuppressive drugs and over immunosuppression. This retrospective study looks at cyclosporin/FK monotherapy in living donor kidney transplants in an era of multiple new immunosuppressive drugs.

METHOD: All living donor kidney transplants done at this centre were evaluated. All patients who were commenced on cyclosporin/FK monotherapy with intention to treat were analyzed with regard to rejections, additional immunosuppression required, graft survival and incidence of CMV disease and malignancies.

RESULTS: Out of 2700 kidney transplants carried out at this centre since the program started in 1968, there have been 235 living related transplants (10%). In line with unit immunosuppressive policy all adult, first kidney transplants were commenced on cyclosporin/FK monotherapy. Complete data were available on 80 recipients who had been commenced on monotherapy with intention to treat, between Jan 1982 and October 2000.Rejections were treated with steroid pulses or ATG, with addition of azathioprine or/and steroids as thought necessary. In this group, currently 61 grafts are functioning (78%). Of the 19 grafts lost 3 were lost due to fulminant rejection in the early post transplant period, while 16 were lost due to a combination of chronic transplant nephropathy and recurrent disease. Of these functioning grafts 33 (54%) continue on monotherapy. This appears to be a self selected group with a rejection rate of less than 20% in this group. 7 patients are on a combination of cyclosporine and prednisolone and three patients, on azathioprine and prednisolone. The remainder are on triple therapy with cyclosporine/FK, azathioprine and prednisolone. The 1 year graft survival is 97.5%, 5yr-83.7% and 10yr-52.8%. The incidence of CMV has been less than 7% and the incidence of malignancies has been less than 5%.

CONCLUSION: cyclosporin/FK monotherapy permits introduction of increasing immunosuppression only when required, leaving a self selected group without the morbidity of long term overimmunosuppression and steroids. However the significant graft attrition rate between the 5th and 10th year suggests a need to reconsider whether the trade-off between long term graft function and low morbidity from monotherapy is an unequal one.

CLINICAL

C80

INDIVIDUALISING IMMUNOSUPPRESSION: THE IMPACT OF THIOPURINE S-METHYLTRANSFERASE POLYMORPHISMS ON AZATHIOPRINE DOSE ONE YEAR AFTER RENAL TRANSPLANTATION

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Understanding the genetic basis of variation in drug metabolism has the potential to improve prescribing in clinical practice. Thiopurine S-methyltransferase (TPMT) is one of the enzymes involved in azathioprine metabolism. It exhibits a number of genetic polymorphisms that correlate with enzyme activity. We undertook a retrospective study to determine whether recipient TPMT genotype predicts azathioprine dose one year after renal transplantation.

Methods

PCR-based assays were developed to genotype for all known TPMT coding variants and for the VNTR polymorphism in the promoter region.

The clinical records of 229 consecutive renal transplant recipients were examined with particular reference to azathioprine dose. 56 individuals were excluded because azathioprine was not given at induction,1 year follow-up was not available, azathioprine was discontinued during the first year because of a fundamental change in immunosuppressive regimen or potential drug interaction, or DNA was not available.

173 individuals were therefore included in the study. In addition to cyclosporin and prednisolone, all received azathioprine according to a single protocol. This stipulated a dose of 1.5mg/kg at induction and throughout the first year after transplantation, unless the white cell count fell below 4.0 x 10^9/l, when it was reduced. No major adverse events related to azathioprine use were recorded.

Results

PCR-SSP genotyping revealed 12 individuals heterozygous for a TPMT coding variant (*3A n=11, *3C n=1; 6.7%). 7/12 TPMT coding heterozygotes decreased their azathioprine dose during the first year because of persistently low white cell counts, compared with 55/161 TPMT wild types, p=0.09. When other factors known to influence white cell count were considered, 6/11 TPMT coding heterozygotes decreased their dose unrelated to CMV infection or ATG, compared with 28/133 TPMT wild types, p=0.01. In this subgroup, mean azathioprine dose at one year was 1.01 mg/kg in TPMT coding heterozygotes compared with 1.26mg/kg in TPMT wildtypes, p=0.05.

VNTR*4 was the most common promoter genotype (present in 128/173 subjects), and repeat frequency ranged from 3 to 8. Amongst TMPT wildtypes, the presence of the promoter VNTR*4 allele was associated with higher mean azathioprine dose at one year (VNTR*4 allele present, 1.20mg/kg; VNTR*4 allele not present, 1.02 mg/kg; p=0.01).

The incidence of acute rejection was unaffected by TPMT promoter or coding genotype.

Conclusion

This study suggests that when azathioprine is initially administered at 1.5mg/kg, both coding and promoter TPMT polymorphisms contribute to the dose tolerated. While regular haematological monitoring remains mandatory, TPMT genotyping may allow individualisation of azathioprine dose after transplantation.

C11 WHAT IS THE VALUE OF SHORT SYNACTHEN TESTS IN PREDICTING EASE OF STEROID WITHDRAWAL? – RANDOMISED CONTROLLED TRIAL

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Withdrawal of steroids from stable renal transplant recipients may not be without risk. Studies of early steroid withdrawal have demonstrated a 10% rate of acute cellular rejection (ACR); some patients may be at risk of subsequently developing chronic allograft nephropathy. Furthermore, some individuals develop a syndrome of malaise, polyarthralgia, muscle aches, depression and occasionally erythematous rashes. This has been termed "steroid withdrawal syndrome" (SWS). A study of withdrawal of prednisolone from patients with failed renal allografts demonstrated that some patients have an abnormal hypothalmo-pituitary-adrenal (HPA) axis for many months.

We proposed that individuals who develop biochemical hypoadrenalism following steroid withdrawal would be more at risk of rejection (acute or chronic) and development of "steroid withdrawal syndrome"

The aim of our study was to investigate the predictive value of short Synacthen tests on individuals withdrawing steroids in the context of a randomised controlled trial. 92 patients were randomised to either withdraw steroids gradually (1mg/month, 44 patients) or to remain on steroids (48 patients). All patients withdrawing prednisolone had standard dose Synacthen tests (250µg synthetic ACTH, serum cortisol at T_0, T_{30mins}, T_{60mins}) performed at baseline, on completion of steroid withdrawal and 3 months post withdrawal. Symptomatic patients had an additional Synacthen test performed when their symptoms resolved. Two Synacthen tests were performed in the control group (19 patients) 6 months apart to confirm no change in adrenal function. All Synacthen tests were performed in the morning and, in the case of individuals on steroids, prednisolone levels were taken to confirm that patients had omitted the last dose. All patients were investigated for other causes of polyarthralgia.

There were two episodes of ACR in the control group and 1 episode of ACR in the withdrawal group. Three patients in the withdrawal group had biopsy proven chronic allograft nephropathy. There was no correlation between hypoadrenalism and the development of rejection (acute or chronic) or the rate of decline in renal function. 8 patients developed SWS in the withdrawal group, no patients developed new symptoms in the control group. Those patients who developed steroid withdrawal syndrome had abnormal Synacthen tests at the time of steroid withdrawal (mean).

	Baseline	30min	60min
Mean Cortisol (SEM) Symptomatic patients	261 (4.9)	496 (9.6)	546 (9.9)
Mean Cortisol (SEM) Asymptomatic patients	340 (6.9)	573 (10.5)	637(13.8)

However, of the patients who developed SWS, two developed chronic allograft nephropathy ($p<0.041$, Fisher's Exact Test).

This study illustrates:

- Late withdrawal of corticosteroids is associated with a significant incidence of symptoms (9%).
- Hypoadrenalism is predictive of the development of symptoms following steroid withdrawal but not rejection or declining renal function.
- Patients with symptoms on steroid withdrawal may be more likely to develop chronic allograft nephropathy.

C34

CHRONIC ALLOGRAFT NEPHROPATHY: A SINGLE CENTRE RANDOMISED TRIAL OF CYCLOSPORIN WITHDRAWAL AND MYCOPHENOLATE MOFETIL OR TACROLIMUS SUBSTITUTION IN KIDNEY TRANSPLANT RECIPIENTS

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Background: The contribution of chronic exposure to Cyclosporin A (CyA) and its role in chronic allograft nephropathy (CAN) remains unclear. We report the long term results of patients with CAN converted from CyA based immunosuppression to either Tacrolimus (FK) or Mycophenolate Mofetil (MMF). Primary endpoints of the study included graft loss, change in isotope GFR and change in the slope of serum creatinine. Other parameters studied were proteinuria, blood glucose control, lipid profile and blood pressure control.

Method: Patients with deteriorating graft function and biopsy-proven CAN were randomised to receive either MMF (n=15) or FK (n=15) with complete withdrawal of CyA therapy. All patients were on triple therapy. FK was commenced at 0.15 mg/kg/day and then adjusted to maintain a level of 10-12 ng/l for the first 3 months and then 8-10 ng/l thereafter. In this group CyA was stopped and azathioprine dose was unchanged. In the MMF group, azathioprine was stopped and MMF was commenced at 2g/day. CyA was reduced from 4 weeks onwards and reduced by a 1/3 each month subsequently. All patients continued on 10mg prednisolone per day.

Results: Mean follow up was 20.2 months (range 13-24). Mean time from transplant to conversion was 78.8 months (range 14-160). There were no rejection episodes during conversion in either group. 2 grafts were lost in the FK group and 1 patient discontinued MMF because of side effects. At conversion serum creatinine, GFR and systolic blood pressures were comparable in each group. In MMF group, serum creatinine declined from 249±18.8 SEM to 170±10.7 SEM ($p<0.001$) and GFR increased from 25±3.2 SEM to 47±6.5 SEM ($p=0.018$). In FK group, serum creatinine increased from 229±16.3 SEM to 230±29.8 SEM ($p=0.861$) and GFR remained stable at 25±2.3 SEM to 25±5.6 SEM ($p=0.674$). Blood pressure was better controlled in both groups, more so on MMF. Lipid profiles were also improved, more so in FK group.

Conclusion:

·Complete substitution of CyA in patients with CAN is safe.

·Although substitution of CyA with another calcineurin inhibitor stabilized renal function there was no significant improvement in GFR at 6 or 12 months.

·Conversion to MMF produced significant improvements in GFR, serum creatinine and blood pressure control at 6 and 12 months.

CLINICAL

C83

CAMPATH 1H IN RENAL TRANSPLANTATION: LONG TERM FOLLOW UP OF THE CAMBRIDGE PILOT STUDY

The Oxbridge Campath 1H study group, to be presented by CJE Watson*
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Introduction
Campath 1H is a humanised CD52 monoclonal antibody. CD52 is an antigen expressed on B ands T cells as well as other circulating mononuclear cells, and administration of this antibody to renal transplant recipients causes profound sustained depletion of lymphocyte numbers. It has been used to treat renal allograft rejection and is also under investigation for the treatment of lymphoid malignancy.

Methods
Recipients of cadaveric renal transplantation received 20mg of Campath 1H at the time of transplantation and a second dose on the following day (day 1). No other immunosuppression was given until day 3 when cyclosporin monotherapy was commenced aiming for a blood concentration of 100ng/ml.

Results
31 recipients of cadaveric renal transplants, median age 39.8, were enrolled into the study between June 1997 and July 1998. 2 received asystolic donor kidneys.
One patient died 11 months post transplant from a myocardial infarction. 2 other grafts were lost, one from recurrent disease (IgA nephropathy), one from chronic rejection.
The uncensored actuarial graft survival rate is 90% at a median 2.7 years.
Prolonged lymphopaenia was observed in all patients. In spite of this only two patients suffered serious infections, one CMV and the other required an ileal resection for TB.
In addition one patient developed a haemolytic anaemia.
Five patients had an acute rejection episode of whom one was switched to tacrolimus; all the other patients continue on cyclosporin.
Median serum creatinine values at 12 and 24 months were 145 µmol/L and 133 µmol/L.

Conclusion
Campath 1H permitted low dose cyclosporin monotherapy in renal transplantation with satisfactory renal function and graft survival. A multicentre trial is currently being established to further evaluate this interesting protocol.

*Participants
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Clinical

C60

MULTICENTRE, RANDOMISED, DOUBLE BLIND, DOSE FINDING STUDY TO EVALUATE THE EFFICACY AND SAFETY OF EVEROLIMUS (RAD) IN *DE NOVO* RENAL TRANSPLANT RECIPIENTS - ONE YEAR RESULTS

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Introduction: Everolimus (RAD) is a potent macrolide immunosuppressive with antiproliferative properties. These are the one year results from a Phase II multicentre, randomised, double-blind dose finding study evaluating the safety, tolerability and efficacy of three oral dose levels of RAD in *de novo* renal transplant recipients.
Methods: 103 patients, undergoing primary cadaveric or HLA mismatched live donor renal transplantation, were randomised to receive one of three doses of RAD (1mg, 2mg or 4mg given orally twice daily) in combination with Neoral® and steroids and appropriate prophylaxis for CMV and PCP. The primary efficacy endpoint was the incidence and time to the first incidence of biopsy-proven acute rejection, graft loss or patient death (whichever occurred first).
Results: At one year, RAD doses of 2mg and 4mg/day showed greater efficacy than 1mg/day in respect to the composite incidence of biopsy proven acute rejection, graft loss or patient death, as well as the incidence of biopsy proven acute rejection (table 1). The severity of acute rejection was dose-related, with a reduction in severity between the 4mg/day and 1mg/day group.

Table 1	RAD 1mg/day (n=34)	RAD 2mg/day (n=34)	RAD 4mg/day (n=35)
Biopsy proven acute rejection, graft loss or patient death	15(44%)	10(29%)	10(29%)
Biopsy proven acute rejection	13(38%)	7(21%)	10(29%)
Graft loss	1(3%)	1(3%)	1(3%)
Death (none considered to be drug related)	1(3%)	3(9%)	1(3%)

RAD was generally well tolerated, but there was a higher frequency of adverse events, dose reduction and drug discontinuation in the RAD 4mg/day group (table 2).

Table 2	RAD 1mg/day	RAD 2mg/day	RAD 4mg/day
Bacterial infection	32%	35%	43%
Viral infection	3%	15%	17%
Fungal infection	9%	6%	14%
Thrombocytopaenia	3%	9%	23%
Leucopenia	3%	6%	17%
Raised cholesterol	13%	22%	18%
Raised TGs	7%	6%	9%

There was no significant difference in the mean creatinine at one year within any group. Abnormal lipids were manageable with lipid lowering agents.
Conclusions: RAD doses of 2mg and 4mg/day had superior efficacy compared to 1mg/day, but more serious adverse events and discontinuations due to adverse events were reported in the 4mg/day group. A dose above 1mg/day and below 4mg/day should be considered for further studies.

C64

NON COMPLIANCE IN RENAL TRANSPLANTATION: DETERMINING THE EXTENT OF THE PROBLEM USING ELECTRONIC SURVEILLANCE.

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Chronic rejection after renal transplantation is the largest cause of graft failure and results in the patient requiring dialysis and returning to the transplant waiting list. The cause of such graft failure is multi-factorial. Donor factors, ischaemic time, recipient factors and possibly immunosuppression, all contribute to the cause of this failure. However, a risk factor, which demands consideration is non compliance to immunosuppression. Non compliance has been postulated to account for up to one third of graft failures. Due to its covert nature, determining the extent of non compliance is not easy. The use of the pill counts, questionnaires, structured interviews, the association of acute rejection and low immunosuppressive levels, are all methods which are utilised to identify non-compliant patients. Although these methods have their merits, they also have their failings and indeed generally underestimate the scale of the problem. Electronic surveillance offers the most accurate approach.

100 stable renal transplant patients (transplanted >1yr) were approached and asked if they would use a pill bottle (Aardex Ltd, Switzerland) with a 'smart' top for their azathioprine/prednisolone. These tops identified and recorded each time the bottle was opened. When the patient was seen in clinic, the information from the top was downloaded onto a computer. In this way, a precise picture of the patients' self-medication practice was assembled. All patients were prescribed azathioprine/prednisolone once per day. Over a three-month study period 64% of patients were found to have at least one day where no azathioprine/prednisolone was taken. In addition, 11% had at least one episode of two consecutive days without azathioprine/prednisolone.

In general, if the patient takes one drug, they will take all the drugs required at that specific time point. In addition, patients show a greater compliance to drugs, which are prescribed only once per day. Consequently poor compliance to azathioprine/prednisolone will be a good indicator of poor compliance to other immunosuppressive medications. This suggests that the size of the problem is immense and completely overwhelms the variations in immunosuppressive regimes.

C33

LARGE INTER-INSTITUTIONAL VARIATION IN REPORTING OF RENAL TRANSPLANT BIOPSIES: A PAN-EUROPEAN STUDY

Dr P. N. Furness Leicester General Hospital, on behalf of the CERTPAP study participants

The Banff classification of renal transplant pathology was developed with two aims; to improve individual patient care and to facilitate inter-institutional comparisons, especially in the context of clinical trials in transplantation.

Published studies show acceptable levels of inter-observer variation, but such studies have invariably involved small groups of pathologists who have worked closely together. To test the schema on a more global scale we recruited 23 leading transplant pathologists, representing most European countries. Replicate sections from 55 biopsies were circulated around these pathologists in groups of 5 cases over two years. No clinical information was given. Participants were asked to grade all the histological features defined in the Banff classification or in the CCTT schema, and several other histological features of less proven value. The results were collated in Leicester and each participant was given regular feedback on whether his/her previous scores were above or below the average for the whole group. The aim was to help participants to 'converge' in their interpretation of the assessment criteria.

Mathematical analysis of inter- observer agreement in these circumstances is difficult, and comparisons with other studies of different design must be done with care. However, all the kappa values for this study were considerably lower than in any previous report, confirming that, despite Banff, inter- institutional variation is still a problem in the interpretation of transplant biopsies.

Evidence of 'convergence' was limited, and could not be proved for most histological features. Closer analysis suggested that some participants had attempted to converge towards the consensus, but others had ignored the feedback and had continued consistently to under- or over- grade features.

There was the expected correlation between a retrospective clinical diagnosis of acute rejection and the grade of tubulitis, interstitial mononuclear cell infiltration and oedema. However, study of a subset of stable 'protocol' biopsies taken at over 6 months post-engraftment showed an unexpectedly strong correlation between subsequent chronic decline in function and these same 'acute' features, tending to support the importance of 'subclinical acute rejection' in the development of chronic allograft nephropathy.

It was not possible to demonstrate a clear advantage between the Banff and the CCTT schemes for the identification of acute rejection, although a simple computer-based inference network performed better than either.

These results have major implications for the design of multi centre trials in renal transplantation; for interpretation of published work in this field; and for the future development of the Banff classification.

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CERTPAP: Convergence of European Renal Transplant Pathology Assessment Procedures. CCTT: Co-operative Clinical Trials in Transplantation

C57

CHRONIC GRAFT NEPHROPATHY (CGN) IS A SEQUEL TO ACUTE INFLAMMATION IN RENAL TRANSPLANT RECIPIENTS TREATED WITH TWO DIFFERENT CALCINURIN INHIBITORS.

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The consequence of acute inflammation are: (i) resolution (no structural damage), (ii) repair (fibrosis) and (iii) chronic inflammation (true chronic rejection). Many "environmental" factors, such as hypertension, lipids etc affect the outcome. However, immunosuppression influences all these factors and also modulates the repair. Hypothesis: chronic graft nephropathy (CGN) is the repair sequel to acute inflammation in the "environment" of immunosuppression. Material & Methods: We analysed our database of an ongoing prospective, randomised study of 231 patients receiving calcinurin inhibiors. The leading candidates for injurious agents were delayed graft function (DGF), acute rejection (AR) and CMV disease (CMV). The leading "environmental" factor was immunosuppression. The end point was the median graft function expressed in Cockroft-Gauld GFR. The repair (fibrosis) was assessed Fibrosis Index (FI) of protocol biopsies. An intragraft expression of TGFβ1 mRNA was quantified using a real-time PCR. RESULTS: GFR are expressed as median.

Statistical analysis was carried out using Mann-Whitney U Test.

Table 1. "No injury" group

	GFR WEEK 1	GFR 1 YEAR	GFR 2 YEARS	GFR 3 YEAR
CYA	54	61	50	51
TAC	60	78	80	78
p value	0.13	0.02*	0.005*	0.002*

Table 2. DGF group (day 3 GFR<25ml/min)

	GFR WEEK 1	GFR 1 YEAR	GFR 2 YEARS	GFR 3 YEARS
CYA	19	50	43	36
TAC	17	57	58	54
p value	0.6	0.2	0.02*	0.01*

Table 3. AR group (histology proven & treated)

	GFR WEEK 1	GFR 1 YEAR	GFR 2 YEARS	GFR 3 YEAR
CYA	33	44	37	40
TAC	24	63	64	63
p value	0.2	0.02*	0.01*	0.12

Table 4. CMV group (requiring gancyclovir therapy)

	GFR WEEK 1	GFR 1 YEAR	GFR 2 YEARS	GFR 3 YEAR
CYA	41	48	46	55
TAC	42	56	68	64
p value	0.6	0.3	0.04*	0.3

The FI in 50 protocol biopsies at 6 months correlated with GFR at 12 & 24 months ($p=0.018$ and 0.0001 respectively). Although the FI at the time zero was 12% for each treatment group, cyclosporin (CYA) patients had higher FI ($p<0.05$) than tacrolimus (TAC) group at 6 & 12 months (36% vs 23% and 44% vs 26% respectively) and higher ($p<0.05$) intragraft expression of TGFβ1 mRNA at 6 months (3.1 vs. 1.9 log copy number). The FI for "No-injury" group was also higher in CYA patients (29% vs 15% and 38% vs 20% - $p=0.03$).

CONCLUSIONS: "No injury" group had the best long-term GFR & low FI. Acute inflammation caused by DGF, AR or CMV healed by fibrosis. Different action of CYA and TAC influenced the FI and GFR. TAC group had best GFRs & FI and superiority in "No-injury" group suggests that CYA-nephrotoxicity heals by fibrosis and not by resolution.

L11

Critical Role for IL-4 and Eosinophils in the Development of Transplant Arteriosclerosis in the Absence of CD40-CD154 Costimulation

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Introduction: Blockade of the CD40-CD154 pathway can inhibit $CD4^+$ T cell activation but is unable to prevent immune responses mediated by $CD8^+$ T cells including virus specific CTLs and allograft rejection. However, even in the absence of $CD8^+$ T cells inhibition of the CD40-CD154 pathways is insufficient to prevent the development of transplant arteriosclerosis. This study investigated the mechanisms of transplant arteriosclerosis in the absence of the CD40 pathway, C57BL6$CD40^{-/-}$ ($H2^b$) recipients were transplanted with MHC mismatched BALB/c ($H2^d$) donor aortas.

Methods: Fully MHC mismatched BALB/c ($H2^d$) donor aortas were transplanted into a BL6/$CD40^{-/-}$ ($H2^b$) recipient. Grafts were analysed by histology, morphometry, and immunohistochemistry on day 30 after transplantation. Intra-graft cytokine and chemokine mRNA production was analysed by competitive RT-PCR on day 14 after transplantation.

Results: Transplant arteriosclerosis was evident in both $CD40^{-/-}$ and $CD40^{+/-}$ mice [intimal proliferation 59±5% ($CD40^{-/-}$) vs. 58±4% ($CD40^{+/-}$)] in the presence or absence of $CD8^+$ T cells [intimal proliferation 46±7% ($CD40^{-/-}$+anti-CD8) vs. 50±10% ($CD40^{+/-}$ + anti-CD8)] confirming that $CD8^+$ T cells are not essential effector cells for the development of this disease. In $CD40^{-/-}$ recipients depleted of $CD8^+$ T cells the number of eosinophils infiltrating the graft was markedly increased [eosinophils/grid 109±24 ($CD40^{-/-}$+anti-CD8) vs. 28±7 ($CD40^{+/-}$+ anti-CD8)]. The increased presence of eosinophils correlated with augmented production of IL-4 within the graft. In order to test the hypothesis that IL-4 and the subsequent recruitment of eosinophils into the graft was responsible for the intimal proliferation, CD8 T cell depleted $CD40^{-/-}$ recipients were treated with anti-IL4 mAb. Inhibition of the functional activity of IL-4 significantly reduced eosinophil infiltration into the graft [12±5 ($CD40^{-/-}$+anti-CD8+anti-IL-4) vs. 109±24 ($CD40^{-/-}$+anti-CD8) eosinophils/ grid], intragraft eotaxin and CCR3 mRNA production and the level of intimal proliferation [18±5% ($CD40^{-/-}$ +anti-CD8+anti-IL-4) vs. 46±7% ($CD40^{-/-}$+anti-CD8)].

Conclusion: Elevated IL-4 production in combination with an eosinophil infiltrate is an important mechanism for the development of transplant arteriosclerosis in the absence of CD40-CD154 costimulation.

L45

The paradox of local complement synthesis in chronic allograft nephropathy

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Chronic allograft nephropathy is now the major cause of renal transplant loss but its pathogenesis remains uncertain. Since T-cell immunosuppressive therapies have not had a major impact on the incidence of chronic rejection, non T-cell dependent mechanisms might be involved. The kidney is capable of synthesizing components of the complement cascade that are potent mediators of inflammation. We have therefore investigated the role of locally synthesized complement C3 in a mouse model of chronic renal allograft rejection.

We pretreated bm12 mice with a depleting regimen of rat anti-mouse CD4 and anti-mouse CD8 mAbs on days -7, -3 and 0 and transplanted them with C57BL/6 (BL/6) kidneys. The donor and recipient mice differ at only a single MHC class II allele, but without treatment we found a graft loss of >50% by d21. In the treated mice, we performed contralateral nephrectomy at d7 post-transplantation and were able to observe the animals over a period of 80 days (n=6). At d80, the transplant kidneys had developed chronic allograft nephropathy, characterised by widespread tubular atrophy and transplant arteriosclerosis leading to vascular occlusion. This coincided with gradual impairment of renal function (rising blood urea nitrogen). In a small group of animals maintained beyond 80 days, death occurred between day 89 and 110 (n=3).

To examine the effect of locally produced complement in this model, we prepared a second group of bm12 recipients (n=6) in the same manner, but used C3 knockout mice congenic with the BL/6 as kidney donors. Data collected over 80 days showed a significant improvement in renal function ($p<0.0001$) in recipients of a C3 deficient kidney compared with recipients of a wild type BL/6 kidney. Histological analysis showed a higher proportion of normal tubules in C3 knockout donor kidneys at d80 (67% vs 23% in controls, $p<0.001$), less expansion of collagen in the extracellular matrix and reduced or absent transplant arteriosclerosis. A mononuclear infiltrate was present in both groups of transplanted kidneys. Immunohistochemical staining showed reduced deposition of C3 in the C3 knockout kidney. The rate of decline of renal function was reduced with C3 deficient grafts compared to control grafts. Interestingly though, immunohistochemistry revealed dense accumulation of rat Ig in the glomeruli of the C3-/- transplanted kidney which was coincident to some glomerulosclerosis and was markedly reduced in wild-type transplanted kidneys. This implied that clearance of therapeutic antibody was reduced in the absence of local C3 synthesis.

The data suggest that locally produced C3 is a causative factor in chronic allograft nephropathy that has not previously been identified, and is a possible target for therapy since no specific strategy exists to modulate chronic rejection. The accumulation of administered antibody in the C3 deficient glomeruli may have led to an underestimate of the protective value of absent local synthesis of C3.

L64

LIVER TRANSPLANT RECIPIENTS GENERATE CHIMERIC CHOLANGIOCYTES AND HEPATOCYTES EXPRESSING LINEAGE-SPECIFIC AND FUNCTIONAL MARKERS

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Bone marrow stem cells possess the capacity to differentiate into hepatocytes in animal models. Human hepatocytes can be chimeric for the Y chromosome after cross-gender bone marrow or liver transplantation, suggesting a similar process of recipient-derived precursors undergoing epithelial differentiation. However, the precise origin of cells in these human studies was not defined and the female livers analysed may have been chimeric for the Y chromosome due to the transplacental passage of male fetal cells. We aimed to clarify the origin of chimeric hepatocytes and cholangiocytes in coincidental cross-gender human liver transplants, and to investigate the expression of functional markers.

We have developed a novel protocol allowing dual Y-chromosome painting, (Fluorescence In Situ Hybridisation with probes decorating the entire chromosome), and immunofluorescence staining of sections of formalin-fixed paraffin-embedded tissue. Applying the technique to the analysis of chimerism in needle biopsies of female-to-male liver transplants demonstrates cholangiocytes and hepatocytes simultaneously displaying bright nuclear Y-chromosome paint signal and protein marker fluorescence. Analysing a chronological sequence of biopsies from each individual liver (n=5) reveals for the first time that chimeric parenchymal cells must be recipient-derived since no male cells are detectable before transplantation in pre-perfusion biopsies. A chronological change in the distribution of chimeric cells is documented. Y-chromosome positive hepatocytes were observed at later time points, and appeared to increase in number in response to the degree of liver injury. Thus, chimeric hepatocytes accounting for 5% of the parenchymal population occurred in a cirrhotic graft two years after transplant, whilst a biopsy taken 7 years after grafting which showed acute cellular rejection, displayed only very occasional Y-positive cells. Dual labelling clearly demonstrates Y-chromosome positive hepatocytes simultaneously staining with albumin antibody, a marker a hepatic function. Chimeric cells displaying Y-chromosome positivity and strong cytoplasmic cytokeratin 7 staining were observed forming proliferating cholangioles in a transplant infected with Hepatitis B virus. Three-dimensional microscopy revealed several Y-chromosome positive cholangiocytes within the same ductal structure suggesting clonal growth.

Recipients of liver transplants therefore generate hepatocytes and cholangiocytes from circulating progenitors infiltrating the graft. In severely injured livers, chimeric progenitors generate nascent cholangiolar proliferations, the sites of hepatic stem cell proliferation and differentiation. Since recipient-derived hepatocytes appear to synthesise albumin, modulating the differentiation of circulating precursors may provide the basis for future clinical therapies based on reconstituting hepatic function.

L18

ALLOREACTIVITY OF NATURAL KILLER CELLS PRECEDES T CELLS: CROSS TALK BETWEEN THE INNATE AND ADAPTIVE IMMUNE SYSTEM?

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INTRODUCTION: Much is known about the role of the adaptive T and B cell response in the rejection process. The innate immune response, e.g. by Natural Killer (NK) cells however, remains a mystery in solid organ transplants. We therefore set out to determine the kinetics of graft infiltrating NK cells and to establish whether they are functional in the early stages post transplantation in terms of cytokine production.

METHODS AND MATERIALS: Fully allogeneic donor BALB/c ($H2^d$) or CBA ($H2^k$) mouse hearts were transplanted into C57BL/6 ($H2^b$) recipients. Cardiac allografts and spleens were harvested from recipient animals at different time points post transplant. Graft infiltrating cells were isolated using collagenase digestion followed by centrifugation through a ficoll gradient for analysis by 2 colour flow cytometry. Spleen cells were treated with the same process with the exception of the collagenase digestion. Cells were stained for DX5 (pan NK cell marker), CD4 and CD8 expression. NK cells were permeabilised by saponin and stained for intracellular IL2 and IFNγ expression.

RESULTS: Rejection of heart grafts takes place between the 9th and 11th days post transplant. In the first 5 days, the majority of the graft infiltrating cells consist of NK cells (fig.1).

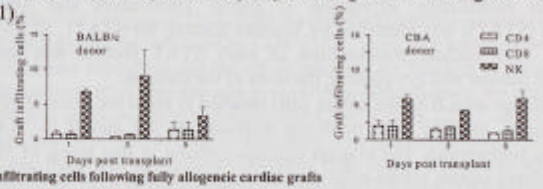

Fig.1: Graft infiltrating cells following fully allogeneic cardiac grafts

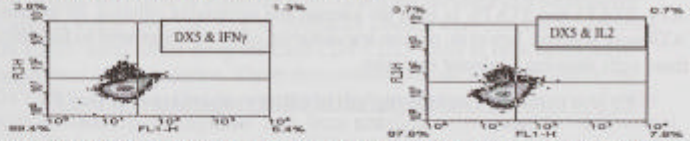

Fig 2:FACS profile of splenocytes>4% staining with pan NK marker, 1.3% of these are expressing *IFNγ (post allogeneic cardiac graft from CBA donor to C57BL/6 recipient).*

Intracellular cytokine analysis on freshly isolated splenic NK (fig.2) and graft infiltrating NK cells (unstimulated ex-vivo) showed greater production of IFNγ than IL2 within 1 day of transplantation. Meanwhile the naïve splenic NK cells do not appear to produce either IL2 or IFNγ cytokines.

DISCUSSION: We have demonstrated the presence of a higher proportion of NK than T cells in the first 5 days post transplantation and that they produce IFNγ. The NK to CD4/CD8 ratio appears to fall with time suggesting that graft rejection is initiated by NK cells in the first few days, after which T cells take over. We postulate that since NK Cell infiltration precedes that of T cells, they may play a crucial role in the rejection process, possibly through the release of cytokines which may have an effect on the subsequent immune response including T cell alloreactivity.

L1

ACTIVE TRANSPLANTATION TOLERANCE *EX VIVO:* STAT LEVELS AND CYTOKINE SECRETION.

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Introduction: An active form of transplantation tolerance can be induced in fully immune competent mice. This tolerance is "infective" in that isolated tolerant spleen cells, when placed into a naive mouse (i.e. competent to reject any allo-graft), transfer the specific state of tolerance for a donor-type heart graft : third party heart grafts are rejected, but F1 hearts (donor x third party) hearts may be accepted, demonstrating spread of active tolerance to co-expressed novel antigens.

Aim and Methods: To probe the molecular regulation of active tolerance in mice, we challenged 2 x 10^7 tolerant spleen cells *ex vivo* with 2 x 10^7 irradiated donor-type spleen cell (stimulators). Allo-**tolerant** responder spleen cells were derived from CBA ($H2^k$) mice rendered tolerant to a BALB/c ($H2^d$) heart graft by CD4 + CD8 blockade. Parallel cultures used allo-**aggressive** responder spleen cells, derived from CBA mice which had rejected a BALB/c skin graft. Levels of secreted γINF, IL2, IL10, and IL4 were measured in the culture supernatants at 16h, 24h and 48h; then at culture day 5 following rechallenge with a second round of irradiated BALB/c stimulator cells. At 3h following this rechallenge, expression of Signal Transducers and Activators of Transcription (STAT)s was identified by Western blotting for STAT1, STAT4, STAT5 and STAT6. Any nuclear translocation of each STAT protein was assessed by comparing the nuclear and cytoplasmic fractions of the cultures.

Results: Challenge with BALB/c spleen cells resulted in rapid secretion of γINF in the rejecting cultures, together with moderate increases in IL2 and IL10. IL4 rose to slightly above background. The tolerant cultures differed in that levels of γINF were low. Unstimulated mouse spleen cells showed minimal background levels of STAT1, STAT4, STAT5 and STAT6. In both the tolerant and aggressive cultures, all measured STATs were induced: however, nuclear translocation of STATs appeared to be reduced in those cells showing a tolerant response.

***Ex vivo* cultures: cytokine (pg/ml) in culture supernatant at Day 5**

	γINF		IL2		IL10		IL4	
	Tol	**Rej**	Tol	**Rej**	Tol	**Rej**	Tol	**Rej**
Expt 1	570	**4,400**	160	**210**	460	**510**	40	**60**
Expt 2	<100	**9,000**	65	**170**	53	**110**	<10	**47**

Conclusions: Responses associated with allotolerance, versus allorejection, can be measured *ex vivo*. These appear to be different from Th2, versus Th1, responses, both in terms of cytokine secretion, and in activation of STATs. By understanding the active regulation of immune tolerance, therapeutic manipulation of the immune response may eventually be extended for the benefit of clinical transplant recipients.

LABORATORY

L17

THE ROLE OF NATURALLY OCCURRING $CD4^+CD25^+$ T CELLS IN THE REGULATION OF ALLORESPONSES IN MAN

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In recent years, there is renewed interest in the role of suppressor cells in the maintenance of immunological tolerance to self and transplant antigens. In most studies, the suppressor population was found within the $CD4^+$ T cell subset. Data from rodent models suggest that naturally occurring $CD4^+CD25^+$ T cells play a vital role in protection against autoimmunity.

In this study, we investigated whether such a suppressor population exists in man and its possible role in regulating alloresponses. We found that $CD4^+$ T cells in peripheral blood of healthy adults always contained a subpopulation which constitutively express CD25. These $CD4^+CD25^+$ cells were phenotypically different from recently activated T cells. Functionally, these cells were hyporesponsive to mitogens and alloantigens compared to their $CD25^-$ counterparts. In addition, $CD4^+CD25^+$ T cells were capable of suppressing the proliferation of and cytokine production by $CD4^+CD25^-$ cells in co-culture. Addition of exogenous IL-2 could reverse the hyporesponsiveness of and abrogated the suppression mediated by $CD4^+CD25^+$ cells. The suppression appeared to be cell:cell contact dependent and not via suppressing the function of antigen presenting cells. To investigate their possible role in regulating alloresponses, we performed limiting dilution analyses using unfractionated $CD4^+$ T cells, $CD25^+$-depleted $CD4^+$ T cells or enriched $CD4^+CD25^+$ cells as responder cells against allogeneic stimulator cells. We found that there was a significant deviation from single-hit kinetics when whole $CD4^+$ T cells were used as responders, indicating the presence of a suppressive population within the $CD4^+$ T cells. Single-hit kinetics were restored by depleting $CD4^+CD25^+$ cells, while using enriched $CD4^+CD25^+$ cells as responders led to exaggeration of the deviation. These observations provide further evidence that $CD4^+CD25^+$ cells may play an important role in regulating alloresponses. Similar results were obtained using $CD4^+$ cells from umbilical venous cord blood in normal infants. Their role in the maintenance of clinical transplant tolerance is currently being investigated.

C38

EQUITY OF ACCESS TO RENAL TRANSPLANT WAITING LIST AND RENAL TRANSPLANTATION IN SCOTLAND

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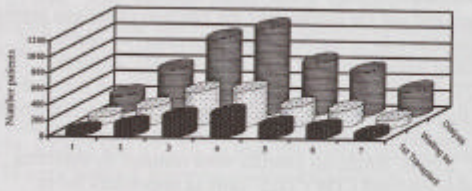
Aim: The acute shortage of donor organs has raised again the issue of equity of access to transplantation and organ distribution. The aim of this study is to investigate whether there are differences in access to either the renal transplant waiting list or renal transplantation in Scotland.

Methods: 4357 adult patients who started renal replacement (RRT) therapy in Scotland between 1st of January 1989 and 31st of December 1999 were identified from the Scottish Renal Registry (SRR). Information regarding date of 1st RRT, date of listing for transplantation, date of 1st transplant as well as demographic data, postcode and transplant details were obtained for each patient by merging data from SRR and UKT databases. Each patient was assigned to a deprivation category (1 to 7) according to the Carstairs 1991 deprivation scores based on postcodes (1= least deprived, 7=most deprived). After statistical advice, Chi-square tests and Cox regression analysis were used as appropriate.

Results: Out of 4357 patient who started RRT in the study period, 1732 (39.8%) were admitted onto the waiting list and 1076 (24.7%) were eventually transplanted. The distribution by social deprivation category is illustrated below.



The pattern of deprivation category distribution seen in the RRT population is different to that in the patients waiting for transplant ($p = 0.001$, Pearson Chi-square), but is similar to those who have received a transplant. However, the length of time spent before admission to the waiting list, is significantly different ($p < 0.05$, Cox regression) according to the deprivation category, age at 1st RRT, gender, transplant centre and linear distance from patients home to transplant centre. The centre effect is significant even after adjustments for all other factors. Once admitted to the transplant waiting list, the age of the patient is the only significant factor governing access to transplantation. There is a statistical trend ($p < 0.0005$, Cox regression) to longer times for elderly recipients

Conclusions: Deprivation category profile of the population on the transplant waiting list is different from that of the RRT population. A higher deprivation category, older age and female gender are all predictors of a longer waiting time prior to access on the waiting list. There is a significant centre effect for waiting list access, which persists despite adjustments for all other factors.

L27

A STEALTH APPROACH TO BONE MARROW-INDUCED TOLERANCE VIA THE INDIRECT PATHWAY

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Objective: Pretransplant exposure to a single donor MHC class I antigen by retroviral transfer of a donor MHC class I gene to recipient BM cells, when combined with anti-CD4 mAb, induces operational tolerance to a fully allogeneic cardiac allograft. We investigated the role of the indirect pathway of allorecognition in this process by generating a vector for expression of a donor MHC class I gene in the cytosol only, rather than at the cell surface.

Methods: The donor MHC class I gene H-2K^b was cloned into the replication-defective retroviral vector MFG (*full MFG-K^b*). To create a vector which would result in protein expression within the cell but not at the surface, the endoplasmic reticulum leader sequence responsible for surface expression of H-2K^b was deleted prior to cloning into MFG (*no-leader MFG-K^b*). The constructs were transfected into pA317 retroviral packaging cells, which were then cloned and high titre retrovirus-producing clones were selected by infection of NIH/3T3 cells and subsequent quantitative PCR for the H-2K^b gene. CBA (H-2^k) bone marrow (BM) cells were transduced by coculture with retroviral producer cells for 72 hours. CBA recipient mice were treated with $5x10^6$ transduced CBA BM cells 27 days pretransplant, in conjunction with two doses of anti-CD4 mAb on days −28 and -27. This was followed by transplantation of a fully allogeneic C57BL/10 (H-2^b) heart on day 0.

Results: The titre of no-leader MFG-K^b supernatant, as assessed by infection of NIH/3T3 cells and quantitative PCR, was 10^6/ml. A full MFG-K^b clone with similar titre was selected as the positive control. Flow cytometry confirmed that NIH/3T3 cells expressed surface H-2K^b after infection with full MFG-K^b, but not after the no-leader vector, although K^b mRNA could be detected by RT-PCR. CBA BM cells transduced with full MFG-K^b, together with anti-CD4 mAb, resulted in long-term survival of 4/6 cardiac allografts (MST >100 days, n=6, p=0.017). No-leader MFG-K^b-transduced BM produced 6/7 long-term surviving grafts (MST >100 days, n=7, p=0.005). Third party NZW hearts were rejected acutely (MST 11 days). Histological examination 100 days after transplantation in the no-leader group revealed well-preserved myocardial architecture & minimal vasculopathy.

Conclusions: These findings indicate that delivery of donor alloantigen via the indirect pathway is certainly sufficient to induce long-term allograft survival in this BM/anti-CD4 model. The absence of surface H-2K^b or other selectable markers on the transduced BM cells may well have facilitated engraftment of the BM cells and thereby generated a long-term supply of donor antigen, an issue which is currently under active investigation. This study has important implications for potential strategies to induce transplantation tolerance by delivery of donor alloantigen.

Parallel Session Two

Wednesday 28 March

Session A

Liver/Heart Session

C59

CADAVER AND LIVING DONOR LIVERS: DIFFERENCES IN INFLAMMATORY MARKERS PRIOR TO TRANSPLANTATION

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Living donor liver transplantation is becoming more commonly practiced because of the shortage of cadaver donor livers. While living donor livers are obtained from healthy individuals, cadaver livers may have suffered inflammatory injury prior to retrieval. The aim of this study is to examine whether differences exist in the leukocyte subpopulations and levels of molecules involved in the inflammatory process between cadaver and living donor livers.

True-cut biopsies were obtained from cadaveric (n=22) and living donor (n=10) livers at the end of the period of cold storage before implantation. Cryostat tissue sections were stained with a panel of antibodies for leukocyte subpopulations, adhesion molecules and MHC antigens. The % area of leukocyte staining was quantitated by morphometric point counting and the other markers assessed on a semiquantative grading scale. Results were correlated with donor factors and intensive care management.

Significantly higher levels of CD45+ leukocytes (7.0 ± 1.9 vs. 2.7 ± 0.8 $p<0.0001$), CD68+ monocytes and macrophages (4.0 ± 1.2 vs. 2.7 ± 0.6; $p=0.0005$) and Fas ligand staining (4.2 ± 2.6 vs. 1.4 ± 1.1 $p=0.0003$) were detected in cadaver compared to living donor livers. Furthermore ICAM-1 was expressed at a higher level in cadaver donor livers (3.0 ± 0.9 vs. 1.7 ± 0.7; $p=0.0004$). This high level of expression of ICAM-1 was associated with infection in the donor ($p=0.013$), longer periods of ventilation ($p=0.01$) and the administration of inotropes ($p=0.03$). While no differences were found in the level of neutrophil infiltration between cadaver and living donor livers, significantly higher levels of neutrophil infiltration were found in cadaver donors with infection.

The results from this study clearly demonstrate that there are identifiable differences between cadaver and living donor livers in leukocyte subpopulations and molecules induced during an inflammatory response. Furthermore these inflammatory changes were found to be associated with events within the donor during the period of intensive care suggesting differences in the quality of organs that may influence outcome.

C50

ENCOURAGING SPLITTING LIVERS: THE UK EXPERIENCE

Miss C J Hamilton, Mr A D Mayer, Mrs F M Seeney on behalf of the UK Transplant Liver Advisory Group, Bristol, UK

BACKGROUND: Liver splitting enables a single liver to be used for transplantation into two recipients, usually an adult and a paediatric patient. Initial experience in the UK suggested that only good quality livers are suitable. To encourage UK liver transplant centres to contemplate splitting a liver, a protocol was introduced that specifies criteria to identify donors whose livers are suitable for splitting. The criteria are that the donor should be aged under 40 years, weigh 50kg or over, have stayed in ITU under 5 days, have no deranged liver function tests, be haemodynamically stable and have a satisfactory macroscopic appearance. When a potential donor meets these criteria the Transplant Unit is specifically asked to consider splitting the liver. However, donors that fall outside the criteria may still be considered suitable for splitting. Reasons for not agreeing to split the liver must be reported to the Duty Office at UK Transplant.

DATA: This study included cadaveric livers offered between 1 January and 31 October 2000 in the UK, those identified as suitable for splitting, the proportion where agreement to split the liver was obtained and the final outcome of these livers. Livers are regarded as being split when both the left and right lobes of the liver are transplanted into different recipients and as reduced when one lobe of the liver is transplanted.

ACTIVITY: There were 674 livers offered for transplant in the period. The Duty Office identified 203 (30%) donors whose liver was suitable for splitting based on the first two criteria. In 42 (21%) cases the centre agreed to split the liver. The main reasons stated for not agreeing to split a liver were that the liver was to be used whole: for a patient requiring a whole liver (45%); for another reason (17%); for a super urgent patient (16%).

Of the 42 livers offered for splitting, 15 resulted in 30 split liver transplants, 19 were used in 19 reduced liver transplants and 7 were transplanted as whole livers. Only 1 liver was not used, due to a tumour.

COMMENT: The criteria are a useful guide for transplant co-ordinators to identify livers that may be suitable for splitting. Based on experience to date, over a 12 month period approximately 240 potential donors could be identified as suitable. This would more than satisfy the need for paediatric transplantation. However, it is not always feasible to split a liver, especially when there is a patient requiring a whole organ.

C114

OUTCOME AND POTENCIAL *EX VIVO* SPLIT LIVER TRANSPLANTATION

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Split liver transplantation (SLT) is one way of optimising scarce donor resources. Since 1998 we have adopted an intention to split policy with simultaneous adult and paediatric transplantation.

Patient and methods: Between Nov /1992 and July 2000 we performed 46 *ex vivo* split procedures resulting in 92 grafts, 13 grafts were exported and 4 imported .83 SLT (48 segment II/III; 2 monosegment II; 2 segment II/III/IV; 20 segment I/IV- VIII; 6 segment IV-VIII; 9 segment V-VIII) were performed in 81patients (32 adults).Donor parameters analysed were :median age - 26.2 (7-55) years, AST- 51 (6-200) U/L .The cold ischaemia time (CIT)was 13 (7-20.6) hours. The outcome analysis was divided into two periods (I=1992-97; II=1998-2000).Factors analysed were: age, sex, indication, UNOS status, blood group, weight, donor/recipient body weight ratio (D/RBW), segments used, cold and warm ischaemic time, blood transfusion, peak (day 1-5)AST, Bilirubin and INR; vascular and biliary complications, primary non function (PNF),bleeding and patient and graft survival outcome.

Results

	PERIOD I (29Tx)	PERIOD II (54Tx)
UNOS status - I	18 (62%)	39 (72.2%)
CIT (minutes)	910 (435 - 1326)	720 (395 - 1086)
AST peak (U/L)	1266 (117 - 10000)	740 (150 - 6961)
Vascular Complications	5 (17.2%)	4 (7.4%)
Biliary Complications	6 (20.7%)	10 (18.5%)
PNF	4 (13.8%)	0
Bleeding	1 (3.45%)	3 (5.5%)
Patient survival (1 year)	72.5%	86.5%
Graft survival (1 year)	60%	80%

The donor pool over 1999 –2000 was a total of 139 donors less than 40 years age, 25(18%)were successfully split , 21 (15%)used as reduced grafts and 93 (67%) as whole grafts, of which 14(12%) utilised as whole or reduced grafts, could have been potential split.

Conclusion - Careful donor and recipient selection with simultaneous transplantation has reduced cold ischaemia time and improved outcome following split liver transplantation.

C1

MANAGEMENT OF POST-OLT BILIARY COMPLICATIONS: SHOULD WE BE MORE AGGRESSIVE?

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AIM: Biliary complications following orthotopic liver transplantation pose a significant problem and often require serial interventions before complete resolution. Our aim was to assess the management of biliary complications and their success within a single liver transplant unit.

METHODS: Data regarding biliary complications was prospectively recorded on a computerised database. We analysed the incidence and the type of biliary complications, the management sequence and its success rate. Success was defined as no need for further biliary intervention. Statistical analysis was performed with the SPSS program version 9.0 using Chi-square and Fisher's exact test where appropriate.

RESULTS: Since November 1992, a total of 301 consecutive liver transplants have been performed. 47 grafts developed biliary complications giving an overall incidence of 15.8%. There was a significantly higher incidence of complications in the hepatico-jejunostomy group (10 out of 42 cases) compared with the duct-to-duct technique (23 out of 200 cases) (p=0.035, Chi square). The number of biliary complications, the management technique employed and their success rates are illustrated in table below.

Type of complication	Total number	Conservative		Non-operative (ERCP/ PTC)		Surgery	
		Number treated	Successful outcome (%)	Number treated	Successful outcome (%)	Number Treated	Successful outcome (%)
Anastomotic leaks	20	15	11 (73%)	7	3 (42%)	4	4 (100%)
Anastomotic strictures	25	5	1 (20%)	22	7 (31.8%)	12	12 (100%)

Patients underwent between one and five interventions until complete resolution of the complications. All anastomotic leaks occurred within 75 days of transplant. Of the 20 cases with initial leaks, 7 (35%) developed a subsequent stricture at a median interval of 164 days (range 49-709 days). The 25 strictures had a bi-modal distribution (14 cases<6 months, 11 cases>6 months). 8 (73%) of those occurring after 180 days post-transplant required surgical management compared to 4 (27%) that occurred before 180 days (p=0.02, Chi square).

CONCLUSIONS: Leaks and early strictures may be successfully treated with a combination of conservative, endoscopic and radiological means, while late strictures should be best treated by surgical intervention.

CLINICAL

C105

SIROLIMUS IN LIVER TRANSPLANTATION FOR MALIGNANCY: 3-YEAR FOLLOW UP OF THE CAMBRIDGE EXPERIENCE

CJE Watson on behalf of the Liver Transplant Unit, Addenbrooke's Hospital, Cambridge.

Introduction

Sirolimus (rapamycin) is a new immunosuppressant licensed for use in renal transplantation in the US being reviewed in the EEC. In contrast to the cyclosporin and tacrolimus, which inhibit cytokine gene transcription, sirolimus inhibits cytokine-mediated proliferation. This anti-proliferative effect extends to inhibition of the growth of tumour cell lines *in vitro* including hepatocellular tumour cell lines. This observation led us to study its effect in patients undergoing liver transplantation for primary hepatic malignancy in the presence of cirrhosis. Transplantation for malignant disease has universally poor results with recurrent disease being common. Current criteria are to only offer transplantation where the tumour is a small (5cm) hepatoma, or if multiple, no more than 3 tumours none larger than 3cm. For the purpose of this study patients with cholangio-carcinoma and patients with tumours larger than 5cm were also included.

Methods

Between 1994 and 1997 17 patients with primary hepatic malignancy and 10 patients without malignancy underwent liver transplantation and were immunosuppressed with sirolimus-based immunosuppression. The immunosuppressive regimen evolved from one of triple therapy of sirolimus, cyclosporin and prednisolone to sirolimus monotherapy as the great potency of sirolimus was appreciated. All regimens aimed to achieve sirolimus monotherapy by 3 months following transplantation.

Results

The 3-year survival of patients with primary liver cancers was 35%, compared to 70% for the non-cancer patients. The principle cause of death in the tumour group was tumour recurrence. No patient with a tumour less than 5cm diameter had recurrence. Three cholangiocarcinomas (5, 6, and 7cm) recurred, as did a 5cm angiosarcoma and 3 larger hepatomas (5, 7, and 8cm). 3 patients in each group died from non-tumour causes, three from chest sepsis and one each from a myocardial infarction, primary non-function and graft versus host disease.

Several patients required operative wound debridement and 30% required subsequent incisional hernia repair, both findings attributed to sirolimus effects on wound healing at the high doses used.

Nine patients discontinued sirolimus therapy, 2 on account of its taste, 2 for peri-articular bone pain, 3 for late acute rejection, 1 each for hyperlipidaemia and sepsis.

Renal function in those who continued on sirolimus therapy was excellent with a median creatinine of 88μmol/L at 3 years.

Conclusion

1. Sirolimus is a potent immunosuppressant in liver transplantation, and monotherapy is adequate for long-term maintenance.
2. Renal function is well preserved on long-term sirolimus monotherapy.
3. The data presented suggest that there is no anti-tumour effect of sirolimus in patients with primary liver cancer undergoing liver transplantation.

C90

ANALYSIS OF FACTORS INFLUENCING PER-OPERATIVE BLOOD USE IN LIVER TRANSPLANTATION

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Blood transfusion requirements during liver transplantation have declined as the procedure has developed due to refinements in technique and use of the Argon Beam Coagulator. We have analysed the peroperative transfusion requirements in adult liver transplants and found a steady reduction in blood use during the early phase of transplantation (1983-1990 n=300, median blood use =11.5 units) and a plateau during the later phase (1991-2000 n=1100, median blood use = 5 units). Despite these improvements the requirement for large volume blood transfusion still occurs in a period when median operative blood loss is no longer falling, suggesting that improvements in technique are unlikely to further reduce operative blood loss. We have prospectively recorded intra-operative blood transfusion and analysed factors which influence blood transfusion requirements in liver transplantation, in an attempt to identify pre-operatively patients likely to require high blood volumes.

Of 1100 adult patients transplanted during the decade 1991-2000, 75% required 10 units of blood or less peroperatively, whilst 54 patients (5%) required greater than 20 units (median=26, range 20-70). The indication for liver transplantation and patient demographics did not differ between these two groups. Three factors were found to have a significant influence on blood requirements: 1) Previous upper abdominal surgery including re-transplantation (except early regrafts) 2) Renal impairment (median blood use = 7.5 units) 3) Portal vein thrombosis (median blood use = 10 units). Furthermore high volume blood transfusion was found to be associated with diminished patient survival (<10 unit transfusion 1 year survival = 85%, >20 unit transfusion 1 year survival = 60%).

These data allow the pre-operative identification of patients likely to require high volume blood transfusion. This may assist in planning blood transfusion services and also allows adjustments of operative technique, for example use of the cell saver, in these high risk patients.

C77

LONGITUDINAL CHARACTERISATION OF HIGH ENERGY PHOSPHATES IN LEFT AND RIGHT VENTRICLES OF HUMAN DONOR HEARTS

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Background: Brain stem death (BSD) induces disproportionate right ventricular dysfunction. We wanted to test if BSD is associated with perturbed energy metabolism in either ventricle and if basal energy stores are further affected by ischaemia and reperfusion.

Methods: Donor hearts were arrested with St Thomas' crystalloid cardioplegia and stored in cold saline. Serial transmural biopsies were obtained from the left ventricle (LV) and right ventricle (RV) as follows: (1) on initial assessment of the donor heart, (2) before explantation, (3) after cold storage, (4) at the end of implantation (warm ischaemia), (5) after 10 minutes of reperfusion. Adenine nucleotides were measured using the luciferase assay. The energy charge (EC) was calculated as ATP+0.5ADP/ATP+ADP+AMP.

Results: 17 BSD donors, 3 live (domino) donors and 23 heart/heart-lung recipients were assessed. A complete study (biopsies 1 to 5) was available in 12 hearts. The cumulated values for all donors and recipients were:

	1	2	3	4	5
LV ATP/ADP	8.07 (4.42)	7.21 (3.21)	9.14 (5.13)	3.82 (1.35)	7.05 (5.38)
RV ATP/ADP	7.44 (3.64)	7.16 (4.58)	11.11 (5.24)	4.88 (2.15)	7.56 (5.46)
LV EC	0.92	0.92	0.94	0.87	0.90
RV EC	0.94	0.91	0.94	0.89	0.91

There was no statistically significant difference between RV and LV energy stores at any time point but the change over time was significant for both RV and LV ($p<0.01$). The 3 domino donors had above average levels of adenine nucleotides.

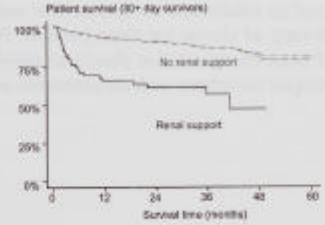
Conclusion: Donor heart dysfunction is not caused by impaired energy metabolism. Moreover, there is no disparity between RV and LV energy stores. Cold ischaemic storage has a protective effect, but a significant energy loss occurs during implantation. Most organs recover their pre-harvesting energy levels after reperfusion. This longitudinal characterisation could assist cardiac preservation efforts.

C18

IMMEDIATE RENAL REPLACEMENT THERAPY AND OUTCOME AFTER CARDIAC TRANSPLANTATION – A MULTIVARIATE ANALYSIS OF RISK FACTORS

I Saeed, CA Rogers, and AJ Murday on behalf of the Steering Group, UK Cardiothoracic Transplant Audit, Clinical Effectiveness Unit, The Royal College of Surgeons of England, UK

Background: Renal failure is a serious complication following cardiac transplantation, and studies suggest that acute renal failure requiring renal replacement therapy in the early post-operative period may be predictive of poor outcome.
Aim: To examine survival in cardiac transplant recipients who did or did not require immediate (within 30 days) haemodialysis/haemofiltration, and examine potential risk factors predisposing to this complication.
Subjects: All adult cardiac transplant recipients transplanted in the UK between July 1995 and June 2000.
Methods: Prospective multi-centre cohort study. Survival curves for patients surviving beyond 30 days were estimated using the Kaplan-Meier method and compared using the log rank test. Stepwise logistic regression was used to examine a range of potential donor and recipient risk factors for haemodialysis/filtration in patients surviving more than 2 days.
Results: Of 1108 cardiac transplants, 128 (11.6%) required haemodialysis/filtration in the first 30 days post-transplant. Risk factors for renal replacement therapy identified were ($p<0.10$) a pre-operative creatinine >150 µmol/l (OR 2.1, 95%CI 1.2-3.7), donor size mismatch >80% (OR 2.4, 95% CI 0.99-5.7), recipient age < 45 years (OR 1.5, 95% CI 0.93-2.4) and recipient diabetes (OR 1.8, 95% CI 0.97-3.5). One and three year patient survival for patients needing haemodialysis or haemofiltration was significantly worse ($p=0.0001$) than for those who did not.



Conclusion: The initiation of renal support in the early post-transplant period is a strong marker of poor short and mid-term survival. Predictors of this serious complication include a preoperative creatinine > 150 µmol/l (a significant risk factor), recipient diabetes, recipient age and donor:recipient size mismatch. Knowledge of these facts provides valuable prognostic information, and may help to refine patient selection for transplantation.

CLINICAL

C17

PAEDIATRIC HEART TRANSPLANTATION IN THE UNITED KINGDOM – A FIVE YEAR REVIEW OF PRACTICE AND RESULTS

I Saeed, CA Rogers, and AJ Murday on behalf of the Steering Group, UK Cardiothoracic Transplant Audit, Clinical Effectiveness Unit, The Royal College of Surgeons of England, UK

Aim: To review the practice and results of paediatric heart transplantation in the UK.
Subjects: All paediatric (16 years) heart transplant recipients, transplanted in the UK between April 1995 and March 2000.
Methods: Prospective multi-centre case-series. Patients were divided into three subgroups according to age: infants (<1 year), children (1-10 years), and teenagers (11-16 years). Survival curves were estimated using the Kaplan-Meier method.
Results: One hundred and thirty-six transplants were performed. Median age at transplant was 7 years (IQ range 2-12). Only 7% of transplants were in infants. Indications for transplantation were: dilated cardiomyopathy (59%), congenital heart disease (24%), retransplant (3%), and miscellaneous (14%). Dilated cardiomyopathy was the major indication for transplantation within each age group; 91% of patients with congenital heart disease had one or more corrective procedures prior to transplant; 50% patients were in-patients at the time of transplant, with 42% requiring inotropic support and 12% ventilated. Median ischaemic time was 205 minutes. Median ITU and hospital stay (respectively) were: 7 and 27 days (infants), 3 and 23 days (children), and 3 and 24 days (teenagers). Overall 30 day, 1 year and 3 year patient survival was 88% (95% CI 82-93), 80% (95% CI 72-87), and 75% (95% CI 66-84). Three-year patient survival by age group was: 80% (95% CI 45-100, infants), 76% (95% CI 64-88, children), and 74% (95% CI 61-88, teenagers).
Conclusion: Results are comparable to or better than those reported by other national/multi-institute studies. The proportionately small number of infant transplants reflects infant donor organ shortages, the poor results of infant transplantation in eras before the study period, and an established acceptance that corrective surgery (despite variable results) is the therapy of choice for these patients. Based on these data, we suggest that the option of heart transplantation should be considered more often when devising management strategies for infants with uncorrectable congenital heart disease.

CLINICAL

Session B

Kidney Session

C88

THE POSSIBILITY OF TRANSMISSION OF CEREBRAL MALIGNANCY IN RENAL TRANSPLANTATION

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Concern has been raised by the reports of cases of apparent transmission of brain malignancy to recipients of organ grafts from donors with such neoplasms. In a current prospective study of cancer incidence and mortality in renal transplant recipients in Britain that is supported by UK Transplant, the possibility of such transmission has been investigated

In a preliminary analysis, 457 patients were identified who had received a kidney from a donor who had died from a cerebral malignancy. Such malignancies were searched for among 457 patients and the number that would be expected was calculated by applying national incidence and mortality rates to the corresponding person years at risk by age group and sex. Person years were measured from the date of the first transplant to the earliest of the following: date of diagnosis of brain cancer, death, the end of the period for which deaths or cancer registrations respectively were judged complete.

Only one case of brain malignancy was found among these 457 recipients, and no death from this cause; the corresponding expected number of cases is 0.21. The difference between the observed and expected numbers of incident cases of brain cancer is, of course, not statistically significant. The single case concerned a man who received a kidney graft at age 45 and was diagnosed with a cerebral malignancy just under a year later. However the histology of the two malignancies was quite different – this being a low grade angiosarcoma in the donor, while the recipient had a glioma.

It is relevant that in the study as a whole involving approximately 30,000 renal transplant recipients, there is no increased incidence of cerebral malignancy (6 observed, 7.8 expected).

This study of malignancy among the recipients of kidneys from donors with primary cerebral neoplasms is the largest in the world. It is currently being updated and the latest figures will be presented at the meeting.

C109

HLA MISMATCHING, DONOR RELATIONSHIP AND OUTCOME OF LIVING DONOR KIDNEY TRANSPLANTS IN THE UK AND ROI, 1989 - 1998.

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Between 1989 and 1998, 1390 living donor kidney transplants were reported to the National Transplant Database. One year follow-up was reported in 1210 (87%) of cases; a further 68 of these were excluded from this analysis because of incomplete data for HLA typing, donor or recipient gender or recipient date of birth. First and repeat (144) transplants were included giving a final dataset of 1142 transplants for analysis.

Multifactorial analysis showed that transplant number, HLA mismatching, and donor age significantly influenced one year transplant survival. Recipients of a second or subsequent graft were twice as likely to experience graft failure within a year than first graft recipients ($p=0.001$). No difference was found between '000' and favourably ('100', '010' and '110' HLA-A, -B, -DR mismatches) matched grafts, but those with non-favourable mismatches had significantly inferior survival to '000' mismatched grafts, $p<0.005$. For donors aged >55 years there was an increased risk of graft failure compared with donors aged 36-45 years (RR=3.4, 95% C.I. 1.4-7.4, $p<0.005$). Factors found to have no influence on one year outcome were donor / recipient relationship, recipient age, year of transplant and gender mismatch.

For genetically related transplants, graft number was the only factor to significantly influence one year survival. There were no 2-DR mismatched transplants from parent donors and only 1.7% of genetically related donors were 2-DR mismatches.

The number of genetically unrelated transplants was small (n=47) but one year transplant survival for those with 2-DR mismatches (n=13) was only 69% (95% C.I. 44-94%) contrasting with 85% for other matches (n=34, 95% C.I. 73-97%, p non sig.). In this group there were 9 failed transplants and 7 occurred in the first 3 months; the cause of failure was reported in 7 cases: 1 hyperacute rejection, 5 irreversible acute rejections and 1 recipient death with a functioning transplant. Six of these failures were from 16 genetically unrelated donor transplants prior to 1996 and 3 were in the 31 transplants since 1996.

For genetically related donors, either a '000' mismatch or 'favourable' match confers significantly improved one year transplant survival over other 0 or 1-DR mismatched transplants. For genetically unrelated donors 2-DR mismatched transplants had a 16% reduced one year transplant survival. There is an established risk of HLA specific sensitisation following HLA mismatched transplantation, which must be considered, especially for younger recipients who may need a repeat transplant.

C39

EARLY POST-OPERATIVE COMPLICATIONS FOLLOWING LIVE KIDNEY DONOR NEPHRECTOMY

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Introduction: The proportion of kidney transplants performed in the United Kingdom from live donors has increased from 5% in 1990 to 15% in 1999. Peri-operative complications following unilateral nephrectomy for organ donation are believed to be low, however, there is a paucity of data from the UK.

Method: This study examined peri-operative adverse events in two UK Transplant Centres between 1997 and 2000. Seventy-one consecutive live donor nephrectomies were reviewed. An open approach through the bed of the 12th rib was used for all procedures. Donor complications were defined and recorded in a standardised format. Pre and post-operative measurements of serum creatinine and blood pressure were also noted. The influence on the complications of age, gender, body mass index, smoking status and transplant centre was assessed.

Results: The median age was 46yrs (range 26-65yrs). Thirty-six donors were male and 35 female, 59 pairings were related and 12 unrelated. Forty-nine donors (69 percent) had one or more peri-operative complications recorded. Nine individuals had minor intra-operative adverse events, and 8 of these donors also developed a post-operative complication. There were no serious or life threatening events.

Post Operative Complication	Number	Percent of Donors
Atelectasis	16	23%
Chest infection	12	17%
Urinary tract infection	9	13%
Wound infection	8	11%
Wound dressing allergy	7	10%
Epidural leak	2	3%

Single incidences of leg paresthesia, wound dehiscence, sub-phrenic collection, colitis, cannula site infection and wound haematoma were noted. No correlation was found between conditional variables and complication rates. Post-operative pyrexia was recorded in the majority of patients. Serum creatinine increased in 69/71 patients, the median increase was 37μmols/l (range 0-97). Constipation requiring treatment was also noted in 66% of donors. The median hospital stay for donors without post-operative complications was 5 days (range 3-7), those with complications 6 days (range 3-12).

Conclusion: This study demonstrates a substantially higher peri-operative complication rate among live donors undergoing nephrectomy than previous reports from the USA and Scandinavia. This may partly reflect more detailed reporting and potential donors should be made aware that minor complications are very common.

C111

INDO-ASIAN EXPERIENCE OF RENAL TRANSPLANTATION IN YORKSHIRE - RESULTS OF A TEN YEAR SURVEY

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In the present study we have analysed equity of access to, and outcome following, renal transplantation in the Indo-Asian ethnic minority in West Yorkshire. This was a retrospective cohort based observational study involving 1) 846 adult patients starting renal replacement therapy 1990-1994, 2) 822 adult patients registered on the cadaveric transplant waiting list 1985-1994, 3) 608 adult patients transplanted 1985-1994. Comparisons were made between the Asian minority and the white non-Asian majority with regard to identification of candidacy for transplantation, rate of transplantation from the waiting list, degree of donor-recipient HLA match, and transplant outcome. At one year from starting dialysis 34% of Asian and 31% of non-Asian patients were registered onto the National Transplant Waiting list held by UK Transplant (non-significant). There was a significant difference in graft rate between the groups: at 3 years 72% of non-Asians and 55% of Asians had been transplanted from the waiting list ($p<0.001$). For those transplanted, HLA matching was superior for white patients: 34% vs 20% of pairings achieving a 000 mismatched or favourably matched graft ($p<0.05$). Five year transplant survival was significantly reduced in Asian patients (71% vs 58%, $p<0.01$). Asian cadaveric donation was identified in only 2 of 608 transplants during a 10 year period. In summary, during the period of this study Asian patients gained access to the transplant waiting list at a similar rate to the non-Asian white majority. Due to difficulties with HLA matching, Asian patients were significantly disadvantaged in terms of receiving a transplant once listed, and post transplant survival was also inferior. Cadaveric donation was extremely uncommon from within the Asian community.

C9

ADEQUACY OF DONOR KIDNEY BIOPSIES: A COMPARISON OF TWO BIOPSY TECHNIQUES

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Pretransplant donor kidney biopsies help to differentiate chronic changes present in the donor kidney from acquired chronic allograft nephropathy or drugs-induced lesions and are potentially useful both for the individual patients and for the therapeutic trials on chronic rejection which incorporate histology in the analysis of the outcomes.
It is clear that, whatever the biopsy technique used (wedge or core needle), the sample it provides must be suitable for assessment and comparable with the postransplant biopsy specimen in term of representativeness of the graft tissue obtained.
In this analysis, we compared 19 needle core and 37 wedge biopsies taken from donor kidneys by evaluating: i) the presence and number of glomeruli; ii) the proportion of sclerosed glomeruli; iii) the number of arteries and arterioles; and iv) the arteriolar hyalinosis index.
All but one (a core needle) biopsy contained renal cortical tissue. Significantly more glomeruli were present in wedge biopsies (median 17) than in core needle biopsies (median 12) (p=0.04) but the proportion of sclerosed glomeruli was not significantly different between the biopsy types (p=0.56).
Statistical analysis regarding sampling of the vessels is shown in the table below. No significant difference in arteriolar number (p=0.2) or hyalinosis index (p=0.56) was identified but, in terms of muscular arteries, significantly more core biopsies were considered to be minimal (a) or adequate (b) samples as compared with wedge biopsies (13/19 vs.1/37 adequate, 18/19 vs. 10/37 minimal) (p<0.001 for both).

Statistic	Arterioles		Muscular arteries	
	Wedge biopsy	Needle biopsy	Wedge biopsy	Needle biopsy
Proportion	37/37(100%)	18/19(95%)	(a) 10/37(27%) (b) 1/37(2.7%)	(a) 18/19(95%) (b) 13/19(68.5%)
Range	1 – 14	0 – 34	0 – 2	0 - 6
Mean ± stdev	4.4 ± 3.2	6.3 ± 7.4	0.3 ± 0.5	2.5 ± 1.5
Median	4	4.5	0	2
Mode	2	2	0	2
P – value	0.2		<0.001	

These data indicate that wedge and core needle biopsies are comparable in sampling glomeruli and arterioles but arteries, which are essential for the baseline evaluation of chronic vascular changes, are significantly better represented in core biopsies. We therefore recommend core needle biopsy as the procedure of choice in sampling representative donor kidney tissue prior to transplantation.

C78

MEASURING INTRARENAL VASCULAR RESISTANCE DURING MACHINE PERFUSION PRESERVATION DOES NOT IMPROVE UPON THE ASSESSMENT OF RENAL VIABILITY MADE ON CLINICAL GROUNDS

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Introduction: A reliable objective test of the viability of ischaemically damaged kidneys prior to transplantation would be very valuable in assessing organs from an uncontrolled non-heart-beating donor (NHBD). Currently in Leicester viability assessment in such instances is made using estimated warm ischaemic time and the macroscopic appearances of the kidney at retrieval. The aim of this study was to assess the potential of intrarenal vascular resistance measurements during hypothermic machine perfusion preservation to determine renal viability.
Methods: Seven pairs of uncontrolled NHBD kidneys were determined at the time of harvesting to be fit (group 1) or unfit (group 2) for transplantation according to their appearances as assessed by the retrieving surgeon. This decision was adhered to irrespective of the results of machine perfusion. All 14 kidneys were then perfused with hyperosmolar citrate solution at a systolic pressure of 60 mmHg and a temperature of 3-7°C for 6 hours. The intrarenal vascular resistance was recorded throughout the perfusion, and the values at the beginning were compared. Statistical analysis was by the Mann Whitney U test.
Results: Eight kidneys were allocated at retrieval to group 1, and 6 to group 2. Of the 8 kidneys transplanted, 5 functioned after a delay (group 1a) and 3 never functioned (group 1b). The median initial resistance for group 1a was 0.70(mmHg/ml/min/100g), 0.67 for group 1b, and 1.32 for group 2 (p=0.003). In those kidneys that did function there was no correlation between best serum creatinine and initial renal vascular resistance (p=0.6)
Conclusion: Intrarenal vascular resistance during machine perfusion is higher in kidneys which are macroscopically unfit for transplantation. However intrarenal resistance does not help to distil viable from non-viable organs in those that are macroscopically judged fit for transplantation.

C102

ASSESSMENT OF LIVE RENAL TRANSPLANT DONORS: GADOLINIUM ENHANCED MAGNETIC RESONANCE ANGIOGRAPHY AND UROGRAPHY IS AS ACCURATE AS DIGITAL SUBTRACTION ANGIOGRAPHY AND INTRAVENOUS UROGRAPHY

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The falling rate of cadaveric renal allograft supply and rising waiting list demand has refocused attention in UK units on the importance of living donor kidney transplantation. Conventional donor assessment involves digital subtraction angiography (DSA), intravenous urography (IVU), and radionuclide functional assessment. Arteriography is associated with significant defined morbidity and has considerable resource implications. Gadolinium-enhanced magnetic resonance angiography (MRA) and urography (MRU) are relatively new alternative techniques potentially able to evaluate renal/vascular anatomy as a 'one-stop' non-invasive outpatient procedure.

This prospective study compared the accuracy of combined MRA and MRU with conventional investigation in the assessment of potential live renal transplant donors. During one year, all medically suitable potential live renal transplant donors underwent both MRA/MRU at one outpatient attendance, DSA as a day case admission and IVU on a different visit. Patients with iodinated contrast allergy were excluded. Informed consent was obtained. MRU was performed using a coronal 2 second HASTE sequence, immediately followed by MRA using a gadolinium-enhanced 3-D breath hold volume acquisition. Conventional techniques were used to perform DSA & IVU. The MRA/U & IVU/intra-arterial(IA) DSA images were assessed independently by 2 radiologists.

Eighteen potential donors were investigated. One patient had an anaphylactic reaction to iodinated contrast during IV urography. Two patients could not tolerate MR scanning due to claustrophobia. Fifteen underwent both full MR and conventional assessment. A total of 40 renal arteries (30 main & 10 accessory) were identified. Results of MRA & DSA agreed in 12 out of 15 patients. For both MRA & DSA the presence of one artery was reported as uncertain. One accessory artery was apparent but not initially reported on DSA. Two accessory arteries were apparent but not initially reported on MRA. These vessels were visible on retrospective review. MRU revealed normal urograms in all patients, in addition to small simple renal cysts in 3 patients. All IVU examinations were reported as normal. Critical evaluation of source MRA images and good arteriographic techniques with selective injections proved essential. The NHS cost of each MRA/U examination was £255, compared to £804 for IA DSA/IVU.

In assessment of potential live kidney donor arterial/urinary anatomy, MRA/U is as accurate as IA DSA and IVU. MRA/MRU avoids possible contrast reaction, exposure to ionising radiation and is a non-invasive combined quick procedure, which costs less. Accessory arteries may be missed by MRA and DSA if meticulous technique and interpretation is not performed.

C124

DOES GADOLINIUM-ENHANCED MAGNETIC RESONANCE ANGIOGRAPHY REPRESENT THE NEW GOLD STANDARD IN THE PRE-OPERATIVE INVESTIGATION OF POTENTIAL LIVING RENAL TRANSPLANT DONORS?

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BACKGROUND

Gadolinium-enhanced magnetic resonance angiography (MRA) is rapidly gaining popularity as an effective imaging modality for the assessment of vascular disease. Its major advantages over the current "Gold-Standard" of digital subtraction angiography (DSA) are that it avoids ionising radiation, the contrast agents are non-nephrotoxic and that is non-invasive and therefore complications of DSA that are related to arterial trauma are eliminated. These advances are particularly important when considering living donor kidney transplantation, as these donors are healthy individuals.

METHODS

A prospective, blinded study was performed to assess the efficacy of MRA in comparison to DSA in identifying renal artery anatomy in potential living renal transplant donors. Images from each study were independently analysed for demonstration of the number of main and accessory renal arteries, and the presence of early arterial branching. Evaluation was performed by separate Consultant Radiologists blinded to the results of the other modality. The results of both imaging modalities were also correlated with operative findings during organ transplantation whenever possible.

RESULTS

During the period April 1998 to August 2000, 25 consecutive donors were evaluated. The donors consisted of 15 females and 10 males with an age range of 30-55 years. The number and position of the main renal arteries identified by DSA were identical to those identified by MRA. All 7 accessory arteries seen on DSA were also identified by MRA but the presence of one artery seen on MRA could not be confirmed by DSA (sensitivity 100%; specificity 98%, positive predictive value 88% and negative predictive value 100%). Six of seven early branches seen on DSA were also seen on MRA but one branch seen on DSA was not visualised by MRA due to a breathing artefact (sensitivity 86%; specificity 100%, positive predictive value 100% and negative predictive value 98%). The operative findings did not contradict the results of imaging on any occasion.

CONCLUSIONS

The results of this study would suggest that gadolinium-enhanced MRA shows comparable efficacy to DSA in the identification of renal artery anatomy, and with a significantly better safety profile, MRA should now be regarded as the new investigation of choice for screening of potential living organ donors. Further evaluation is continuing to assess whether MRA should indeed be considered the "Gold-Standard" for investigation of all vascular disease.

CLINICAL

C4

IS IT WORTH FORMING A WAITING LIST ALLIANCE? FIGURES FROM SCOTLAND-NORTHERN IRELAND ALLIANCE FOR THREE YEARS

G.C. Oniscu, W.Plant, P. Pocock, J.L.R. Forsythe on behalf of the Scotland-Northern Ireland Alliance

BACKGROUND: The Scotland-Northern Ireland Kidney Allocation Alliance was created in August 1998 shortly after a new allocation scheme was implemented in the United Kingdom. The purpose was to optimise the transplant service through increased regional exchange, higher quality matched kidneys and better organ distribution.

METHODS: An analysis was performed on prospectively collected data regarding retrieval and transplant activity. The degree of HLA matching, the cold ischaemic time (CIT) and the balance of exchange were analysed for a two-year period following the introduction of the new alliance. A comparison with the last year pre-alliance was carried out.

RESULTS: Despite a reduction in the number of donors, there was a 17.7% increase in the number of transplants performed and a higher organ exchange. 79% of the kidneys in the first alliance year and 77% in the second one were exported from the retrieving centre compared with 55% in the pre-alliance year, ($p<0.05$, Chi square). The quality of HLA matching is illustrated in table 1. There was a significant increase in the number

Year of activity	Number of transplanted kidneys	Tier 1 (%)	Tier 2 (%)	Tier 3 (%)
Pre-alliance	158	15 (9.5%)	83 (52.5%)	60 (38%)
Year 1	164	29 (18%)	91 (56%)	42 (26%)
Year 2	186	38 (21%)	114 (61%)	34 (18%)

Table 1. Quality of HLA matching for each of the three years.

of favourable matched kidneys (tier 1 and tier 2), ($p=0.01$, Chi square). There was no significant difference between the mean CIT for the three study periods, nor between the CIT for locally used versus Alliance exchanged kidneys ($p>0.05$, two sample Student-t test) (table 2). A large centre tends to be a net importer of kidneys from the Alliance as well as UKT, while small and medium sized centres tend to balance their exchange within the two-year period.

Period	Locally used kidneys Mean CIT	Exchanged within alliance Mean CIT	Imported from UKT Mean CIT
Pre-alliance	1225	1174.4	1582.4
Year 1	1263.1	1376.6	1332.2
Year 2	1305.4	1355.2	1439.3

Table 2. Cold ischaemic time for the three-years according to kidney origin (minutes).

CONCLUSIONS: The introduction of a regional kidney allocation alliance has improved the degree of HLA matching and increased the exchange of organs, without a significant increase in the cold ischaemic time. There is an imbalance of kidney distribution between large and smaller participating centres which did not correct with time.

Thursday 29 March

Parallel Session Three

Session A – Laboratory Session

L10

PECAM-1 (CD31) Expression on Donor Endothelial Cells attenuates Transplant Arteriosclerosis in Aortic Allografts

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Introduction: PECAM-1 (CD31) is expressed on the surface of endothelial cells, platelets, monocytes, neutrophils, and certain T cell subsets. It has been shown *in vitro*, that PECAM-1 is critically involved in the transendothelial migration process of leukocytes. We therefore wanted to test the hypothesis that a reduced number of leukocytes transmigrating into a PECAM-$1^{-/-}$ aortic allograft would result in less damage to the graft by activated infiltrating cells.

Methods: Fully mismatched PECAM-$1^{-/-}$ (C57BL/6/$H2^b$) or PECAM-$1^{+/+}$ (C57BL/6/$H2^b$)(wildtype) grafts were transplanted into BALB/c ($H2^d$) recipients. Grafts were analysed by histology, morphometry, and immunohistochemistry on day 30 after transplantation. Intra-graft cytokine mRNA production was analysed by competitive RT-PCR on day 14 after transplantation.

Results: A significant increase in the level of intimal proliferation was observed in PECAM-$1^{-/-}$ (C57BL/6/$H2^b$) abdominal aortic allografts transplanted into BALB/c ($H2^d$) recipients compared to that found in PECAM-$1^{+/+}$ (C57BL/6/$H2^b$) grafts 30 days after transplantation [PECAM-$1^{-/-}$: 57±5 % vs. PECAM-$1^{+/+}$: 36±6 % ($p< 0.005$; n=6)]. Absence of PECAM-1 expression on donor endothelial cells did not reduce the overall number of graft infiltrating cells significantly, but instead resulted in a significant increasing infiltration by macrophage ($CD11b^+$) and significantly elevated intragraft mRNA expression of iNOS. We also investigated the kinetics of donor endothelial cell replacement in the PECAM-1 deficient aortic grafts. Replacement of donor endothelial cells was only seen in the allogeneic situation, commenced 14 days after transplantation and was complete by day 30.

Conclusion: These data suggest that PECAM-1 expression on donor endothelial cells may play a protective role for the development of transplant arteriosclerosis, possibly by preventing excessive macrophage infiltration.

L20

A CLINICALLY RELEVANT TOLERANCE PROTOCOL USING NON-DEPLETING ANTI-CD4 ANTIBODY INDUCES $CD25^+$ REGULATORY T CELLS

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Introduction

The ability to identify T cells with immunosuppressive or regulatory properties as specific unresponsiveness develops *in vivo* would be a major benefit for the development of novel strategies for tolerance induction in clinical transplantation.

Aim

In this study we investigated the use of pre-treatment with a non-depleting anti-CD4 antibody (YTS177) and DST; this pre-treatment has been shown to allow the acceptance of primary cardiac allografts without the need for additional treatment. We wished to ascertain whether regulatory cells could be isolated following pre-treatment alone, and to characterise the phenotype of these cells further.

Results

Mice were pre-treated with YTS177 and DST and, twenty-eight days later, $CD4^+CD25^+$ cells isolated from their spleens by flow cytometry. These cells were administered to T cell depleted syngeneic recipients together with $CD4^+CD45RB^{high}$ cells from naïve mice. These reconstituted mice then received donor-specific skin grafts. Mice reconstituted only with $CD45RB^{high}$ cells rejected their skin grafts acutely (MST 20 days), but, in clear contrast, co-transfer of $CD25^+$ cells from pre-treated mice prevented rejection, with the majority of mice accepting their grafts (MST >60 days). Graft rejection was not affected by co-transfer of $CD25^-$ cells from naïve mice, confirming that regulatory cells in this system develop as a consequence of the pre-treatment protocol. Analysis of the proportion of $CD25^+$ cells at the time of adoptive transfer revealed a small increase in pre-treated animals compared to naïve controls (11.9% versus 10.1%, $p<0.05$).

Conclusion

The present study demonstrates that $CD4^+CD25^+$ regulatory T cells with the ability to override normal rejection responses are generated in recipient mice exposed only to donor antigen plus non-depleting anti-CD4 antibody. We find evidence for a small increase in the proportion of $CD25^+$ cells following pre-treatment, and these cells are clearly distinct from their naïve counterparts. This well-characterised model of transplantation tolerance should allow us to refine the phenotypic identification of these regulatory cells, which in turn might provide a relevant screening tool for tolerogenic protocols being considered for clinical application.

L43

ANTI-CD4 AND DST INDUCED TOLERANCE: THE ROLE OF REGULATORY CELLS

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The development of operational tolerance is an important goal in transplantation and a considerable body of evidence suggests that T cell mediated regulation may be an important and even essential component of such tolerance.

We have previously established an *in vivo* model in which mice administered a donor-specific blood transfusion (DST) under the cover of a depleting anti-CD4 antibody accepted primary cardiac allografts for over 100 days (termed long term survivors, LTS) and demonstrated prolonged survival of subsequent donor-specific skin grafts. Tolerance was donor alloantigen specific as third party cardiac grafts were rejected.

The aim of this study was to extend these observations by attempting to identify cell surface markers that could enrich for a population of donor alloantigen-specific regulatory cells. We purified sub populations of $CD4^+$ T cells (from LTS), sorted on the basis of $CD25^+$ or $CD45RB^{low}$ phenotype and, in a skin allograft model, investigated their ability to regulate rejection mediated by $CD45RB^{high}$ $CD4^+$ T cells from naive animals.

T cell depleted mice reconstituted with $1x10^5$ $CD45RB^{high}$ $CD4^+$ T cells from naive animals rejected BL10 skin grafts acutely (MST= 20 days). In contrast co-transfer of $5x10^5$ $CD45RB^{low}$ $CD4^+$ T cells (from LTS) suppressed rejection mediated by $CD45RB^{high}$ $CD4^+$ T cells (from naive animals) with 5/6 animals accepting donor strain skin grafts for > 100 days. This effect was abrogated by administration of an anti-IL-10 monoclonal antibody. $CD45RB^{low}$ -mediated suppression was antigen-specific as third party skin grafts were rejected. In contrast, $CD45RB^{low}$ $CD4^+$ T cells isolated from naive animals were unable to suppress rejection. In a subsequent experiment T cell depleted mice were reconstituted with $1x10^5$ $CD45RB^{high}$ $CD4^+$ T cells from (naive animals) and $5x10^5$ $CD25^+CD4^+$ T cells (from LTS). 7/9 mice accepted skin allografts indefinitely (>100 days). Significantly, an equivalent number of $CD25^+CD4^+$ T cells isolated from naive animals were unable to provide immunoregulation. Regulatory activity was a specific function of $CD25^+CD4^+$ T cells from LTS, as $CD25^-CD4^+$ T cells were unable to suppress rejection.

These data demonstrate that among $CD4^+$ T cells isolated from LTS the $CD45RB^{low}$ and $CD25^+$ subsets can effectively suppress rejection mediated by alloaggressive-cells *in vivo*. The observation that the corresponding population isolated from naive animals is unable to regulate indicates that the regulatory potential of these cells arises as a consequence of the induction of operational tolerance.

L52

INHALED NITRIC OXIDE DURING PERFUSION OF THE NON-HEART-BEATING-DONOR LUNG AMELIORATES WARM ISCHAEMIC INJURY POST TRANSPLANTATION.

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Aims. Lungs from non-heart-beating donors (NHBD) are a promising source of extra organs for transplantation. However, poor post-transplant lung function is a potentially fatal consequence in up to 20% of current heart-beating donor lung transplants. In clinical non-heart-beating renal transplantation programmes, a high rate of delayed or primary non-function is seen due to warm ischaemic injury, which would be fatal in the pulmonary setting. We therefore aimed to determine whether inhaled nitric oxide would ameliorate warm ischaemic lung injury, thus allowing prolongation of the warm ischaemic interval prior to retrieval and studied this in our existing pig non-heart-beating lung donor model.

Methods. Landrace cross Yorkshire White pigs of approximate weight 50kg were anaesthetised throughout and euthanased without regaining consciousness. Experimental donor lungs were retrieved 1 hour (**N1**, n=6) or 2 hours (**N2**, n=6) and (**N2NO**, n=5) after hypoxic death. In-situ **left** lungs were ventilated with 100% oxygen and perfused for 20 minutes with deoxygenated and neutrophil depleted blood; in addition, **N2NO** lungs had nitric oxide added at 20 p.p.m. to the ventilating oxygen during blood perfusion. The **left** lungs were subsequently transplanted, total ischaemic times 8.1h (**N1**), 9.1h (**N2**), 9.3h (**N2NO**).

Results: Assessment. After 15 minutes' assessment, pulmonary vascular resistances were lower in **N1** than **N2**, mean 8 Wood units (s.d. 4) *v* 11 (4) respectively, p=n.s. (t test). Addition of nitric oxide significantly reduced this in **N2NO** to7 (2), p=0.05 *v* **N2** (t-test). Oxygenation was acceptable in all groups, but after two hours' ischaemia was significantly lower; **N1** 58 (8) kPa, **N2** 47 (5), p=0.02 (t test). Oxygenation improved significantly to 58 (8) kPa in **N2NO**, p=0.02 *v* **N2** (t test).

Transplantation. Over the 12-hour follow-up post transplantation, **N1** blood perfused lungs showed better vascular function than **N2**, **N1** PVR mean 23 (11) *v* **N2** 50 (32), p=0.05 (t-test). With nitric oxide, PVR in **N2NO** was reduced to 27 (24) Wood units; this was no longer significantly different to **N1**, but failed to differ significantly from **N2**, p=n.s. (t test). Differences in mean oxygenation between groups were not statistically significant; **N1** 52 (15) kPa *v* **N2** 58 (16) *v* **N2NO** 58 (13), p=n.s. (ANOVA) and all levels were consistent with good oxygenating function.

Conclusions. A significant deterioration in assessed function was seen in non-heart-beating donor lungs as warm ischaemic intervals prior to retrieval increased from one to two hours. Some of these differences were also significant post transplantation. Addition of nitric oxide for the brief period of ventilation during assessment significantly improves function during assessment. Post transplantation, the significant increase in pulmonary vascular resistance seen with increased warm ischaemic interval is reduced to a non-significant level by the ventilation with nitric oxide.

L72

ROLE OF NITRIC OXIDE IN ISCHAEMIC PRECONDITIONING OF THE LIVER

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Background: Nitric oxide (NO) may mediate some of the protective effects of Ischaemic preconditioning (IPC) against ischaemia reperfusion injury (IRI) of the liver. **Methods:** Sprague Dawley rats were subjected to 45 mins lobar ischaemia followed by 2 hr reperfusion (IR). L-arginine or L-NAME were administered to stimulate or block NO synthesis. Study groups (n=6) had, (1) sham laparotomy, (2) IR, (3) IPC with 5 min ischaemia and 10 min reperfusion before IR, (4) L-arginine before IR, (5) L-NAME + IPC before IR. Liver function tests, serum nitrites and tissue ATP were analysed. Nitric oxide synthase (NOS) distribution was studied using NADPH diaphorase histochemistry and the endothelial cell and inducible isoforms of NOS (eNOS and iNOS) identified using immunohistochemistry. Data was analysed using analysis of variance (ANOVA) for multiple comparisons and paired Student's t test. **Results:** IR induced substantial liver injury as assessed by significant increase in liver enzymes and was associated with reduced tissue ATP. IPC and L-arginine treatment reduced liver enzymes and increased ATP levels, and increased nitrites, whereas L-NAME treatment prevented these effects. NOS detected with NADPH diaphorase staining was associated with sinusoidal endothelium and was induced by IPC. Immunohistochemistry showed that both iNOS and eNOS were expressed in the liver after IPC. **Conclusions:** IPC induced NOS and attenuated IRI. These data strongly suggest a role for nitric oxide in IPC.

	Group 1 (sham)	Group 2 (IR)	Group 3 (IPC)	Group 4 (L-arginine)	Group 5 (L-NAME+IPC)
Serum ALT (u/L)	414±268	5173±215*	1380±320*†	1257±990*†	5962±317‡
Serum AST (u/L)	548±267	3103±190*	1537±113*†	1501±731*†	3653±211‡
ATP (µmol/g liver tissue)	20.0±0.5	5.0±0.1*	10.0±0.8*†	8.0±0.6*†	4.3±0.2‡
Serum NO_2^-/ NO_3^- (µM)	16.9±3.7	18.4±2.3^{NS}	93.8±24.3*†	84.0±4.7*†	17.3±1.7‡

Mean±SD; *P<0.05 vs sham; †P<0.05 vs IR; ‡P <0.05 vs IPC and L-arginine; ^{NS}Not significant.

FRESHLY ISOLATED AND RECENTLY ACTIVATED CD4$^+$ CD25$^+$ T CELLS AS IMMUNOREGULATORY CELLS IN VITRO

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Background: The CD4$^+$25$^+$ T cell subpopulation may be critical in controlling self-reactive T cells in vivo. This study further characterises the phenotype and function of CD4$^+$25$^+$ T cells *in vitro* as a prelude to investigating their role in the induction and maintenance of transplantation tolerance *in vivo*.

Methods: Responder and regulatory T cells were prepared from BALB/c, B6, (BALB/cxB6)F1 and TCR transgenic DO.11.10 (H2^d) and OT-1 (H2^b) mice. The B9 T cell clone provided responder cells in some experiments. APC were obtained from B6, BALB/c and FI mice. $cOVA_{257-264}$, $cOVA_{323-339}$, HY/Dby:NAGFNSNRANSSRSS and anti-CD3 were used as antigens or mitogen. CD4$^+$25$^+$ T cells were isolated by MACS or FACS; T cell responses were measured by proliferation .

Results: Freshly isolated (FI) CD4$^+$25$^+$ T cells from either normal or DO.11.10 mice, failed to proliferate following TCR triggered stimulation using cognate peptide or CD3 mAb, whilst FI-CD4$^+$25$^-$ T cells proliferated well under the same conditions. In cell mixing experiments FI-CD4$^+$25$^+$ T cells inhibited FI-CD4$^+$25$^-$ T cells. Thus FI-CD4$^+$25$^-$ T cells appear to be naturally occurring anergic and regulatory T cells. FI-CD4$^+$25$^+$ T cells from BALB/c and B6 mice were able to suppress the response of FI-CD4$^+$25$^-$ T cells from DO.11.10 mice and the HY specific H2A^b restricted clone B9 to anti-CD3 but not the respective cognate peptides. This implies that FI-CD4$^+$25$^+$ T cells require the signals via their TCR to exert suppression. To investigate antigen specificity and the mechanisms of suppression by FI-CD4$^+$25$^+$T cells, three- and four-cell model experiments were set up. FI-CD4$^+$25$^+$ T cells from $cOVA_{323-339}$ specific DO.11.10 mice were used as suppressors, FI-CD8$^+$25$^-$ T cells from $cOVA_{257-264}$ specific OT-1 mice as responders and spleen cells from either BALB/c, B6 or F1 mice as APC with the cognate peptides. Although suppression was seen in experiments using a mixture of APC from both parental strains (four cell model), suppression was very much more marked in the three cell model, using APC from F1 mice. Suppression was therefore antigen non-specific; the results obtained from the four-cell model indicate that responder T cells could serve as direct target cells of regulator T cells. The possible inhibitory function of APC induced by FI-CD4$^+$25$^+$ is under investigation. CTLA-4 blockade failed to abrogate the suppression by CD4$^+$25$^+$T cells in vitro.

FI-CD4$^+$25$^+$ T cells did not respond either to Con A or IL-2 alone, but they did proliferate well in the presence of both. This allowed the generation of two types of recently activated (RA) CD25$^+$ T cells, by culturing either FI-CD4$^+$25$^+$ or FI-CD4$^+$25$^-$T cells with Con A and IL-2 in the presence of APC. RA-CD25$^+$ T cells derived from CD4$^+$25$^+$T cells were still anergic and had even stronger regulatory function. In contrast, RA-CD25$^+$ T cells derived from culture of CD4$^+$25$^-$T cells were responsive. Moreover, to exhibit their suppressive effects, RA-CD25$^+$ T cells derived from culture of FI- CD4$^+$25$^+$ T cells still required signals via their TCR. FACS analysis showed expression of CD38 but not CD69 was up-regulated on RA-CD25$^+$ T cells derived from FI- CD4$^+$25$^+$ T cells.

Skin graft experiments involving in vivo depletion of CD25$^+$ cells or adoptive transfer of T cells enriched or depleted of CD4$^+$25$^+$T cells, are now underway, aimed at understanding the contribution of this immuno-regulatory population to induction and maintenance of transplantation tolerance.

Conclusions: This is the first study showing that following TCR ligation CD4$^+$25$^+$T cells are able to suppress CD8 T cells responses in an antigen non-specific manner. Both FI- or RA- CD4$^+$25$^+$ T cells are anergic T cells following TCR stimulation and are powerful suppressors in vitro. How best to manipulate this population to suppress graft rejection in vivo needs to be explored.

L44

ALLOGRAFT REJECTION DESPITE CD40-CD154 BLOCKADE.

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Introduction. CD40Ligand (CD154) delivers a co-stimulatory signal pivotal to T cell dependent immune responses. Blockade of the CD40-CD154 pathway has proved effective at permitting long-term allograft acceptance in several rodent as well as primate models. However, this strategy does not always induce indefinite graft survival. The aim of this study was to determine whether CD154 blockade-resistant allograft rejection was mediated by $CD4^+$ or $CD8^+$ T cells.

Results. We have previously demonstrated that fully MHC mismatched C57BL/10 (B10; $H2^b$) cardiac allografts survive indefinitely in CBA/Ca (CBA; $H2^k$) recipients following administration of an anti-CD154 mAb (MR1, 500μg i.p. on day 0,2,4). The Median Survival Time (MST) of B10 grafts was >100 days compared to a MST of 7 days (n=6) in mice treated with a control antibody. In contrast, the majority (>75%) of CBA recipients treated with MR1 rejected BALB/c ($H2^d$) cardiac allografts (MST 69 days, n=9). Moreover, all B10.BR ($H2^k$) recipients treated with MR1 rejected B10.S(7R) ($H2^s$) cardiac allografts (MST 25 days; n=7). As several studies have recently shown that $CD8^+$ T cells are resistant to CD154 blockade, we investigated whether allograft rejection was mediated by CD154 blockade-resistant $CD8^+$ T cells. Indeed, depleting $CD8^+$ T cells in MR1 treated mice greatly improved the survival of both BALB/c and B10.S (7R) grafts. However, $CD8^+$ depletion in combination with MR1 therapy was insufficient to induce indefinite survival of all allografts. Furthermore, this combined treatment did not prevent the development of chronic vascular changes in cardiac allografts that survived >100days. Computer-assisted analysis of elastin-stained coronary arteries was used to assess severity of luminal occlusion. At day 100, BALB/c grafts showed 13.4±9.5% occlusion (n=5) and B10.S (7R) grafts showed 25.1±7.1% occlusion (n=4). To ensure CD40-CD154 interactions were blocked throughout the duration of the experiments, we studied an extended course of MR1-therapy in the absence of $CD8^+$ T cells. We found that the administration of additional doses of MR1 had no significant effect on graft survival or the development of vasculopathy compared to the short-course MR1-treatment.

Conclusion. These data demonstrate that despite CD40-CD154 blockade allografts can be rejected. The rejection is partly mediated by $CD8^+$ T cells resistant to CD154 blockade. However, even in the absence of $CD8^+$ T cells, anti-CD154 mAb mono-therapy was insufficient to prevent allograft rejection. These data suggest that there is also a population of $CD4^+$ T cells that are resistant to anti-CD154 therapy and capable of mediating allograft rejection.

This work was supported by National Kidney Research Fund.

L29

THE NITRIC OXIDE DONOR, FK409, PREVENTS MUCOSAL VILLOUS MICROCIRCULATORY DISTURBANCES AND LUNG INJURY INDUCED BY INTESTINAL ISCHAEMIA-REPERFUSION INJURY

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Background. Although outcomes are improving, long-term survival of small bowel transplants remains hampered by acute and chronic rejection, which may result in part from ischaemia-reperfusion (I/R) injury. Suppressed nitric oxide (NO) production may contribute to a range of microcirculatory disturbances. Therefore, this study used fluorescent *in vivo* microscopy to investigate the effects of the NO donor, FK409, on the villous microcirculatory disturbances following intestinal I/R injury. Remote organ injury, in particular to lung tissue, frequently accompanies experimental models of intestinal I/R injury and it is plausible that preventing injury to the site exposed to ischaemia may also inhibit remote organ injury. Therefore, the effects of FK409 on lung injury were assessed histologically.

Methods. Experiments were carried out on anaesthetised adult male PVG rats. Animals were assigned to untreated ischaemia (n=12), FK409 treated ischaemia (n=12) or non-ischaemic sham control (n=12) groups. The superior mesenteric artery was clamped for 30 min in the ischaemic groups only. Treated animals received FK409 (10 mg/kg; i.v) 30 min prior to the induction of ischaemia and 30 min post-reperfusion. The mucosal surface was visualised after clamp removal via an incision in an exteriorized ileal segment. FITC-BSA (1mg/kg, i.a) or acridine orange (1mg/kg; i.a) were used to quantitate macromolecular leak (MML) and leukocyte adhesion respectively. MML from, and numbers of adherent leukocytes within individual villi were determined every 15 min for 2 hr. Heart rate and mean blood pressure (mBP) were monitored throughout the experiment, at the end of which, lungs were removed and histologically assessed.

Results. Eleven of 12 untreated animals subjected to intestinal I/R injury failed to survive the 2 hr reperfusion period. Increased MML and leukocyte adhesion was observed ($p<0.001$) and blood flow stasis eventually ensued. Respiratory distress was a common feature and lungs removed from these animals demonstrated alveolar collapse and consolidation with oedematous walls and a marked neutrophil infiltrate. In contrast, all 12 of the FK409 treated animals survived. Villous blood flow was maintained throughout reperfusion despite a decrease in mBP ($p<0.001$). MML and leukocyte adhesion were also reduced ($p<0.001$). Normally aerated alveoli with thin walls were present and only a few neutrophils were observed in the lungs from these animals.

Conclusions. FK409 significantly reduced leukocyte adhesion, maintained blood flow and prevented mortality after intestinal I/R. FK409 may therefore be of value in reducing localised tissue damage and improving the short and long-term outcome after small bowel transplantation. The observation that FK409 reduced lung injury suggests that it is also effective in inhibiting remote organ injury associated with intestinal I/R. It may attenuate remote tissue damage in other situations in which intestinal ischaemia is predictable, such as abdominal aortic aneurysm repair.

L23

EFFECT OF DONOR ALPHA-GAL GENE KNOCKOUT AND HUMAN CD46 TRANSGENE EXPRESSION ON HYPERACUTE REJECTION OF PANCREATIC ISLET XENOGRAFTS (MURINE TO PRIMATE)

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INTRODUCTION: There is currently great interest in finding alternative sources of tissue for islet transplantation, and recent descriptions of culture techniques to greatly expand the islet yield from murine pancreas makes the mouse a serious potential source for xenogeneic donor islets. However, the first hurdle to be overcome is hyperacute rejection, since we previously reported rapid destruction of C57Bl6 mouse islets transplanted under the renal subcapsular space of cynomolgus monkeys, associated with natural antibody binding and complement fixation. Now, we report the effect of donor targeted α-gal gene knockout (removing a major target for natural antibody) and preliminary results of human CD46 transgene expression (allowing human complement regulation).

METHODS: Handpicked intact α-gal knockout and CD46 transgenic mouse islets were transplanted in separate groups under the renal subcapsule of cynomolgus monkeys along with a separate control group of normal C57Bl6 mouse islets. The islets were retrieved after 24 hours and assessed "blind" by histology using light and electron microscopy. Immunoperoxidase staining was used to show the distribution of α-gal antigen and human CD46. In vitro studies of natural antibody binding and complement fixation following exposure of mouse islets to human serum were also undertaken.. Finally the *in vitro* interaction between the genetically altered and control islets exposed to fresh xenogeneic blood was assessed using thrombelastography and histology.

RESULTS: Controls Histological examination of freshly isolated islets from controls showed scattered Gal alpha positive cells but the majority of islet endocrine cells mice were α-gal negative. Transplanted xenogeneic control islets appeared to suffer the same hyperacute rejection process as before and electron microscopy confirmed destruction of islets by necrosis. In vitro incubation in human serum resulted in binding of IgM and IgG with C3, C4 and C5b-9 deposition. Exposure of isolated control islets to fresh human blood resulted in accelerated clotting and subsequent histological examination showed severe damage in 54% of islets. **α-gal KO mice**. All cells were negative for α-gal in α-gal KO islets (n = 5). Transplanted mouse α-gal KO islets into primate recipients (n=5) were not protected from early destruction. In vitro incubation in human serum resulted in binding of IgM and IgG with C3, C4 and C5b-9 deposition equal in intensity to controls. Exposure of α-gal KO islets to fresh human blood caused rapid clotting with thromboelastography parameters unchanged from controls and histological evaluation showed severe destruction in 56% of islets. **CD46 transgenic mice** Human CD46 expression was strong on the transgenic islets when compared to pancreatic acinar tissue. Histological examination after transplantation into cynomolgus monkeys (n=2) showed that early destruction was little changed in one but markedly reduced in the second. Incubation in human serum resulted in IgG, IgM, C3 and C4 deposition equal in intensity to controls but C5b-9 deposition was reduced. Clotting studies are awaited.

CONCLUSION: Our findings suggest that the α-gal epitope does not play a significant role in xenorecognition of pancreatic islets. Preliminary observations with CD46 transgenic islets are encouraging and suggest that complement plays a more significant role in islet xenograft destruction.

LABORATORY

Session B

Kidney Session

C108

Listing of dialysis patients for transplantation

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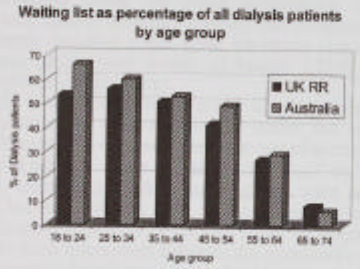
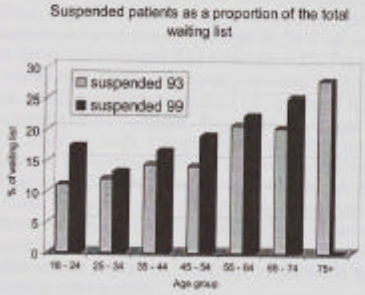
The UK Renal Registry has linked with UKTSSA to analyse waiting list data in areas covered by the Registry. Although the UK Renal Registry does not cover the whole UK population, the waiting list has been related to the dialysis population covered by the Registry. This co-operation provides a unique UK data set



The above figure only includes data from centres on the UK Renal Registry and is an approximation. In the UK only 50% of dialysis patients in the 18 – 44 age group were active on the waiting list. The Australian data has been taken from the ANZDATA report and excludes patients suspended from the waiting list.



In 1993, 16% of the total number of UK patients on the waiting list were suspended and this had risen to 19% on the 1st January 1999. As expected the proportion of suspended patients rises with age. The reason for the rise in suspension of the 18-24 year olds is unknown

C106 **Diabetes, transplantation and waiting lists**

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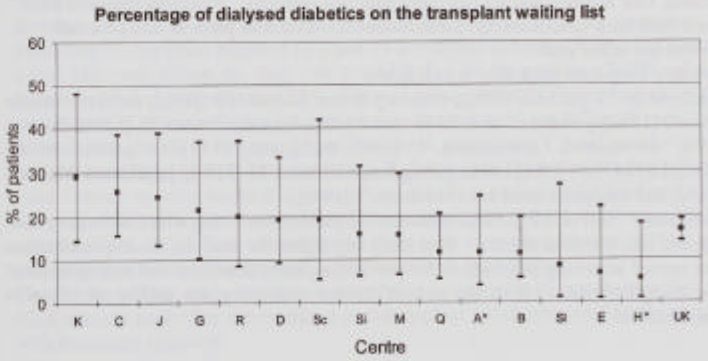
The UK Renal Registry has linked with UKTSSA to analyse waiting list data in areas covered by the Registry. Although the Renal Registry does not cover the whole UK population, within those areas covered the waiting list population has been related to the denominator of the dialysis population
The table below shows there was a marked increase between 1983 and 1998 in the incidence of diabetic patients who were transplanted. As a proportion of all transplanted patients this was 2.2, 6.4, 8.7 % in 1988, 1993, 1998 respectively.

Age group	1988	1993	1998
18-24	0	2	2
25-34	10	36	36
35-44	11	71	80
45-54	7	64	74
55-64	3	50	66
65-74	1	13	25
75+	0	0	0
Total	32	236	283

This may be a reflection of the increased percentage of diabetics entering the renal replacement therapy programme or it might additionally be a change in attitude to transplanting diabetic patients.



* indicates 2 centres with a probable inaccurate count of the dialysis population

The graph shows the variation by dialysis centre in the percentage of dialysing diabetics on the active transplant list. The lines on the graph indicate the 95% confidence intervals. These numbers are small and the 95% confidence intervals are wide. Only 18% of diabetics on dialysis are on the active waiting list compared with 28% of non-diabetics.

C40

MORBIDITY IN RECIPIENTS WITH RENAL ALLOGRAFTS FUNCTIONING FOR OVER TWENTY YEARS.

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Introduction: Long-term graft survival is associated with significant morbidity most of which is attributed to long term immunosuppression.
Method: The data base and patients notes were scrutinised to identify patients who were transplanted in the pre-cyclosporin era and who have a graft functioning for twenty years or more.
Result: Since March1968 over 2700 renal transplants have been carried out in this unit. In the period till December 1980 three hundred and forty four patients received three hundred and ninety one renal allografts.
Fifty-five patients are known to be alive with a functioning transplant, of these 49 patients (26 males), are currently available for review 20-30 years after transplantation with a mean follow up period of 22.9 years. This represents a one year survival of 54.5% and 20 year survival of 20.2%. The recipient mean age at time of transplantation was 29.8 years. There were 42 (86%) cadaveric and 7 (14%) live donor transplants. All patients are maintained on azathioprine (50 – 150 mg / day) and prednisolone (5-12.5 mg / day). The current mean serum creatinine is 146 umol/l (83 –334) with a mean GFR of 57 (14-112). Twenty-six patients had one episode of acute rejection, four had two and six had more than two episodes. Rejection episodes were treated with intra venous methyl prednisolone however four patients also received radiotherapy to the graft.
The major long-term morbidity is as follows:
Hypertension 39 patients (80%), coronary artery disease 10 (20%), cerebro-vascular disease 9 (18%), peripheral vascular disease 3 (6%), hyperlipidaemia 31 (63%), diabetes 4 (9%), osteoporosis / osteopaenia 15 (31%), malignancy 17 (35%), gastro intestinal disorders 23 (47%) , infections requiring hospitalisation 25 (51%), psychiatric disorders 8 (16%) and transplant renal artery stenosis 7 (14%).
Conclusion: It is no longer uncommon to see patients surviving with functioning renal allografts into the third decade. This study highlights the need for the implementation of an agreed screening programme for prevention, early detection and management of these complications. With the aim of further improving the quality of life after transplantation.

C61

AN ASSOCIATION BETWEEN POST-TRANSPLANT DONOR HLA SPECIFIC ANTIBODY PRODUCTION AND RENAL TRANSPLANT FAILURE

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Introduction: An association between post-transplant production of donor specific antibodies and the incidence of rejection has been demonstrated although some data have been difficult to interpret due to the poor definition of HLA specific antibodies. We have used enzyme-linked immunoabsorbent assays (ELISA) which enable increased sensitivity of detection and more accurate characterisation of HLA specific antibodies to re-evaluate their role in transplant outcome.
Method: 115 consecutive primary cadaveric renal transplants were carried out in a single centre during 1990. 17 patients without post-transplant serum samples were excluded from the study. Patients with primary function were given cyclosporin monotherapy and the mean number of HLA mismatches at the HLA-A, -B and –DR loci were 0.8, 0.97 and 0.38 respectively. Pre- and post-transplant (1month, 6 month, annual) sera were selected from each patient and screened using the LAT™M (One Lambda) ELISA based assay to detect the presence of HLA class I and class II specific antibodies. Definition of the HLA specificities was performed using the ELISA based kits QuikID (GTI Inc) and PRA-STAT (SangStat Medical Corp) and complement dependent cytotoxicity using a selected panel. The results were correlated with transplant outcome.
Results: 98 patients were donor HLA specific antibody negative pre-transplant. Post-transplant 85 remained negative (-/-) and 13 developed donor HLA specific antibodies (-/+). Ten year follow up data was available for all 98 patients: 18.8% of the -/- transplants failed as oppose to 92.3% of the -/+ group ($p<0.005$ Fisher Exact). Of the 13 -/+ patients, 7 developed class I donor specific antibodies (4 pre-failure, 3 post-failure), 5 developed class II donor specific antibodies (4 pre-failure, 1 functioning transplant) and the remaining patient developed class II donor specific antibodies pre-failure and class I donor specific antibodies post-failure. Of the 6 patients who produced class II donor specific antibodies, 2 were HLA-DR specific (DR9, DR53) and 4 were HLA-DQ specific (DQ1, DQ3). It is of interest that there is an equivalent number of HLA class I and class II donor specific antibodies associated with graft failure.
Conclusions: This study has demonstrated that even in a well matched patient group HLA specific antibodies are produced post-transplant which are significantly associated with transplant rejection.

C99

SEQUENTIAL PROTOCOL BIOPSIES FROM RENAL TRANSPLANT RECIPIENTS SHOW AN INCREASING EXPRESSION OF ACTIVE TGFβ

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Chronic allograft nephropathy (CAN) is a major cause of graft failure after renal transplantation. The underlying pathological mechanisms involve abnormal repair processes, with increased deposition of extracellular matrix. The cytokine transforming growth factor beta (TGFβ) is thought to play an important role and there have been reports that it is increased after transplantation. However not all studies have measured active TGFβ, and in some the biopsies taken were for acute events that may have had direct effects on TGFβ expression. This study set out to measure expression of active TGFβ in protocol renal transplant biopsies.

Biopsies were taken from forty renal allografts at the time of transplantation and then, using ultrasound guidance at 1 week and 6 months post-transplant. Sections were stained with an antibody to human active TGFβ and then analysed using semi-quantitative confocal fluorescence microscopy. Data were expressed as the ratio of the mean fluorescence of the experimentally stained tissues (excluding the tubule lumen) to the corresponding value from control sections.

There was very little variation in active TGFβ expression between patients in their pre-perfusion biopsies. Expression increased by 1 week and then very significantly by 6 months ($p<0.0001$, Wilcoxon). Patients who suffered delayed graft function had increased TGFβ expression at both time-points post-transplant. There was no difference according to donor type, acute rejection or immunosuppressive drug (cyclosporin or tacrolimus). There was no correlation between the amount of TGFβ expression at any time-point and isotope GFR at 12 months.

This study has demonstrated in a group of stable kidney transplant recipients that there is a definite increase in the renal expression of active TGFβ compared to pre-transplant levels. Because of the small numbers it has not been possible to ascertain if established risk factors for CAN affect this expression, and further patients are being studied to clarify this.

C72

ACCURACY OF CALCULATED GLOMERULAR FILTRATION RATE COMPARED TO ISOTOPE GLOMERULAR FILTRATION RATE IN PATIENTS WITH DETERIORATING GRAFT FUNCTION.

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Background: Radioisotope techniques provide the gold standard assessment of glomerular filtration rate (GFR) in both renal failure patients and kidney transplant recipients, but are expensive and time consuming. Most studies in kidney transplant recipients use either estimation of creatinine clearance (CCr) using timed urine collections or one of a number of formulae devised to predict GFR. To determine the accuracy of these measurements in patients with deteriorating kidney allograft function we compared estimated urinary creatinine clearance, three formulae, Cockcroft-Gault (CG), MDRD study equation (levy et al., Ann. Int. Med 1999) and Nankivell (Nankivell et al., Transplantation, 1995) to isotope GFR measurements.

Methods: GFR of 30 patients with rising creatinine and biopsy proven chronic allograft nephropathy were analysed. GFR was measured using [^{51}Cr] EDTA. Predicted GFR was calculated using the three prediction equations and 24 hours urinary CCr estimation. All measurements were corrected for body surface area. The accuracy of the prediction equations was assessed using the methods of Bland and Altman.

Results: 40 GFR examinations were analysed from patients aged 47.9±11.7 years (range 24-67), with BMI of 26.5±4.7. Mean serum creatinine was 222±70 mmol/l (median 205 mmol/l). Mean isotope GFR was 26.8±10.4 mls/min/1.73m^2. The mean calculated GFR using CG, MDRD and Nankivell equations were 38.8±11.5, 27.6±10.7 and 40.0±11.9 respectively. Mean urinary CCr was 42.0±19.6. The Bland-Altman analyses of results showed that CG, Nankivell and CCr methods overestimated GFR, while MDRD values closely approximated (mean differences from isotope GFR were 8.8, 10.5, 21.0 and 1.1 ml/min respectively).

Conclusions: In patients with deteriorating allograft function both the CG, Nankivell and CCr equations have substantial bias and overestimate GFR. The MDRD equation is less prone to bias.
All four calculations show significant variability, which limits their usefulness in the prediction of GFR.
The MDRD equation appears to be the most accurate of the three prediction equations in this group of patients. However, the limitations of the MDRD equation should be borne in mind and it is advisable not to use it at lower values of GFR.

CLINICAL

C21

THE COMPARISON OF KIDNEY TRANSPLANT SURVIVAL OUTCOMES BETWEEN CENTRES

Johnson RJ[1], Armstrong SA[1], Collett D[2], Belger MA[1], Briggs JD[3] on behalf of the UK Transplant Kidney and Pancreas Advisory Group, Bristol, UK
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Centre comparison is as important for renal transplantation as for other specialties, particularly in terms of the outcome achieved for patients. Direct comparison of unadjusted survival rates over the last 20 years has shown wide centre variation lessen with time. However, it is important that centre comparisons are based on risk-adjusted survival which allows for differences in the case-mix of transplanted patients at each centre. A number of statistical methods can be used to obtain risk-adjusted centre outcomes and several have been investigated for UK renal transplant centres.

This risk-adjusted analysis is based on first adult cadaveric kidney only grafts from heart beating donors for the years 1994 to 1996. All UK renal transplant centres are included. One-year transplant outcome was known for 3275 (98%) of these grafts, allowing a binary outcome (functioning/failed) to be modelled. A multifactorial mixed effects logistic model was developed for these data which adjusts for known risk factors: recipient age, waiting time and diabetes, donor age and cause of death, HLA matching, local or imported kidney and donor-recipient gender match. Estimated centre effects and associated confidence intervals are derived from the model. Further analysis provides a ranking of centres, each rank having associated confidence limits.

The results are encouraging: there is no evidence of a significant centre effect for this cohort of transplants. There is a high degree of overlap between the interval estimates for both the centre effects and the ranks of the centres.

This analysis is based on one-year outcome of first adult grafts. Work is now under way to extend the analysis of named centres to three-year outcome, regraft survival and short-term outcome of more recent grafts. However, lack of follow-up data from some centres precludes presentation of these further analyses at present.

C67

The challenge of cardiovascular disease after renal transplantation (RTx) – a planned multidisciplinary approach.

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Cardiovascular disease (CVD) is 3 to 5 times more common in RTx patients than in subjects without a history of renal disease. Obesity, hypertension and dyslipidaemia are all common after RTx. We wished to establish the effectiveness of implementing simple protocols to detect and intervene for the major CV risk factors, using a Novartis-funded health promotion nursing post.

In 100 consecutive renal and renal-pancreas Tx patients (under our long-term care 1998 – 2000) we used ambulatory BP monitoring (ABPM) to characterise outpatient BP, and performed regular lipid screening. We also counselled the patients about exercise, weight gain, diet, alcohol, and smoking. In our unit, we embark upon secondary CVD prevention in all RTx patients, and primary CVD prevention in all diabetics, and in all patients with a predicted 10-year risk of CVD (via Framingham algorithm) > 15%. Target total cholesterol for successful treatment is 5.0 mmol/l.

17% of patients were smoking before RTx, and 13% post-RTx, despite advice. Weight gain at 12 months was an average of 4.8kg with a shift in the percentage of patients in the healthy BMI range into the overweight range We found that in 20% of patients there was a discrepancy between clinic and ABPM results – 4% true "white coat hypertension" (ie over-diagnosis of hypertension) and 16% low clinic BP values (ie under-diagnosis of hypertension).

In 70 other RTx we used sustained (18 months) therapy with Atorvastatin for post-transplant dyslipidaemia. There were 46 males and 24 females; 12 were diabetics. Renal allograft function was stable.

Time after A	Cholesterol (M ± SD) (mmol/l ;	Triglyceride (M ± SD) mmol/l ; mg/dl)	P (Kruskal-Wallis) Cf to baseline
Baseline	7.02 (0.16)	2.46 (0.16)	
18 months	4.63 (0.11)	1.96 (0.13)	< 0.01

No patient had to discontinue Atorvastatin therapy because of side-effects. The dose of Atorvastatin required to achieve target cholesterol was 10 mg in 62 / 70; 20mg in 4 / 70; 30mg in 2 / 70 and 40mg in 2 / 70. Mean Cyclosporin A levels were slightly increased 6 months after commencing Atorvastatin (121.7 iu/l) compared with levels at baseline (108.4 iu/l) however this did not reach statistical significance (p=0.07).

Using ABPM to measure BP accurately, and Atorvastatin to treat dyslipidaemia, these two important CV risk factors can be tackled effectively and safely. Getting patients to achieve lifestyle changes though is much more challenging.

C94

UKTSSA RECURRENT DISEASE REGISTRY: THE FIRST 2 YEARS

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The UKTSSA recurrent disease registry was set up in 1999 with the aims of determining the incidence of the various types of recurrent disease in UK renal transplant recipients and defining their impact on graft function and survival. Here we report the findings from the first 2 years of the registry. Two sources are used for identification of cases. A question is included in the UKTSSA annual follow-up form and pathologists have been asked to report cases directly to UK Transplant. All biopsies are reviewed independently by two pathologists. From January 1999 to December 2000, there have been 74 notifications. However, not all renal transplant units are as yet reporting cases so this figure does not represent the UK incidence for these two years. Full pathological review has been performed for 51 to date, and the following data is based on these cases.

Only 55% of transplant centres have provided notifications. There is considerable variation in the apparent incidence of recurrent disease between the units that have reported cases; the incidence varies from 1.5 to 14.3% of transplants performed. Following review, 8 submitted cases were thought not to be recurrent disease, reducing the highest incidence in a single unit to 10%. Most of the rejected diagnoses were of focal segmental glomerulosclerosis (FSGS), where the reviewing pathologists were reluctant to accept small sclerosed segments as evidence of this condition without heavy proteinuria or FSGS in a biopsy of the native kidney. The commonest diagnosis is IgA nephropathy (IgAN) that accounts for 39.5% of recurrent disease, followed by FSGS (11.6%), membranous nephropathy (MN; 11.6%) and vasculitis/ pauci-immune glomerulonephritis (9.3%). All cases of recurrent FSGS presented in the first 18 months post-transplantation, whilst MN and IgAN were diagnosed later (see table). Recurrent disease resulted in loss of the transplant kidney within the first month in four patients, due to anti-phospholipid syndrome (2), haemolytic uraemic syndrome and anti-GBM disease (primary disease Alport's syndrome).

Diagnosis	Months from transplantation to biopsy: median (range)
IgA nephropathy	60 (11 – 118)
FSGS	4 (1 – 18)
Membranous nephropathy	33 (27 – 92)
Vasculitis/pauci-immune GN	42 (2 – 96)

Initial findings suggest that recurrent disease may affect 5-10% of grafts. The apparent variation between units is likely to reflect differences in diagnostic stringency and failure of notification rather than true variation in disease incidence. Some conditions cause early graft loss but further follow-up is required to determine the impact of the commonest recurrent diseases on graft outcome. We hope that this preliminary report will stimulate interest in the Register and lead over the next few years to more complete notification of cases of recurrent disease. Should this happen it will be possible to build up a comprehensive database of recurrent disease in the UK.

L3

DIFFERENTIATED FUNCTIONS OF HUMAN MONOCYTIC U937 CELLS FOLLOWING CD31 LIGATION.

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Introduction: Chronic allograft rejection involves recruitment and differentiation of blood monocytes into tissue macrophages. Monocyte recruitment involves adhesion to endothelial CD31 (PECAM-1) at the vascular endothelial junction. Reciprocal adhesion of the *monocytic* CD31 may play a role in maturation and viability of monocytes at an early stage of alloactivation.

Aim and Methods: The human monocytic cell line, U937, is $CD31^{+}$ve and $Fc\gamma R^{+}$ve. We have asked if ligation of CD31 induces modulation of U937 function by measuring **(i)** TNFα secretory responses and **(ii)** apoptosis. These two parameters were also used to look for specific effects on monocyte function mediated by the protein phosphatase calcineurin, by protein or lipid kinases, or by G-protein-coupled receptor signalling.

WM59, an anti-CD31 monoclonal antibody (mab), was immobilised onto plastic for cross-ligation of cell surface CD31: corresponding controls used an immobilised, isotype-matched murine IgG1, MOPC-21. Phorbol myristate acetate (PMA) was used to induce monocyte differentiation and secretion of TNFα. Apoptotic indices were calculated from FACS analyses of annexin V and propidium iodide stained cell populations.

Results:

1. TNFα secretion in response to PMA was not influenced by anti-CD31, nor by the MOPC-21 control mab.

2. During serum starvation, crosslinking of CD31 protected against apoptosis: similar protection was found in control monocyte cultures treated with MOPC-21.

3. Neither anti-CD31, nor MOPC-21, gave protection against "differentiation-induced apoptosis" associated with PMA treatment.

4. Of six specific inhibitors tested at therapeutic doses, only the phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002, altered U937 cell responses to PMA, manifest by increased apoptosis and decreased TNFα secretion.

Discussion: Immobilised IgG1 will cross ligate FcγR on U937 cells. Since both the anti-CD31 and control IgG1 mabs protected against apoptosis, we have no evidence that CD31 ligation confers additional benefit over FcγR ligation. Differentiation-induced apoptosis was insensitive to either mab treatment, indicating involvement of different apoptotic pathways, and/or increased strength of pro-apoptotic signalling. LY294002 is an inhibitor of both PI3K and DNA-PKcs, the catalytic subunit of DNA-protein kinase which functions in repair of DNA double strand breaks. One, or both, of these enzymes appears to be required for monocyte differentiation and survival.

L7

THE EFFECT OF DIFFERING IMMUNOSUPPRESSIVE REGIMES ON mRNA CYTOKINE EXPRESSION IN A RAT RENAL ALLOGRAFT MODEL OF CHRONIC REJECTION.

Miss Donna Green, Mr JS McGrath, Mr K Graetz and Mr M Shehata The Nottingham Transplant Unit, Nottingham City Hospital, Nottingham, U.K.

Background: The rate of graft attrition after the first year post-transplantation remains unchanged despite impressive advances in 1-year graft survival rates. Chronic rejection represents the leading cause of late graft loss and there are currently no effective measures for its prevention or treatment. We have previously demonstrated differing functional and morphological changes in a rat renal allograft model treated with differing immunosuppressive regimes. The current study explored the cytokine responses in each of the treatment group utilising semi-quantitative reverse transcriptase-PCR.

Methods: Orthotopic renal transplantation was performed from F344 donors into unilaterally nephrectomised LEW recipients (n=48) in addition to isograft controls (n=8). Transplanted kidneys were retrieved at 2 and 4 months and stored in liquid nitrogen. Experimental groups included monotherapy (cyclosporin (CyA) or tacrolimus (FK)), dual therapy (mycophenolate (MMF) and cyclosporin, mycophenolate and tacrolimus, cyclosporin and SDZ-RAD) and untreated. mRNA was extracted from each sample and expression of mRNA for IL-1, TGF-β, PDGF, IFN-γ, TNF-α and bFGF was studied using RT-PCR technique. Semi-quantitative analysis of products was performed using gel electrophoresis and computer-aided band analysis.

Results:

With the exception of CyA treated rats, there was a late rise in IL-1 expression in all allograft groups. TGF-β expression was elevated in all allografts and isografts with most marked increase in untreated allografts and least marked in CyA-treated allografts. Differences in PDGF expression were subtle between the groups, with allograft expression being very similar to that of isografts. IFN-γ expression was markedly elevated in untreated and FK-treated allografts. TNF-α expression was increased in FK and FK+MMF-treated allografts but not in other MMF treated allografts. Untreated and CyA+RAD-treated allografts had marked increase in expression of bFGF.

Conclusions:

In the rat renal allograft model of chronic rejection described:

1. Treatment with differing immunosuppressant regimes is associated with characteristic differences in cytokine mRNA expression.
2. Furthermore, these differences are associated with variations in graft histology between treatment groups.
3. The cytokine system forms part of a complex network and it is therefore difficult to link association with direct causality in this study in relation to graft histology and cytokine mRNA expression.

LABORATORY

L8

CYTOKINE mRNA EXPRESSION IN THE RAT RENAL ALLOGRAFT MODEL OF CHRONIC REJECTION: THE EFFECT OF CYCLOSPORIN WITHDRAWAL AND CONVERSION TO ANOTHER IMMUNOSUPPRESSIVE AGENT.

Miss Donna Green, Mr JS McGrath, Mr K Graetz and Mr M Shehata The Nottingham Transplant Unit, Nottingham City Hospital, Nottingham, U.K.

Background: The rate of graft attrition after the first year post-transplantation remains unchanged despite impressive advances in 1-year graft survival rates. Chronic rejection represents the leading cause of late graft loss and there are currently no effective measures for its prevention or treatment. Cyclosporin has been implicated in the onset and progression of the disease and there has been considerable interest in cyclosporin withdrawal regimes. We have previously demonstrated differing functional and morphological changes in a rat renal allograft model treated initially with cyclosporin (CyA) prior to CyA withdrawal and conversion to one of the newer immunosuppressive agents (mycophenolate (MMF) / tacrolimus (FK) / SDZ-RAD). The current study explored differences in cytokine expression in each of the treatment group utilising semi-quantitative reverse transcriptase-PCR.

Methods: Orthotopic renal transplantation was performed from F344 donors into unilaterally nephrectomised LEW recipients (n=32) in addition to isograft controls (n=8). Transplanted kidneys were retrieved at 2 and 4 months and stored in liquid nitrogen. All allotransplanted animals received CyA for the first two months after transplantation followed by two months of either CyA, MMF, FK or SDZ-RAD. mRNA was extracted from each graft and expression of mRNA for IL-1, TGF-β, PDGF, IFN-γ, TNF-α and bFGF was studied using RT-PCR technique. Semi-quantitative analysis of products was performed using gel electrophoresis and computer-aided band analysis.

Results:

Withdrawal of cyclosporin at 2 months led to increased expression of IL-1, TGF-β and IFN-γ in all allografts. The expression of PDGF showed only minor variations between the groups. bFGF expression was reliably elevated in animals converted to FK with modest increases in the MMF and RAD groups.

Conclusions:

In the rat renal allograft model of chronic rejection described:

1. Differences in mRNA cytokine expression can be detected on withdrawal of cyclosporin and conversion to another agent.
2. Such differences were not detected in animals maintained on CyA.
3. Furthermore, variations in graft histology were seen between the treatment groups.
4. The cytokine network is a complex system and the association between graft histology and cytokine expression does not necessarily imply causal effect.

LABORATORY

L9

ANTI-CD4 TREATMENT PREVENTS AUTOIMMUNE DESTRUCTION OF TRANSPLANTED PANCREATIC ISLETS IN THE NOD MOUSE

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Islet transplantation is a therapeutic strategy for the treatment of insulin-dependent diabetes mellitus (IDDM) that aims to restore good metabolic control and to prevent diabetic complications. Two processes may result in immune-mediated islet damage: allogeneic rejection and ongoing autoimmune destruction. The success of islet transplantation is dependent upon suppression of both responses, either independently or together.

The non-obese diabetic (NOD) mouse is a model of IDDM in which diabetes develops spontaneously in female mice, or may be induced in irradiated male mice by transfer of syngeneic, diabetic splenocytes. Development of the disease is accompanied by a peri- and intra-islet cellular infiltrate comprising CD4+ and CD8+ T cells, dendritic cells and macrophages. Experimental IDDM may be prevented, but not reversed, by treatment with a non-depleting anti-CD4 monoclonal antibody (YTS 177).

Anti-CD4 treatment is effective at inducing tolerance in some models of allogeneic tissue transplantation. We have investigated its efficacy, in conjunction with syngeneic islet transplantation, as a therapy for established diabetic disease both in the spontaneous female NOD mouse model and in the male transfer model.

Islets were isolated from non-diabetic donors using collagenase digestion together with individual islet selection. They were transplanted, by laparotomy with general anaesthesia, under the left renal capsule of syngeneic recipient mice that had become diabetic 1-2 weeks previously. Approximately 800-1000 islets, from four donors, were sufficient to achieve transient normoglycaemia for 2-10 days before the transplanted islets succumbed to autoimmune destruction. Recurrence of disease was accompanied by infiltration with T cells and macrophages. When islets were transplanted together with a short course of anti-CD4 treatment, normoglycaemia was achieved in 60% of diabetic mice, and they remained normoglycaemic for at least 6 weeks. Histological examination revealed good preservation of transplanted islets with minimal cellular infiltration. A short course of anti-CD4 antibody alone, at 1-2 weeks after development of diabetes, had no influence on the disease in the absence of transplanted normal islets.

This study suggests that immunosuppressive therapy based on manipulation of CD4 T cells is a successful approach for preventing autoimmune destruction of transplanted islets and it may reduce the requirement for adjunctive treatment of allograft rejection.

L15

NATIVE AND ALTERNATIVELY SPLICED VARIANTS OF IL-2 mRNA IN TWO-WAY MIXED LYMPHOCYTE REACTIONS.

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It is now widely accepted that the alloantigen-induced interactions between the donor's immune cells transferred by transplanted organ and the recipient's immunocompetent cells determine the outcome of organ transplantation. 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). In spite of immunosuppression, IL-2δ2 mRNA and IL-2δ3 mRNA were still observed 3, 6 and 12 months post-transplantation. Both scheduled and unscheduled biopsy samples rarely express the native IL-2L form of IL-2mRNA. The role of alternative splicing of IL-2 mRNA in alloreactivity and transplantation is largely unexplored. It is unclear how the interaction between pre-existing kidney donor T-cells and recipient's T-cells determines the kinetics, level and longevity of expression of both IL-2L and IL-2δ2 and IL-2δ3 mRNA.

The aim of this study was to mimic the alloantigen driven donor/recipient lymphoid cell interaction in vitro and follow the kinetics and type of IL-2 mRNA splicing. For the two-way mixed lymphocyte reaction (MLR) the non-irradiated peripheral blood mononuclear cells (PBMC) from two donors were mixed. Equal aliquots (2x10e7 cells) were incubated with or without 20U/ml of recombinant IL-2 for one, two, three, six or seven days. To analyse the effect of Cyclosporine A (CsA) on differential splicing of IL-2 mRNA, 200ng/ml of CsA was kept in cultures for two days. Cells were harvested on day 2 or washed and cultured without CsA for another day (day 3) or four more days (day 6). SYBR Green® I based real-time quantitative RT-PCR assays were developed to measure IL-2L mRNA, IL-2δ2 and IL-2δ3 mRNA. The IL-2L mRNA was expressed on day 1, peaked on day 3 (1.8 x10e4 copies/25ng of reverse transcribed RNA) and showed 2-5 fold decrease on day 6 and 7. Both CD4+ and CD8+ T-cells, purified by negative selection using *MACS* beads (Miltenyi Biotec Ltd), expressed IL-2L, IL-2δ2 and IL-2δ3 mRNA. The ratio IL-2δ2/IL-2L or IL-2δ3/IL-2L was 1000:1 and spliced variants peaked on day 3. In IL-2 treated cultures the observed peaks for IL-2, IL-2δ2 and IL-2δ3 mRNA were shifted to day 2 and were 5-10 fold higher than in normal MLR. CsA induced a hundred-fold reduction of the IL-2L mRNA response on day 3. The IL-2δ2 and IL-2δ3 mRNA were not detected. During the observed period, the levels of spliced variants of IL-2mRNA in MLR never reached levels seen in kidney biopsies, suggesting that maybe the kidney milieu and not the alloantigen stimulation controls the alternative splicing of the IL-2 pre-mRNA.

L26

EFFECT OF BILE SALT SUPPLEMENTATION ON BILE PRODUCTION DURING EXTRACORPOREAL LIVER PERFUSION

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Introduction: On an extracorporeal liver circuit currently being tested for normothermic organ preservation, we discovered cholestasis and inspissation occurs after 10 hours of perfusion in a previous set of experiments. We hypothesized that this was secondary to bile salt depletion, and that by augmenting the circuit with bile salts, thereby replicating enterohepatic circulation, we could maintain bile flow over a 48 - hour period.

Methods: In the porcine model, livers were perfused with oxygenated, autologous blood at 38C for 48 hours by centrifugal pump to the hepatic artery, and gravity drainage to the portal vein. Plasma and bile samples from the previous experiments were tested for deconjugated bile acids using Gas Chromatography Mass Spectroscopy as described by P.T.Clayton et al. Bile production throughout the perfusion period was recorded and histology assessed. The circuit was then supplemented with bile salt in the form of a taurocholate infusion (2g/100ml at 5ml/min) and results assessed in the same manner.

Results: Data from five unsupplemented liver perfusions confirmed our hypothesis with a clear decline in all 3 major porcine bile acids (chenodeoxycholic, hyocholic and hyodeoxycholic acid) coinciding with a decline in bile production at a mean of 10 hours. This effect reversed by supplementing when taurocholate was added continuously to the perfusate. Bile production continued throughout the 48 hour of the perfusion maintaining physiologic serum and biliary bile acid profiles.

Conclusions: Bile production throughout a prolonged perfusion periods can be maintained by replicating enterohepatic circulation. Inspissation and subsequent damage to the canaliculus is prevented. This allows for the potential use of an extracorporeal circuit to preserve donor livers and assess their viability prior to transplantation.

L32

HYPEROXIA POST-TRANSPLANTATION IMPROVES THE SURVIVAL OF INTRAPORTALLY TRANSPLANTED ISLET GRAFTS IN DIABETIC RATS.

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Hypoxia in the portal vein may compromise the survival of intraportally transplanted pancreatic islets. We therefore compared the outcome of intraportal islet transplantation in rats housed under hyperoxic or normoxic conditions post transplantation. 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 (33.2±1.4 vs 9.5 mmol/l in controls). After 7 days 500, 700 or1000 islets were transplanted into the liver via the portal vein and the animals housed for 48 hours under hyperoxic (100% O_2) or normoxic (21% O_2) conditions.

In normoxic diabetic rats, the smallest graft size to consistently restore normoglycemia measured at 6 weeks after transplantation was 1000 islets (non-fasting plasma glucose, 9.8±0.8 mmol/l). In contrast in hyperoxically housed rats, a graft size of 700 islets restored normoglycemia in 8/9 animals compared to only 3/9 in normoxic animals (plasma glucose, 10.5±2.6 vs 24.4±4.1 in normoxic rats, $p<0.05$). Even a graft of 500 islets restored normoglycemia in 5/8 hyperoxically housed animals compared to 0/7 in normoxically housed rats. The glucose tolerance of the hyperoxically treated rats receiving 700 islets was similar to that of normoxic animals receiving 1000 islets; the AUCs were 1129±105 and 1263±69 mmol/120 min in hyperoxic and normoxic animals respectively, compared with 650±50 mmol/120 min in non-diabetic controls. The islet-cell mass was quantified morphometrically in liver sections collected post-mortem after 6 weeks. The total islet area in hyperoxically treated rats receiving 700 islets was not significantly different from normoxic recipients of 1000 islets and was 534, 510±62,110 μ^2 (compared to 660, 237±99, 320 μ^2 in normoxic animals). The average size of the islets was the same. These results indicate that hyperoxia post-transplantation increases the number of islets that survive the engraftment process and allows normalisation of plasma glucose levels with a smaller number of transplanted islets.

LABORATORY

L37

HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) IS UP-REGULATED DURING ISCHAEMIA/REPERFUSION INJURY IN RAT CARDIAC ISOGRAFTS

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Background: During procurement, preservation and reperfusion, organ transplants are subjected to a repetitive series of low oxygen tension. Hypoxia stabilises the heterodimeric transcription factor HIF-1 which, after translocation to the nucleus, mediates transcription of target genes involved in a variety of adaptive responses to low oxygen. HIF-1 regulates hypoxia induced genes that are also critically involved in inflammatory reponses in organ grafts (e.g. VEGF, ET-1, iNOS, PDGF, FGF). We have shown elsewhere that HIF-1α is expressed under normothermic hypoxia but not hypothermic hypoxia in a variety of cell types. We are investigating the effects of hypoxia and kinetics of HIF-1α expression in the setting of an isogeneic transplant model.

Methods: Adult Lewis rats were used for heterotopic cardiac transplantation. For constant experimental conditions second ("warm") ischemia time was intentionally kept at 1 h with topical cooling of the graft. In group 1 (n = 5), hearts were harvested and specimens immediately fixed in formalin and snap frozen in liquid nitrogen (native hearts); in group 2, hearts were transplanted without cold ischemia (n = 5); in group 3 (n = 5), cardiac grafts were subjected to prolonged cold ischemia (10 hrs) only; in group 4 (n = 10), grafts were subjected to prolonged ischemia (10 hrs) and subsequently transplanted. Graft function was assessed upon laparotomy after 24 hrs and scored according to a standardised protocol (0 to 4). Specimens were analysed by histology (H&E) and immunohistochemistry (HIF-1α).

Results: Compared to grafts with prolonged ischaemia, function of immediately transplanted hearts was significantly better (3.7 ± 0.3 vs 2.5 ± 1.2, $P < 0.05$). Native hearts showed HIF-1α staining in the nuclei of cardiomyocytes as well as endothelial staining of intramyocardial arteries. In hearts preserved for 10 hrs there was swelling of cardiomyocytes, but no increase of staining for HIF-1α was detected. Hearts transplanted without cold preservation showed mild infiltration of inflammatory cells consisting of predominantly neutrophils in conjunction with staining for HIF-1α in endothelial cells and cardiomyocytes. In contrast, grafts stored for 10 hrs and then transplanted, showed extensive myocardial necrosis with no HIF-1α staining. However, there was HIF-1α immunostaining in the area of infiltrating inflammatory cells.

Conclusion: This is the first report that HIF-1 is present during the early events of graft injury in an isogeneic transplant setting. We observed in cardiac grafts that after prolonged cold ischaemia there is a loss of HIF-1 protein expression in cardiomyocytes and HIF-1α staining associated with infiltrating inflammatory cells. This altered distribution of HIF-1 protein expression suggests that HIF-1 may function as a potent regulating factor in the process of ischaemia reperfusion injury.

L39

THE EFFECTS OF RAPAMYCIN AFTER CYCLOSPORIN DOSE REDUCTION ON PROFIBROTIC GENE EXPRESSION IN BOTH GLOMERULI AND INTERSTITIUM FROM RENAL TRANSPLANT RECIPIENTS WITH CHRONIC ALLOGRAFT NEPHROPATHY.

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Introduction: Overexposure to Cyclosporin (CyA) is a risk factor for chronic allograft nephropathy and thus CyA dose reduction has been advocated. Experimental evidence suggests that Rapamycin (Rapa) may retard the development and progression of CAN. The aim of this study was to determine the molecular impact of the addition of Rapa after CyA dose reduction in both the glomerular and interstitial compartments of renal allografts with CAN.

Methods: Thirty-one renal transplant recipients with CAN were prospectively randomised to receive either a 40% dose reduction in CyA (control, n=15), or a 40% dose reduction in CyA with the addition of Rapa 2mg/day (n=16). Patients had a renal allograft biopsy on recruitment and again 6 months later. Glomeruli were plucked from the surface of each biopsy core and these as well as a small sample of interstitium underwent total mRNA extraction using oligo DT dynabeads. Complementary DNA was synthesized by reverse transcription and the polymerase chain reaction was used to amplify specific mRNA species. These were quantified using an ELISA technique and levels compared to the expression of the housekeeping gene GAPDH.

Results: Both groups were well matched in terms of patient characteristics and mean (±sd) CyA trough levels after dose reduction (Rapa 68±21 vs Control 56±19 ng/ml). Mean Rapa trough levels were 7.1 (3.9) ng/ml. Profibrotic gene expression is shown below.

Gene	Control n=15		Rapa n=16	
	Pre	Post	Pre	Post
Glomerular Timp-1	0.72 (0.23)	1.44 (0.60)**	0.48 (0.29)	1.67 (1.05)**
Interstitial Timp-1	0.55 (0.37)	0.68 (0.39)	0.47 (0.38)	0.66 (0.43)
Glomerular Timp-2	0.37 (0.33)	0.41 (0.2)	0.30 (0.13)	0.55 (0.33)*
Interstitial Timp-2	0.30 (0.17)	0.27 (0.26)	0.18 (0.21)	0.21 (0.19)
Glomerular TGFβ	0.48 (0.33)	0.25 (0.14)*	0.32 (0.13)	0.32 (0.18)
Interstitial TGFβ	0.13 (0.12)	0.22 (0.11)	0.10 (0.12)	0.16 (0.12)
Glomerular Collagen III	0.64 (0.33)	0.78 (0.20)	0.52 (0.16)	0.95 (0.43)*
Interstitial Collagen III	0.65 (0.24)	0.83 (0.38)	0.56 (0.20)	1.21 (0.98)*
Glomerular MMP-2	0.03 (0.04)	0.12 (0.14)	0.02 (0.02)	0.11 (0.09)*
Interstitial MMP-2	0.02 (0.04)	0.08 (0.07)	0.02 (0.04)	0.09 (0.11)

Values expressed as mean (sd) in arbitrary units. *p<0.05, **p<0.01, student's t-test vs pre-trial value.

Conclusion: Glomerular but not interstitial TGFβ expression was decreased following CyA dose reduction. Nevertheless elevations in both glomerular TIMP-1 and MMP-2 suggest a persistent increase in the turnover of extracellular matrix here. Neither glomerular nor interstitial TGFβ expression fell significantly after the addition of Rapa. However this increased glomerular Timp-1, Timp-2, MMP-2 and collagen III as well as interstitial collagen III mRNA expression suggesting that the addition of Rapa in this setting may result in an accumulation of extracellular matrix.

L41

A NOVEL METHOD FOR FUNCTIONAL ASSESSMENT OF THE NON-HEART-BEATING DONOR LUNG OFFERS IMPROVED PRESERVATION

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Aims. Lungs from non-heart-beating donors (NHBD) are a promising source of extra organs for transplantation. However, poor post-transplant lung function is a potentially fatal consequence in up to 20% of current heart-beating donor lung transplants. Reference to non-heart-beating renal transplantation suggests a very high rate of warm ischaemic injury causing delayed or primary non-function, which would be fatal in the pulmonary setting. We therefore aimed to develop an isolated ventilation and perfusion technique to assess the ante-mortem function and degree of warm ischaemic organ injury suffered by non-heart-beating donor lungs prior to retrieval.

Methods. Landrace cross Yorkshire White pigs of approximate weight 50kg were anaesthetised throughout and euthanased without regaining consciousness. Experimental donor lungs were retrieved 1 hour (**NHBD1**) (n=6) or 2 hours (**NHBD2**) (n=6) after hypoxic death without Eurocollins perfusion. In-situ **left** lungs were ventilated with 100% oxygen and perfused for 20 minutes with deoxygenated and neutrophil depleted blood. The **left** lungs were subsequently transplanted, total ischaemic times 8.1h (NHBD1) and 9.1h (NHBD2). As with our current clinical and previous experimental practice, **Control** organs (n=6) were retrieved using standard techniques after cardiac arrest by aortic cross-clamping and cold modified Eurocollins solution perfusion of the pulmonary artery. The control **left** lungs were transplanted with a cold ischaemic time of 7.0h; control **right** lungs were assessed by in-situ ventilation and perfusion as above.

Results: Assessment. After 15 minutes' assessment, pulmonary vascular resistances were significantly lower in both NHBD1 and NHBD2 than controls; mean 8 (s.d. 4) Wood units *v* 11 (4) *v* 35 (17) respectively, p=0.006 (t-test). Oxygenation was acceptable in all groups, but after two hours' ischaemia was significantly lower; control 60 (3) kPa, NHBD1 58 (8), NHBD2 47 (5) p=0.0002 *v* control, p=0.02 *v* NHBD1 (t test).

Transplantation. Over the 12-hour follow-up post transplantation, both NHBD1 and NHBD2 blood perfused lungs showed significantly better vascular function than controls, NHBD1 PVR mean 23 (11), NHBD2 32 (11), control 58 (23) Wood units respectively, p=0.0001 (ANOVA). Differences in mean oxygenation between groups were not statistically significant; NHBD1 52 (3) kPa *v* NHBD2 58 (7) *v* control 58 (7), p=0.08 (ANOVA) and all levels were consistent with good oxygenating function.

Conclusions. The assessment technique allows functional assessment and subsequent transplantation with improved function of the NHBD lung over control retrieval with Eurocollins perfusion. Function remains acceptable with a warm ischaemic period of two hours. It also offers the potential to detect poor pre-existing lung function or occult warm ischaemic damage.

L46

IMPROVED RENAL PROTECTION AGAINST WARM ISCHAEMIA WITH NEWLY DEVELOPED PRESERVATION SOLUTIONS

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This experiment evaluates the protective role of two newly developed preservation solutions in the prevention of warm ischaemic and reperfusion injury in a rat kidney model. The new solutions were based upon PBS140, an accepted renal preservation solution. Glucose, potassium and various anti-oxidant agents were added to both solutions. Solution II also had lactobionate as an additional impermeant. Results were compared with PBS140 and UW.

Male Wistar rats (280-380g) were anaesthetized by intraperitoneal injection of inactin (120 $mg.kg^{-1}$). An Intravenous infusion was set up at $6ml.hr^{-1}$ (NaCl, 125$mmol.l^{-1}$ and HCO_3, 25 $mmol.l^{-1}$) 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 one of the solutions (PBS140, UW, solutions I and II) kept at room temperature (23°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 and composition from the left kidney were observed for 4 hours. The results are summarized below for one-hour post-ischaemia compared to pre-ischaemic control.

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	2.26 ± 0.36	205 ± 25	1024 ± 98
	1 hr	18.05 ± 6.23	144 ± 24	426 ± 35
UW	Pre-ischaemia	1.67 ± 0.13	180 ± 26	1094 ± 127
	1 hr	26.91 ± 7.68	104 ± 25	365 ± 16
Sol I	Pre-ischaemia	2.52 ± 0.7	190 ± 14	902 ± 156
	1 hr	22.11 ± 3.7	*196 ± 51	*480 ± 53
Sol II	Pre-ischaemia	3.11 ± 0.6	221 ± 25	942 ± 130
	1 hr	35.19 ± 8.4	*200 ± 47	409 ± 20

Significant improvement in inulin clearance and urine osmolality was observed with the new solutions ($p<0.05$). Whilst, a significant drop of inulin clearance noted in the 1st hour in PBS140 and UW groups, this was within 10% of pr-ischaemic control in the new solutions (103% and 91% respectively). The new solutions provided improved protection of renal function against severe warm ischaemia in our model

L51

A NEW AND IMPROVED SOLUTION FOR LIVER PERFUSION AND PRESERVATION.

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OBJECTIVES: Further developments in perfusion and preservation solutions are required to improve organ quality and extend functional lifetime. We have compared PBSL, a newly developed phosphate buffered sucrose based solution with five other preservation and perfusion solutions used in clinical practice using our isolated perfused rat liver model.

MATERIALS & METHODS: All the experiments were carried out under a Home Office license and according to normal guidelines of animal care. Rats were anaesthetized, the bile duct cannulated, and liver flushed with cold either PBSL (Phosphate Buffered Sucrose for Liver), UW (University of Wisconsin), Celsior, HTK (Histidine Tryptophan Ketoglutarte), or HL (Histidine Lactobionate) solutions via the aorta and portal vein. After flush the liver was removed and stored in 60 ml of preservation solution at less than 4°C for 24 hours. The liver was re-perfused at 37°C at a rate of 15 ml/min and the following observations of the liver function were made.

RESULTS:

	PBSL (n=18)	*UW (n=12)*	*Celsior (n=6)*	*HTK (n=5)*	*HL (n=6)*
Bile flow (µl/min/g)	87.21±3.32	62.4±3.57*	68.62±2.72*	69.1±2.84*	71.22±1.4*
O_2 Consumption (µmoles/min/g)	0.67±0.03	0.71±0.04	0.54±0/05	0.48±0.041	0.48±0.96
LDH release (U/L)	411±22.38	612±10.24*	618±25.56*	668±48.19*	566±22.27*
ALT release (U/L)	42.88±4.1	62.11±6.6*	86.84±3.9*	78.89±6.4*	56.62±4.8*
AST release (U/L)	39.82±4.3	68.22±7.9*	88.53±9.15*	75.54±6.4*	44.49±6.6
Portal pressure (kPa)	3.2±0.07	3.1±0.05	3.25±1.1	3.1±0.05	3.2±0.8
Weight change after storage (%)	-2.94±0.41	-3.55±0.47	1.8±0.74*	1.37±0.56*	-1.98±0.81
Weight change after reperfusion (%)	-0.09±1.43	4.49±0.42*	3.91±1.59*	2.26±0.92*	3.4±1.39

Mean±SEM *$p<0.05$ (one way analysis of variance)

CONCLUSION: PBSL provided better preservation than the tested solutions in the isolated perfused rat liver model. This solution would be expected to provide better quality preservation in clinical use.

LABORATORY

L54

TGF-β1 codon 25 GC polymorphism is linked to rate of development of chronic vascular rejection in renal transplant recipients.

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Chronic rejection (CR) of renal allografts remains and intractable problem and there are no predictive genetic markers of patients at high risk. A number of immunological and physiological factors including episodes of acute rejection, CMV infection, hypertension, delayed graft function and hyperlipidaemia may influence CR by modulating TGF-β1.

We have shown in a prospective study that plasma TGF-β1 levels in the first year post engraftment are linked to subsequent chronic vascular rejection (CVR) at 2 years (JASN 10:1999; 768A).

The aim of this retrospective study was to investigate the association of TGF-β1 genotypes for codon 10 and codon 25 with rate of development of CVR. Patients transplanted between 1994-1999 who developed biopsy proven CVR (41) and a control transplant group of 71 patients transplanted in the same period but without clinical signs of chronic rejection were studied. TGF-β1 genotypes were determined by PCR RFLP.

Recipients who develop CVR by 2 years have a significantly higher frequency of the codon 10 C allele (54.8% v 35.7%, p=0.01 Fisher's exact test) and a significantly higher frequency of the codon 25 C allele (14.5% v 4.3%, p=0.02). In patients who are diagnosed with CVR, there is a significant difference in the median time to diagnosis of CVR according to TGF-β1 genotype codon 25 (GC) versus (GG) 30 versus 61 weeks respectively, log rank test p=0.029. This effect is evident within the first year post transplantation and is not apparent with codon 10 genotype (p=0.289).

Both TGF-β1 codon 10 and codon 25 genotypes are associated with the incidence of CVR. Interestingly, the codon 25 GC genotype is also significantly associated with a more rapid rate of development of CVR in the renal transplant population which suggests an important influence of TGF-β1 on the underlying pathophysiological mechanisms of fibrosis.

L55

Improving the quality of kidneys from NHBD, using Streptokinase: an animal model

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Introduction

Warm ischaemia is a crucial factor in the causation of tissue damage in NHBD kidneys. In machine-perfused (MP) NHBD kidneys, a significant proportion of kidneys are rejected at viability assessment because of poor-perfusion or enzyme characteristics (33.9 % of 56 MP kidneys). It is believed that intravascular thrombosis occurs after cardiac arrests at a variable rate and this feature causes poor flow characteristics on testing the kidney. We looked at the use of Streptokinase in a porcine model of NHBD to evaluate the effect on MP characteristics and kidney tissue preservation. The porcine study involved two groups of pigs, both of which had cardiac arrest induced, one received heparin with perfusion & the other heparin with Streptokinase.

Materials & methods

Thirteen female Landrace Yorkshire-white pigs of 3-4 months age were entered into the study. At donor pig procurement, the carotid artery & internal jugular vein were cannulated. Following approx. 70 minutes warm ischaemia time (WIT), intravascular flush was carried out, by the perfusion of 4 litres Marshall's solution (4°C temp) with heparin (1000 IU/L) ± Streptokinase (1.5 Million units/L) via the carotid artery. Blood was vented through the internal jugular vein. After retrieval, the renal artery of both kidneys were cannulated & placed in the locally developed MP system. The kidneys were machine-perfused for 4 hours. Hourly recordings (pressure, flow, temperature) were made & specimens of kidney effluent were taken for GST estimation. Flow rates in MP was fixed for both sets of animals & the affect on pressure were noted.

Results

	Streptokinase	Non-Strep	Mann Whitney U
Flow (mls/min/100g) - T4	53.0 ± 3.6	52.8 ± 6.7	p = 0.598
Wt increase (%)	43.7 ± 5.3	70.9 ± 7.7	p = 0.017
Mean pressure – T0	114.2 ± 8.0	136.8 ±11.2	p = 0.114
Mean pressure index –T4	0.722±0.04	1.013±0.05	p = < 0.0001
Resistance –T4	1.41±0.07	2.49±0.27	p = < 0.0001
GST/100g – T4	10.8±1.7	24.2±5.1	p = 0.019

Conclusion

Both groups had identical WIT's & flow rates on MP. The pressure required for this was marginally better for the streptokinase group at initiation of perfusion (Mann Whitney U, p = 0.114). However this improved significantly with the Streptokinase versus the non-Streptokinase group (p = 0.0014). In addition glutathione-S-transferase (GST) values were significantly better in the streptokinase group. Some limited histological evidence is also available, which confirms the GST findings, i.e. less tissue damage with Streptokinase.

L56

DE-NOVO SOLID ORGAN MALIGNANCY FOLLOWING RENAL TRANSPLANTATION IS ASSOCIATED WITH DONOR-SPECIFIC HYPORESPONSIVENESS AND IL-10.

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Malignancy is a well-recognised complication of long-term immunosuppression following organ transplantation. The incidence of malignancy is likely to increase in years to come as older patients are being considered for transplantation and more potent immunosuppressive agents are being used. Here we test the hypothesis that patients who develop malignancy following renal transplantation will show donor-specific hyporesponsiveness (DSH) and produce regulatory cytokines such as IL-10.

This is a single centre retrospective study of 44 cadaveric renal allograft recipients with a functioning graft for more than 5 years, 8 of whom have developed a solid organ malignancy post-transplantation. The response of each recipient against donor splenocytes and third party cell was tested in mixed lymphocyte culture. A relative response index of less than 20% was used to define DSH and reflects the recipient's proliferative response to the donor compared to third party stimulation. Analysis of Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-10) cytokines have been analysed by ELISA and RT-PCR.

We have identified DSH in 54% (24/44) of patients studied overall, but 75% (6/8) of patients with malignancy show DSH. All patients studied were long-term graft survivors and the common feature is high IL-10 production. However, in contrast to patients without malignancy, the predominant cytokine produced by patients with malignancy is IL-10 and the mean level produced is four-fold higher than any other cytokine. We have shown a significant positive correlation between donor responsiveness and serum creatinine, IL-2 and IFN-γ production and negative correlation with IL-4 production. Patients with DSH, but do not have malignancy produce significantly higher levels of IL-4, and lower levels of IL-2 than those who remain response to their donors.

Our data suggest that IL-10 may not only facilitate long-term graft survival, but may be an important factor in the development of malignancy following renal transplantation. Donor-specific hyporesponsiveness is not exclusive to patients with good graft outcome and therefore cannot be used on its own to tailor immunosuppression. Patients with stable graft function and a low IL-2 and high IL-4 profile may be candidates for reduction of immunosuppression to prevent long-term complications such as malignancy.

L60

A SMALL, BIFUNCTIONAL SYNTHETIC PEPTIDE FOR NON-VIRAL GENE DELIVERY TO HEPATOCYTES VIA THE SERPIN-ENZYME COMPLEX RECEPTOR

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Receptor-targeted DNA vectors offer many advantages over virus vectors for gene therapy, especially in a transplant setting where non-immunogenicity of vectors is likely to be important.

We have investigated the use of small, bifunctional synthetic peptides to target the serpin-enzyme complex receptor (SECR) for gene delivery to hepatocytes. These peptides consist of an amino terminal chain of 16 lysines for electrostatic binding of DNA, and the SECR-binding motif of human α_1-antitrypsin (phe-val-phe-leu-ile or FVFLI in the one letter code) for targeting. Two synthetic peptides designated polylysine anti-trypsin 1 (PAT1) (K_{16}FNKPFVFLI) and PAT2 (K_{16}CSIPPEVKFNKPFVFLI) were evaluated for gene delivery to the HUH7 human hepatocyte carcinoma cell line.

Both PAT1 and PAT2 bind to and condense DNA into small particles (200-300nm) as shown by laser-scattering techniques. However, only PAT2 is effective for gene delivery, presumably an account of the greater distance between the K16 chain and the FVFLI motif. Gene delivery by PAT2/DNA complexes is chloroquine dependent, can be blocked completely by free ligand (CSIPPEVKFNKPFVFLI), and is highly efficient (e.g. approximately 5-fold more effective than lipofectamine).

The PAT2 peptide represents a highly efficient and readily standardised DNA vector for hepatocytes, with potential for gene delivery to the liver in transplantation.

L61

DEVELOPMENT OF TECHNIQUES FOR *IN VIVO* GENE DELIVERY TO THE LIVER USING A RECEPTOR-TARGETED NON-VIRAL DNA VECTOR SYSTEM.

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Gene delivery to the liver has many potential clinical applications, especially in transplantation. An elusive first step is the development of simple, safe, and efficient *in vivo* gene delivery. Although adenoviruses are excellent DNA vectors for hepatocytes, they have many disadvantages. A particular problem in a transplantation setting is immunogenicity, 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. Here we report a comprehensive analysis of gene delivery to the isolated lobes of the rat liver, via local perfusion through a branch of the portal vein or of the bile duct.

Initial vector localisation studies demonstrated excellent penetration of vector/DNA complexes into the hepatic lobule by either the portal venous or bile duct route. However, the DNA/vector complexes were rapidly lost from the liver (in less than 15 minutes) when delivered by the vascular route.

Quantitative studies on gene delivery were performed using DNA plasmids containing the luciferase reporter gene, homogenates of the treated liver lobes being assayed for luciferase enzyme activity. Gene delivery via the bile duct was consistently ~10 fold higher than via the portal vein. All subsequent studies were therefore performed using gene delivery via the bile duct.

DNA vector complexes were substantially smaller (as measured by laser scattering techniques) in 5% dextrose as compared to phosphate buffered saline (~80nm versus ~700nm). Possibly as a consequence of this, gene delivery in 5% dextrose was found to be ~10 fold higher than in PBS. Dose-response studies with locally and systemically administered chloroquine (to assist endocytic escape of vector/DNA complexes) have been completed. Time studies demonstrated that 2 hour exposure to vector/DNA complexes via the bile duct is optimal.

L66

Rapamycin has no effect on the progression of established allograft vasculopathy in rats

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Aims: Progressive allograft vasculopthy and organ fibrosis (chronic rejection) remains an important cause of late organ transplant failure and no therapeutic agent has been shown to have an effect on this process. Rapamycin is a novel immunosuppressant that has been shown to inhibit the development of de novo intimal hyperplasia and fibrosis in animal models but to date the effect of Rapamycin on established allograft vasculopathy in humans or animal models remains unclear. The aim of this study was to measure the effects of Rapamycin on established allograft vasculopathy as well as to measure its effect on the expression of genes that control extracellular matrix turnover in established chronic rejection.
Methods: Thoracic aortas from F344 rats were transplanted heterotopically into the abdominal aorta of Lewis recipients. Six animals per group started Rapamycin (0.5mg/kg/day) at 8,12, and 16 weeks with six receiving no treatment as a control. Grafts were harvested at 24 weeks and analysed histologically. mRNA was extracted from frozen tissue and expression of fibrosis associated genes was studied by means of quantitative reverse transcriptase-polymerase chain reaction.
Results: Between eight and 24 weeks weeks untreated allografts developed significant intimal thickening and expansive vascular remodelling. Levels of gene expression for species important in extracellular matrix remodelling are listed below. Values are expressed as a ratio of the house keeping gene ß Actin.

Rapamycin commencement	8 Weeks	12 Weeks	16 Weeks	Control	P Value
Intimal Medial Ratio	0.13±0.22	0.18 ±0.06	0.16 ±0.1	0.26 ±0.22	0.925
MMP 2	2.4±0.48	2.05 ±1.18	2.23 ±0.47	2.83 ±0.20	0.27
MMP 9	3.83±0.97	4.62 ±1.45	3.76 ±1.47	4.25 ±0.7	0.38
TIMP 1	1.58±0.20	1.99 ±0.53	1.45 ±0.22	1.42 ±0.06	0.13†
TIMP 2	2.35±0.35	2.35 ±0.21	2.20 ±0.50	2.52 ±0.23	0.49
Collagen 3	2.11±0.25	2.69 ±0.82	2.02 ±0.39	1.80 ±0.12	0.17†
TGF β	3.74±0.54	2.97 ±1.53	3.40 ±0.29	3.57 ±0.30	0.46

Mean values ± St. Deviation, P=Kruskall Wallis. † Collagen 3, TIMP 1 significantly higher in 12 week group by Mann Whitney.
Conclusion : These data suggest that Rapamycin has no significant effect on allograft vasculopathy and may in fact increase pro-fibrotic gene expression in allografts with early allograft vasculopathy.

L68

UNSUSPECTED ACUTE REJECTION IS ASSOCIATED WITH UPREGULATION OF TGF β1 mRNA EXPRESSION.

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BACKGROUND

Whilst modern immunosuppressive agents have significantly reduced the frequency of clinical acute rejection episodes, the chronic graft nephropathy rate has remained unaltered leading to the hypothesis that there is a significant incidence of subclinical rejection stimulating the fibrotic process. The aim of this study was to determine the incidence of clinical and subclinical acute rejection by means of renal biopsies and to analyse TGFβ1 mRNA expression in these biopsies using a novel real-time quantitative PCR system.

METHODS

Clinical acute rejection episodes were confirmed by biopsy and treated appropriately. Protocol renal biopsies were performed at baseline (time of transplant) and at 3, 6 and 12 months follow-up. Rejection identified in the protocol biopsies was NOT treated. Following extraction and reverse transcription, TGFβ1 mRNA expression in the protocol biopsies was quantified using a Lightcycler (Idaho Technology) real-time PCR system.

RESULTS

At the time of analysis 46 patients had complete follow-up to 12 months. Twenty three (50%) suffered no acute rejection episodes, 13 (28.3%) were treated for acute rejection and 10 (21.7%) had subclinical rejection. TGFβ1 mRNA expression is shown below as Log10 copies/μl RNA with values expressed as mean±s.d.

Pathology	Baseline	3 months	6 months	12 months
No Rejection	1.69±1.40	1.66±1.42	1.84±1.49*	2.10±1.35
Clinical Rejection	1.88±0.98	2.00±1.66	2.54±1.54	2.37±1.31
Subclinical Rejection	1.73±0.90	2.43±0.90	3.14±1.21	2.83±1.21

* $p<0.05$ Students *t* test: no rejection versus untreated rejection.

CONCLUSIONS

We identified a high incidence of subclinical rejection and demonstrated that TGFβ1 expression is greatest in this group. The identification of TGFβ1 upregulation in early protocol biopsies may present a marker for the subsequent development of chronic graft nephropathy and allows a potential window for therapeutic intervention.

L69

THE GLOBAL PERSPECTIVE ON TRANSPLANT INFLAMMATION: ATTACK VERSUS DEFENCE, REPAIR AND REGENERATION

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BACKGROUND

Investigation of the inflammatory process, which characterises renal allograft rejection, has traditionally concentrated on the 'attack' side of the equation. The aim of this study was to determine some aspects of the relative roles of defence, repair and regeneration mechanisms that counterbalance the injurious process.

METHODS

Plasma von Willebrand factor (vWF), selenium, transforming growth factor beta ($TGF\beta_1$) and hepatocyte growth factor (HGF) levels were determined in 40 primary cadaveric renal transplant recipients receiving calcineurin inhibitor-based immunosuppression (cyclosporin A or tacrolimus).

RESULTS

The results are summarised as medians. * $p<0.05$ Students *t* test: Baseline versus 3, 6 and 12 months.

Feature	Parameter	Normal Values	Baseline	3 months	6 months	12 months
Attack	vWF (% normal)	100	167	184	168	164
Defence	Selenium (μmol/l)	0.8-1.4	0.7	0.92*	0.88*	0.95*
Repair	$TGF\beta_1$ (ng/ml)	1.56-3.24	10.3	18.55*	38.85*	52.4*
Regeneration	HGF (pg/ml)	120	234.0	179.9*	177.7*	181.5*

Vascular endothelial activation (>50% normal) is evident at a constant level. Antioxidant status is impaired in the uraemic patient but then normalises. $TGF\beta_1$ expression increases sequentially with time whilst there is continuous decreased production of the renotropic growth factor HGF.

CONCLUSIONS

Further studies are ongoing to examine the relative influence of these mechanisms on both graft function and the development of chronic graft nephropathy. Attention to these natural 'balancing' mechanisms may allow an opportunity for therapeutic intervention and control of the inflammation.

L71

A COMPARISON OF DENDRITIC CELLS MIGRATED FROM HUMAN LIVER AND SKIN

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There is evidence of organ specific acceptance in the absence of immunosuppression following liver transplantation and it has been suggested that liver dendritic cells (DCs) play a role. We have developed a novel method to isolate DCs from human liver, modified from a skin DC isolation described by Larsen et al, in which thin pieces of liver tissue were cultured overnight without cytokine supplement, allowing DCs to migrate out before being purified by density gradient centrifugation. A similar method was used to isolate skin DCs so that they could be compared. The cells were characterised by immuno-cytochemistry, flow cytometry and their ability to stimulate naïve cord blood lymphocytes tested in a mixed lymphocyte reaction. The function was compared by ELISA measurement of IL-10 and IL-12p70 in DC cultures.

In culture liver DCs had short processes initially and developed typical veils after further culture. Cells from normal liver were monocyte like and expressed CD14 (50%), CD11b, CD11c but not CD68, as well as HLA-DR and co-stimulatory molecules. After culture cells developed a distinctive morphology, lost expression of CD11b and CD14 but increased CD83 and CD86. Cells derived from diseased liver contained a subset with a more mature morphology that expressed CD68 intracellularly. Skin cells expressed similar myeloid DC markers, but a majority of cells were CD1a and S100 +ve. The liver DCs were able to activate naïve T cells efficiently. Chemokine receptor expression was consistent with DCs leaving tissue i.e. CCR7 and CXCR4 high with low CCR5 expression. Pure liver DCs produce IL-10 (414pg/ml+/-50), whilst skin DCs do not, DCs from diseased liver produce increased IL-10, but not significantly (673+/-143). None of the DCs produce IL-12p70 in the conditions tested so far.

In conclusion, DCs of intermediate maturity can be isolated from human liver with minimum manipulation. They have a similar phenotype both in terms of cell type and maturity to skin DCs isolated by the same method, although CD1a is not expressed. Initial assessment of function suggests that liver DCs can, as expected, efficiently stimulate naive T cells. It is of interest that liver DCs produce IL-10 a cytokine associated with tolerance.

C2

MRI OF POTENTIAL RENAL DONORS - MRA, MRV AND MRU

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Purpose: To assess the feasibility of performing magnetic resonance angiography (MRA), venography (MRV) and urography (MRU) as a single examination in potential renal donors and to evaluate the diagnostic accuracy of MRI compared with the surgical findings.

Method: Forty-two subjects were evaluated with a 1T Siemens MR system in a body array coil. Sequences were performed during breathold with no special preparation. Renal anatomy was assessed by axial scans followed by MRA, MRV and MRU. Images were displayed using maximum intensity projection and multi-planar reformatting.

Results: Surgical correlation was possible in 29 of the 42 potential donors. Surgery was performed on the left in 19 donors and on the right in 10. MRI findings that resulted in the choice of right-sided surgery included double left renal arteries in seven, and the presence of complex left venous anatomy in two. A right kidney was also selected for donation due to the presence of right renal cysts. The MRU demonstrated a dilated calyx and cortical scarring in one case, resulting in donor exclusion. In 27/29 donors MRA and surgery were in agreement with regard to the number of hilar arteries. MRA missed one small polar artery, which was ligated at surgery. MRV was accurate in depicting the main and extra-renal veins in all cases.

Conclusion: MRI has the potential of becoming the primary imaging technique in the assessment of potential renal donors, eliminating the use of ionising radiation, reducing the number of examinations and the cost of imaging.

CLINICAL

C7

INDIVIDUAL VERSUS GROUP PSYCHOTHERAPY: ADDRESSING EMOTIONAL PROBLEMS AMONGST TRANSPLANT PATIENTS

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Introduction: Kidney transplant patients have been shown to develop emotional problems. These issues are more complex than previously suggested and can be directly related to compliance behaviour. Psychotherapy has been shown to be effective in addressing developmental delay, coming to terms with the past and can be a catalyst for change. In general, group psychotherapy has been shown to be more cost effective than individual therapy. In this study we have compared the efficacy of the two modalities of therapy in recipients of kidney transplants.

Methodology: Recipients of cadaver kidney transplantation were randomised (using computer generated numbers to ensure even gender and age distribution) into two groups, to receive a 12 week course of *Systemic Integrative* group or individual psychotherapy. There was no control (no therapy group) for ethical reasons. The *Beck Depression Inventory* (BDI) was utilised as a measure of change in emotional state, pre-transplant, at 3 and 6 months. A higher score on BDI was suggestive of psychological dysfunction (total possible score was 3). All recipients during the recruitment period were offered the opportunity of participation in the study.

Results: To date, 54 patients have completed a twelve week course of psychotherapy, 34 in individual and 20 in group therapy. The mean age was 38 years, there were 31 males and 23 females. At the end of 12 sessions, overall, patients in the individual therapy had significantly lower BDI score versus patients in group therapy. In general, patients who have received pre-transplant intervention show improved scores. Our findings suggested that the treatment outcome was determined by patient-specific variables, namely, length of time on hemodialysis, age at onset of renal failure, pre-morbid personality traits, participation in pre-transplant psychotherapy and employment status. In both groups, patients who received hemodialysis for 3 or more years scored higher on the BDI relating to feelings of sadness, failure and low self-esteem. Gainful employment was a major factor in a lower rating on BDI and resulted in the maximum benefit at the end of therapy.

Conclusion: The focus of psychotherapy is centred upon a 'return to normal'. However, normality is defined in the past and serves to maintain low mood. Improved mood states are associated with individual psychotherapy, which focuses on easing patients from premature 'medical' retirement. Negative pre-morbid personality traits are amplified during renal disease and their relational aftermath is an issue amongst all patients. We conclude that individual psychotherapy was found to be a more effective modality.

C8

PROMOTING ORGAN DONATION AMONGST ASIANS: A PUBLIC INITIATIVE

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Introduction: The demand for kidney transplants across the UK far outweighs availability. This problem is particularly prevalent amongst the Asian population, as fellow Asians with whom they are most likely to be compatible make reluctant donors. The effects of nationwide initiatives to understand this issue have been limited. We arranged a public forum amongst the local Asian community to promote awareness and determine attitudes towards organ donation and kidney transplantation, with a view to generating future research and initiatives.

Methodology: During the forum, respondents were asked to complete a confidential, self-rating, self-administered, pre-piloted questionnaire. A single sociologist was at hand to assist with the filling of questionnaires.

Results: Over 300 persons from all walks of life attended the forum. Ninety-two questionnaires were distributed and eighty-two were returned fully completed. The findings were analysed in age bands (20-60 yrs). All respondents demonstrated awareness and were in favour of organ donation and kidney transplantation, particularly when a family member was involved. However, the majority did not carry organ donor cards. There was a more inhibited attitude towards organ donation with age (40 yrs upwards) attributed to concerns regarding their own health, predicted success of transplant and post-transplant convalescent period. There appears to be a relationship between pre-existing religious belief and positive attitude towards donation. A hesitancy regarding donation was particularly evident in younger Muslims (20s age band). Religious leaders were considered well placed to promote these issues. The majority of respondents were familiar with the National Organ Donor Register. Younger women had a more positive attitude towards transplantation versus older women and men in all age bands. Across the spectrum all respondents strongly viewed 'required request' and 'presumed consent' laws as a personal affair and were opposed to legislation.

Conclusions: The majority of respondents that did not carry donor cards, despite being aware and in favour of organ donation and transplantation. This attitude does not bode well for Asians awaiting kidney transplants. However, the positive response to donation when a family member is involved suggests that there is potential for live donation. Indeed, these findings suggest that Asians remain passive or are not emotionally connected to this issue. Nomination of religious leaders as 'moral integrators' as well as 'moral prescribers' of these issues into Asian culture (music, art, dance and literature) may help rectify this problem. It appears that men occupy the socio-economic power base of Asian life and have been targeted by awareness campaigns. However, those women who described themselves as 'wives' and 'mothers' might be centrally placed in the primary family social networks to facilitate such integration and remain an under utilised resource. We are extending our study to other cities in the UK.

C16

METHODS OF CLASSIFYING THE PRIMARY INDICATION FOR LIVER TRANSPLANTATION: IMPLICATIONS FOR ESTIMATING PATIENT AND GRAFT SURVIVAL IN THE UK.

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Introduction: Many liver transplant recipients have 2 or more indications for surgery. We examined to what extent disease-specific patient and graft survival depends on the method of classifying the primary indication for transplantation.
Methods: We examined patient and graft survival after liver transplantation in 3112 adult (15 years and above) first graft recipients between March 1994 and March 2000 in the UK & Ireland. Initially, we identified the primary indication for transplantation by taking the first of the 3 possible diagnoses that could be recorded. Subsequently, we considered all 3 possible diagnoses by creating mutually exclusive diagnostic groups that reflect the importance of the recorded diagnoses for the patient's prognosis. First, all patients who had been listed as super-urgent were grouped as (1) acute transplants. Second, all remaining patients, who had cancer recorded in any of the diagnostic fields were considered to have (2) hepato-biliary cancer. Subsequently, all remaining patients with a diagnosis of (3) metabolic disease were placed into the next group, and this process was repeated for all patients who had a recorded diagnosis of (4) cholestatic disease, and of (5) cirrhosis. After these 5 steps, the remaining patients were grouped as (6) other non-acute indications.
Results: The method of identifying primary recipient disease hardly affected the patient numbers and survival estimates, except for patients identified as having hepato-biliary cancer. By considering only the first of the 3 possible diagnoses, we identified 96 patients with hepato-biliary cancer, who had a 1-year patient survival of 76% (95% confidence interval 67%-85%) and graft survival of 67% (58%-77%), and a 4-year patient survival estimate of 27% (12%-42%) and graft survival estimate of 22% (8%-36%). By considering all 3 possible diagnoses on the basis of the above-described classification system, we identified 270 patients with cancer who had a 1-year patient survival estimate of 75% (70%-81%) and graft survival of 71% (65%-77%), and a 4-year patient survival estimate of 42% (32%-53%) and graft survival of 42% (32%-53%).
Conclusion: We found that the number of patients identified as having been transplanted for hepato-biliary cancer considering all diagnoses was almost 3 times higher and that the estimates for long-term patient and graft survival were considerably better than when identifying patients with cancer by taking only the first recorded diagnosis. This implies that one should take the method of identifying the primary identification for transplantation into account when interpreting disease-specific estimates for patient and graft survival.
CLINICAL

C19

PROMOTING NON-HEART-BEATING-DONORS (NHBDS) FOR RENAL TRANSPLANTATION - DEVELOPMENT OF RELIABLE VIABILITY TESTS

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Objective: The use of Non-Heart-Beating-Donors for renal transplantation has been controversial over the last decade. After the acceptance of brain death criteria in 1972, this organ source was generally replaced by controlled Heart Beating Donors. The increasing demand for kidneys over recent years however has renewed interest in these organs. In 1988 the South Thames Region started a NHBD program. This was evaluated in 1995 since the overall outcome was significantly poorer than the HBD program. It was mainly due to a high Primary Non Function (PNF) rate associated with inadequate donor selection criteria. A new NHBD programm was subsequently started in St. George's Hospital in March 1995 using strict criteria for donor and recipient selection.
Patients and Methods: Between March 1995 and May 2000, 34 kidneys from NHBDs in Category I – III according to the Maastrich classification were retrieved and transplanted. Only donors under the age of 55 were considered, but uncontrolled donors (Category I and II) were included. Places of retrieval were: Intensive care units, St. George's Accident & Emergency Department and several hospices. Warm ischaemic times varied between 15 and 70 minutes. Category III donors were crash - retrieved with or without aortic *in situ* perfusion. A femoral cut down for cannulation with a double baloon catheter and cold *in situ* perfusion with Marshals solution was used in Category I and II donors. Grafts were stored on ice and cold ischaemic times did not exceed 24 hr.
Results: 27 out of 34 transplants (79.4%) started functioning within 4 weeks and were successful, with recipients independent of dialysis, with a median follow up of 40 months. So far none of the functioning grafts have been lost. The Delayed Graft Function (DGF) rate was 20/27 (74.1%) and the Primary Non Function (PNF) rate (kidneys that never functioned) was 7/34 (20.6%).
Conclusions: NHBDs are a valuable source of kidneys and can extend the donor pool by up to 40%. Donor selection criteria and good surgical retrieval techniques are required to obtain a low PNF rate. Kidneys from uncontrolled NHBDs (Category I and II) are particularly difficult to assess prior to transplantation and PNF rate in this subgroup can compromise the outcome of the NHBD program overall. Machine perfusion assessment or other viability parameters might enable the identification of suitable organs for transplantation and thus could decrease the PNF rate. In St. George's Hospital we recently started Mashine Perfusion (MP) of NHBD kidneys. So far 4 grafts of Category I donors have been transplanted after MP. Two patients are dialysis - independent with serum Creatinine concentrations under 200 umol/l, 5 weeks after transplantation. The follow - up of the other 2 patients is currently less than 2 weeks.

C23

THE LIVER FAST TRACK SCHEME IN THE UK

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Background: The UK operates a Fast Track Scheme (FTS) for offering livers available with short notice to all 7 transplant centres in the UK simultaneously. Introduced on 1 November 1997, the scheme was subsequently revised on 11 October 1999 to only offer donated livers with a cold ischaemic time exceeding 4 hours. The aims of this study were: to summarise the fast track offer activity; to assess the usefulness of the scheme; to compare the survival rate of transplants using fast track organs with non fast track organs.

Methods: The number of livers offered and the reasons stated for offering through the UK FTS between 1 November 1997 to 30 June 2000 were collated. Reasons were categorised as either donor or recipient reasons. Three month Kaplan-Meier graft survival rates were calculated for first routine cadaveric liver transplants in the UK resulting from fast tracked and non fast tracked organs from the UK and Republic of Ireland.

Results: 169 whole livers and lobes have been offered through the UK scheme. 67 (40%) were accepted resulting in 57 transplants in the UK. This represents approximately 3% of all liver only transplants in the UK during this period. A further 7 livers were placed overseas. Since the introduction of the rule revision the average number of transplants per month using fast tracked organs has reduced from 2.1 to 1.7. 53% of livers were offered for recipient reasons such as 'no suitable recipients' and 'recipient unfit'. 47% were offered for donor reasons such as 'fatty liver' and 'donor unsuitable-size'.

The 3 month graft survival rate for transplants resulting from 1269 non fast track organs over the same period was the same as for 53 fast track organs: 94% (95% CL 93-95%) compared with 94% (95% CL 87-100%), Log-rank test p-value=0.99.

Conclusion: The UK Fast Track Scheme has provided organs for 57 liver transplants in the UK that may not have been possible if the offering process had not been speeded up by simultaneously offering the organs to centres. With 54% of these transplants using organs fast tracked for recipient reasons and three month graft survival for fast track organs being comparable with that for non fast track organs, the scheme does not appear to be placing poor organs.

C26

THE DECREASE IN ORGAN DONATION IN GREAT BRITAIN

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Background: Each year since 1990 the number of solid organ donors in Great Britain has decreased as has deaths in the population. The donation rate i.e. the number of donors in relation to deaths from road traffic accidents and cerebrovascular accidents has also fallen over this time period. The aim of this study is to further investigate the reduction in organ donation in the hope that this can lead to initiatives which will increase donation rates.

Methods: The analysis covered the period 1 January 1990 to 31 December 1998 during which time there were 7380 cadaveric solid organ donors. For each donor, the cause of death, year of donation and the type of donor unit were obtained from the UK National Transplant Database. For the same period the office for National Statistics provided information on death rates and causes of death for the general population of Great Britain.

Results: The number of solid organ donors decreased by 18% from 896 in 1990 to 732 in 1998. There was a reduction both in road traffic accident (RTA) deaths and in the number of donors when expressed as a fraction of RTA deaths. RTA deaths fell from 4821 in 1991 to 3484 in 1998 while the number of donors per thousand RTA deaths fell during the same time period from 40.7 to 30.1. In 1990 organ donors resulting from RTA deaths accounted for 27% of the total and this fell to 14% by 1998.

The number of donors resulting from cerebrovascular accident (CVA) deaths increased from 446 in 1990 to 547 in 1995 and subsequently fell to 476 in 1998. The total number of deaths due to CVAs decreased between 3% and 6% each year from 1991 to 1994 and between 1% and 3% each year from 1995 onwards. Throughout the early to mid 1990s the donation rate among those dying from CVAs increased but from 1996 onwards the rate fell. In 1990 there were 59.9 solid organ donors per 10,000 CVA deaths. This figure rose to 80.9 in 1995 and had fallen to 74.1 by 1998. In 1990 50% of all solid organ donors died from CVAs while this figure increased to 65% in 1995 and remained constant thereafter.

Conclusion: This analysis emphasises the considerable reduction in organ donor numbers during the 1990s among those dying both from RTAs and CVAs. This fall was in part due to the decrease in RTA and CVA deaths which occurred throughout 1990 to 1998. Although reducing organ donor numbers, this reduction in death rate is clearly a highly encouraging trend with regard to the general population. However, there was also a reduction in organ donors when expressed as a percentage of deaths. This applied throughout the period of analysis for RTA deaths and from 1996 onwards for CVA deaths. The reduction in organ donor numbers from these two causes of death has therefore been due in part to the reduced death rates but also to a change in the management of RTA and CVA patients following their admission to hospital.

C27

PREDICTING A PATIENT'S WAITING TIME TO HEART TRANSPLANT IN THE UK

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Background: Patients accepted for heart transplantation face an uncertain wait. Knowledge of the expected waiting time for each candidate can help patients and clinicians to discuss management. This study aimed to: identify factors influencing waiting time, estimate patient-specific waiting times, produce software for use in clinical practice and evaluate the accuracy of the software.

Methods: Data on 1165 adult patients, listed for a first heart transplant in one of 7 UK centres during the period 1990 to 1997 were analysed. All UK 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. A multiple variable survival model was used to access the predictive value of: patient sex, age at listing, blood group, weight, height, primary diagnosis, CMV status at listing, number of previous heart transplants received and time already spent on the active waiting list. The analysis was stratified by centre to produce centre specific waiting times as well as National estimates. Software was developed which reports the chances of receiving a transplant within 3, 6, 9 and 12 months depending on patient characteristics. An additional 247 registrations were used to test the accuracy of the software.

Results: The following factors were identified as influential factors: blood group, weight, height, primary disease and the current time spent on the active transplant waiting list. Patients with blood group A or AB do not wait as long for a transplant as blood group O patients. Patients with a disease other than cardiomyopathy wait longer than those with congenital heart disease. Patients who are 66kg or over in weight and who have been listed for under 3 months receive a transplant quicker than lighter patients but heavier patients who have waited for over 6 months wait longer than their lighter counterparts.

Using the additional 247 listings, at 3 months post-listing 59% of patients were no longer waiting, of which 47% were predicted correctly by the model - by 12 months, 93% were no longer waiting, of which 83% were predicted correctly.

Conclusions: Desktop software, based on readily available patient data, has been developed which predicts the chance of receiving donor hearts by 3, 6, 9 and 12 months after listing.

C28 THE INCIDENCE OF ANAEMIA AND ITS RELATIONSHIP TO GFR IN RENAL ALLOGRAFT RECIPIENTS.

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The incidence of anaemia and its relationship to GFR is unknown in renal allograft recipients. In pre-dialysis and dialysis patients, anaemia is an important complication of renal failure that accounts for many uraemic symptoms and contributes to the development of cardiovascular morbidity. The Lotess study (Long Term Efficacy and Safety Surveillance of Neoral) has enrolled over 8,000 transplant patients from more 90 UK transplant units. It, therefore, provides a large cohort of patients to analyse complications and co morbidity in a transplant population. This study has examined the incidence of anaemia and its relationship to GFR in 5,923 adult renal allograft recipients treated with cyclosporine immunosuppression. Anaemia was defined as a haemoglobin of less than 12.5g/dl in males and less than 11.5g/dl in females. Patients were followed for a period of 48 months. The data has been censored for use of erythropoeitin, ACE inhibitors and Angiotensin II receptor antagonists. Results are expressed as mean ± SEM. The incidence of anaemia at each time point following transplantation is outlined below:

	Visit (months)	0	6	12	24	36	48
F	Incidence (%)	42	31	32	33	35	36
	GFR (ml/min)		42±1	42±2	41±1	39±1	38±2
M	Incidence (%)	45	33	31	35	34	36
	GFR (ml/min)		52±1	48±1	46±2	46±1	45±1

In the UK approximately 40% of patients are anaemic at the time of transplantation. The incidence of anaemia in both females and males is approximately 35%. The average GFR in anaemic patients is 40ml/min in females and 48mls/min in males. The incidence of anaemia remains remarkably constant over the first 48 months following transplantation. The relationship between haemoglobin and GFR is outlined below:

	HB g/dl	<8	8-9	9-10	10-11	11-12	12-13	13-14	14-15
F	N	236	500	1123	2369	3658	3794	2918	1507
	GFR ml/min	28±1	30±1	37±1	40±1	46±2	50±1	54±1	55±2
	N	512	1024	2103	3652	5037	5694	4858	2936
M	GFR ml/min	23±1	36±2	38±1	43±1	51±1	56±2	61±1	64±2

A Pearson Correlation showed a strong correlation between GFR and haemoglobin for both male and female patients with anaemia (2 tailed significance P<0.0001). We were unable to demonstrate a correlation between haemoglobin and GFR in non anaemic patients (p=0.14). In conclusion this study has demonstrated a high incidence of anaemia in renal allograft recipients. As with the pre dialysis population there is a strong correlation between haemoglobin and GFR in anaemic patients. We hypothesise that correction of anaemia in renal allograft recipients may improve their quality of life and cardiovascular co-morbidity.

C29 NON-IMMUNE ACUTE GRAFT INJURY AFTER LUNG TRANSPLANTATION AND THE RISK OF SUBSEQUENT BRONCHIOLITIS OBLITERANS SYNDROME (BOS).

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Primary graft dysfunction, characterised by diffuse alveolar damage (DAD), remains a major cause of early morbidity and mortality after lung transplantation. Evidence from animal models suggests that this non-immune acute graft injury enhances HLA expression in the lung and furthermore in human renal transplantation, early allograft injury increases the risk of subsequent graft loss due to chronic rejection. We hypothesised that this non-immune acute injury in the lung allograft may by increasing organ immunogenicity and promoting fibrotic repair impact not only on early survival but also on longer-term survival by increasing the incidence and rate of onset of Bronchiolitis Obliterans Syndrome.

In our centre, recipients undergo routine transbronchial lung biopsy in the first week after transplant. Patients who die in the immediate postoperative period undergo autopsy. We retrospectively identified early lung biopsies from 291 lung transplants performed between 1987 and 2000, (129 single; 106 bilateral sequential and 56 heart-lung transplants). 55 (19%) recipients had histologically proven DAD, their 30 day survival was significantly worse 62.5% than for recipients without DAD 87.5%, p<0.0001 (Chi Squared). For survivors beyond 3 months, all serial FEV1 measurements were reviewed and the time that criteria for BOS was reached was noted. The incidence of subsequent BOS was not significantly different in those with and without DAD on early biopsy, 46% and 59% respectively, p=0.22 (Fisher Exact). Neither did BOS occur any earlier in the DAD group, 953 (152-1393) days compared to 665 (52-4299) days in the non-DAD group, p=0.48 (Wilcoxon Survival Analysis). When all deaths within 30 days were excluded, there was no difference in actuarial survival between recipients with and without DAD, p=0.12 (Wilcoxon Survival Analysis). The development of non-immune acute graft injury after lung transplantation has a very poor early prognosis. However any recipients who survive beyond 30 days can expect comparable long-term survival and similar BOS-free time to recipients without non-immune acute graft injury.

CLINICAL

C31

SHOULD OBESE PATIENTS BE OFFERED KIDNEY TRANSPLANTS?

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Introduction: Obesity is an independent risk factor for most types of surgery however its influence on the outcome of renal transplantation is not clear. Some transplant units have a policy of only placing recipients on waiting lists if their body mass index (BMI) is <30 kgm^2 but current literature is contradictory.

Aim: To study the differences in outcome in obese and non-obese patients following renal transplantation.

Method: The outcome of consecutively matched obese and non-obese patients were analysed retrospectively for mortality, complications and graft function at a single institution between 1994-1999. Obesity was defined as having a pre-operative BMI > 30kg/m^2.

Results: Seventy-nine non-obese and 43 obese patients were identified. The mortality at 1, 3 and 5 years post renal transplant was 0%, 1.7% and 7.5% in the non-obese patients and 0%, 13.6 % and 22 % in the obese patients ($p<0.05$). Wound infections, urological complications, bleeding and acute renal vasculature thromboses were all more common in obese when compared to non-obese patients (36.8% vs 23.1% not significant). Obese patients had significantly more cardiovascular complications when compared to non-obese patients (15.8% vs 1.6% $p<0.05$). Graft survival at 1,3 and 5 years post transplant was 94.4%, 88% and 78% in the non-obese patients and 93%, 78% and 70% in the obese patients that was not significant.

Conclusions: Pre-operative obesity is associated with a significant increase in mortality and cardiovascular complications following renal transplantation. Obesity however, is not associated with a significant difference in graft survival. These observations suggest that weight reduction to a BMI<30 kgm^2 and intensive cardiovascular screening should both be a prerequisite prior to transplantation.

C48

ANALYSIS OF RENAL REGRAFT SURVIVAL: IS MORE IMMUNOSUPPRESSION REQUIRED?

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Traditionally renal regraft survival has been worse than 1st grafts with a high rate of immunological graft loss in the first year. This has led to the use of more intensive immunosuppressive regimes in regraft recipients. Whether this is necessary for all patients, or just a high risk sub-group, remains to be determined. This study analysed the outcome of 1st (n=420, 83%) and second or subsequent (n=95, 17%) renal cadaveric allografts performed in a single center in the period 1990-98. All donor/recipient pairs had negative flow cytometric cross match prior to transplant. 1st graft recipients received triple immunosuppressive treatment (CyA/Aza/Pred). In addition, regrafts received induction antibody. Patient and graft survival, creatinine and rejection episodes were compared. Donor characteristics and mismatching were also considered.

Results

	1st graft	Regraft	
1yr survival	83%	81%	n.s.
5yr survival	70.5%	68%	n.s.
Acute rejection episodes	1.38	1.36	n.s.
Creatinine 1yr (mmol/l)	162	164	n.s.
Creatinine 5yr (mmol/l)	137	142	n.s.
Donor age >60yrs	17.7%	12%	n.s.
0-0-0 mismatches	2.1%	16.8%	P<0.01

The comparable survival seen is in contrast to the regraft survival in the cohort transplanted 1984-88, prior to the introduction of flow cytometric cross matching (69% vs 81% 1yr survival, $P<0.05$). Although in 1990-98 overall survival was similar it was still possible to identify a high risk sub-group of regrafts with a worse outcome. A high peak panel reactivity (>70%) prior to regraft predicted a poor 5yr survival compared to a PRA <70% (56% vs 79% respectively, $P=0.06$). Early immunological loss of the first graft (<1yr) also predicted a worse outcome (51% vs 76% 5yr regraft survival, $P=0.1$). This is despite favourable donor age and matching characteristics in the regraft recipients (see table).

In conclusion, there is a sub-group of regraft recipients who, despite more intensive immunosuppression have a worse outcome. The majority, however, have a similar outcome to 1st graft recipients. Although it is not possible to rule out a treatment effect, this suggests that increased immunosuppression may be unnecessary for this group. Studies are needed to confirm this.

C49

QUALITY OF LIFE IN RENAL TRANSPLANT PATIENTS WITH FUNCTIONING GRAFTS; THE STORY UNDERNEATH

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Aim: To measure the subjective QOL(Quality of Life) of patients with functioning kidney grafts and associate it with risk factors including the use of maintenance steroids.

Patients and methods: 103 renal transplant patients with functioning grafts at least 1year post-transplant were interviewed by using 3 instruments of measurement of QOL: Kidney Transplant Questionnaire (KTQ) - a disease specific questionnaire, SF36 - a generic health questionnaire for chronic illness, and EORTC health thermometer. Patients with an acute infection, acute rejection or cardiac event in the last 4 weeks were excluded from the interview.

Results: 54.4% of patients were receiving maintenance steroids. The total KTQ score was 138.8(±24.5). A worse total KTQ, was associated with a creatinine over 200mm/l (p=0.02), treatment with steroids (p=0.03) but not with sex, age group and mode of dialysis treatment pre-transplant. A worse appearance score in KTQ was more common in females (p=0.04), patients on steroids (p=0.1), and patients with a creatinine over 200mm/l (p=0.05). A worse score in the physical dimension of the SF-36 was associated only with an age over 55 years (p=0.006), but not with other variables. The use of steroids was associated with a worse score in the emotional dimension of both KTQ (p=0.08) and the SF-36 (p=0.08). The perception about their health measured by SF-36 was worse in patients on steroids (p=0.01). Patients with a creatinine over 200mm/l had less vitality (p=0.04) than the rest. The mean Health Thermometer score was 69.85 (±18.4) and was significantly reduced with increased age (p=0.04), creatinine over 200mm/l (p=0.07), CAPD use before the transplant as opposed to haemodialysis or no dialysis (p=0.04), and the use of maintenance steroids (p=0.04).

Conclusion: Kidney transplant patients have a good subjective QOL as measured by various scoring systems. Their age seems to be affecting only the physical dimension of those scores. In contrast the use of maintenance steroids seem to be adversely affecting the quality of life in multiple dimensions (physical, emotional, appearance) as well as the overall perception of the patients about their health prospects. Patients with a creatinine over 200mm/l seem to have more physical problems, less vitality and worse overall health. Good kidney function on a steroid free regimen seems to be the ideal solution for a good quality of life following renal transplant.

C52

DO PRODUCTS OF ALTERNATIVELY SPLICED VARIANTS OF IL-2 mRNA REPRESENT THE ENDOGENOUS IL-2 RECEPTOR BLOCKER? A NEW AVENUE IN THE MAZE OF TRANSPLANT IMMUNOLOGY.

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Recently, the recombinant truncated IL-2δ2 and IL-2δ3 proteins generated by alternative splicing of IL-2 mRNA were shown to inhibit IL-2 induced proliferation of T cells *in vitro*. The hypothesis that the generation of cytokine variants by alternative splicing might give rise to receptor antagonists as part of important autoregulatory mechanism in modulating immune response remains unconfirmed. We have previously shown that both pre- and post-transplantation, scheduled and unscheduled kidney core biopsy samples predominantly express alternatively spliced variants of IL-2 mRNA (IL-2δ2 mRNA-missing exon 2 and IL-2δ3 mRNA-missing exon 3). We were not able to find the correlation between the expression of alternatively spliced variants of IL-2mRNA and the histologically verified rejection. **AIMS** of this study were to further analyse the relationship between the levels of native (IL-2L) and spliced variants of IL-2 mRNA in sequential kidney core biopsies and the clinical outcome of transplantation. Parameters for clinical outcome were graft function (GFR), clinical acute rejection (AR), infections (CMV) and chronic graft nephropathy (CGN). A SYBR Green® I based real-time Quantitative RT-PCR assays were developed to quantify IL-2L, IL-2δ2 and IL-2δ3 mRNA. Clinical data were collected prospectively on our database. Renal function was expressed as Cockroft-Gauld GFR. **RESULTS**: The RNA was extracted from a total of 421 biopsies in 89 patients. There were 370 protocol biopsies and 51 unscheduled samples. There were 4 distinct groups: Group 1 (n=41) did not express any variant of IL-2mRNA at any time, Group 2 (n=26) expressed IL-2δ2and/or IL-2δ3 mRNA, Group 3 (n=15) expressed native IL-2L mRNA only , and Group 4 (n=7) expressed both IL-2L and spliced variants IL-2δ2 and IL-2δ3 mRNA. Kruskal- Wallis and Pearson Chi-Square tests were used for statistical analysis.

Table 1. Cockroft-Gauld GFR (median ml/min) in relation to mRNA expression.

	3 days (n=89)	1 week (n=89)	3 months (n=86)	6 months (n=88)	1 year (n=87)	2 years (n=47)	3 years (n=36)
Group 1	21	39	51	58	56	43	38
Group 2	39	53	60	54	64	64	69
Group 3	16	20	41	40	44	58	43
Group 4	23	32	55	54	60	46	45
p value	0.01*	0.01*	0.07	0.20	0.29	0.05*	0.005*

Table 2. Incidence of AR, CMV and CGN in relation to mRNA expression.

	AR	CMV	CGN
Group1	42%	32%	37%
Group 2	35%	23%	35%
Group 3	60%	47%	40%
Group 4	57%	71%	57%
p value	0.3	0.08	0.7

CONCLUSIONS: The results of this first ever study, investigating alternatively spliced variants of IL-2LmRNA in a clinical scenario, show that patients with IL-2δ2 and IL-2δ3 mRNA have better GFR. These findings support the concept that IL-2δ2 and IL-2δ3 proteins may indeed act as natural IL-2R blockers and might have beneficial effect on renal allograft function.

C53

COMPARISON OF NORMOTHERMIC PERFUSION TO COLD STORAGE IN LIVER PRESERVATION

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Introduction: Perfusion during preservation is currently not used clinically. Standard liver preservation in cold storage results in energy depletion followed by ischaemic injury with further injury upon reperfusion. By using oxygenated sanguinous perfusion at body temperature, the livers energy stores can be maintained during preservation. Therefore, this should prevent both the progressive decline in organ viability and the ischaemia-reperfusion injury associated with cold preservation. Furthermore, a functioning liver during the preservation period allows for a method to assess viability prior to transplantation.

Methods: Porcine livers were preserved in either UW solution by standard cold storage or perfused with oxygenated autologous blood on an extracorporeal circuit. Both groups were subsequently tested on the circuit during a 24-hour reperfusion phase. Perfusate samples, flow dynamics, bile production, and histology were compared.

Results: Livers preserved by perfusion displayed superior synthetic function (factor V, bile), metabolic capacity (galactose elimination kinetics, glucose utilisation, pH control) and perfusion flow dynamics (total flow, hepatic artery resistance). Significantly less global liver injury was seen in hepatocellular enzymes, histologic scoring, and markers of reperfusion injury.

Conclusions: Continuous perfusion is a superior method of organ preservation when compared to simple cold storage. In addition, perfusion during preservation offers the ability to assess organ viability prior to transplantation, which is not feasible during cold storage.

C54

RECOVERY OF NON-HEART BEATING DONOR LIVERS UTILISING NORMOTHERMIC MACHINE PRESERVATION

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Introduction: The inclusion of non-heart beating donor (NHBD) livers in current transplantation programs is not possible as no method of viability assessment or recovery of function during preservation is achievable using standard cold storage techniques. Normothermic sanguinous machine perfusion has been shown to restore depleted ATP levels, may reduce ischaemia/reperfusion injury and allows for accurate assessment of an organs risk of primary non function after transplantation. We aim to compare warm perfusion with cold storage in UW solution as a mechanism of preservation in a porcine model of NHBD liver transplantation.

Methods: Two groups of porcine livers, having undergone 60 minutes of warm ischaemia in the donor, were harvested and preserved either in cold (4°C) UW solution or perfused with whole blood on an extracorporeal circuit for a 24 hour period. Both groups were then reperfused utilising the circuit as a surrogate for transplantation, and haemodynamic perfusion characteristics and perfusate sampling used to assess ischaemia/reperfusion injury and organ function.

Results: Normothermically preserved NHBD organs displayed significantly better synthetic function in terms of bile production (mean 6ml/hr) and factor V production which were comparable to normal livers. Glucose utilisation and galactose elimination kinetics, as well as perfusion haemodynamics were also superior in this group. Preservation/ischaemia injury was far more marked in the cold preserved group which after 1 hour of reperfusion showed no evidence of viability (bile production 0ml/hr) with complete haemolysis of the perfusate. This was supported by histological and immunohistochemical analysis of wedge biopsies.

Conclusions: Normothermic machine perfusion offers a superior method of preservation for NHBD livers, and a possible mechanism for the inclusion of these marginal organs in the current donor pool. It may also allow for assessment of viability and extended preservation periods.

C55

A NOVEL MARKER OF ISCHAEMIA/REPERFUSION INJURY AND A MECHANISM FOR VIABILITY ASSESSMENT IN LIVER TRANSPLANTATION: β-GALACTOSIDASE

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Introduction: Glycohydolases are a group of enzymes contained predominantly within lysosomes, which are released during kupffer cell activation or death. One of these, β-galactosidase, has been proposed as a marker of ischaemia/reperfusion injury in the liver, as kupffer cell activation represents a primary event in the injurious reperfusion cascade. This is compared to more traditional markers of global liver damage in a porcine model of liver preservation/reperfusion injury, and correlated with markers of liver function, to assess its potential role as a marker of liver viability during machine perfusion.

Methods: Porcine livers were harvested and exposed to 24 hours of cold UW storage or the same time period of normothermic machine perfusion. Both groups of livers were reperfused utilising the circuit as a surrogate for transplantation, and perfusate sampled to assess levels of ß-galactosidase (UV spectrophotometry) as well as traditional markers of liver injury and function.

Results: A sharp rise in ß-galactosidase was seen on reperfusion of cold preserved livers (to a maximum of 3000U/ml) which preceded the rise in transaminases and GGT. This contrasted dramatically with normothermically preserved livers, where the level never exceeded 250U/ml. The disparity in glycohydrolase levels was more marked than any other measured parameter representing liver damage. Good correlation between ß-galactosidase levels and liver viability was also evident, with a return to baseline levels towards the end of a 24 hour reperfusion period in organs displaying continued function.

Conclusions: The measurement of ß-galactosidase is a simple and cheap test to assess ischaemia/reperfusion injury in liver transplantation. It appears to be more sensitive than traditionally used markers of global liver injury, with a faster and larger increase in the face of this type of liver damage. It may well be useful in the viability assessment of a liver during machine preservation prior to transplantation, so that rational decisions regarding the use of organs can be made.

C56 AN ECONOMIC COMPARISON OF TACROLIMUS AND CYCLOSPORIN MICROEMULSION IN KIDNEY TRANSPLANTATION.

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Background: An extensive database regarding medical resource utilisation has been collected throughout a prospective, randomised study investigating risk factors for graft failure. The aim was to compare both the clinical outcomes and the relative cost effectiveness of a tacrolimus-based (Prograf ®) therapy with a cyclosporin-based therapy (Neoral ®).

Methods: Analysis includes 179 patients transplanted between 1996-1999 that consented to participate in the study. They were randomised to receive either tacrolimus (TAC) (starting dose of 0.2mg) (n=90) or cyclosporin microemulsion (CYA) (starting dose of 8mg/kg) (n=89). In addition patients received azathioprine and steroids. The end points of data collection were death with a functioning graft, death on dialysis following a graft failure and for the survivors - the time of analysis (February 2000). Pharmaco-economic data was collected to compare the relative cost-effectiveness of the two treatments from a hospital's perspective. Only direct medical costs were considered; the costing was based on an intent-to-treat principle. Medical resource utilisation data (treatment medication, concomitant medication, and length of initial hospitalisation as well as re-admissions, requirement for dialysis, diagnostic tests and additional surgical procedures) were costed alongside the associated clinical outcome measures (patient survival, graft survival, patients with a rejection-free graft). Sensitivity analyses examined the impact of cost variations of the medical resources on the overall costs.

Results: The median follow-up was 2.7 years. There were 5 deaths and 7 graft failures in the TAC group and 10 deaths and 11 graft losses in the CYA group. The median time to graft loss (excluding deaths with functioning graft) was 484 days (range 158-1031) in TAC group and 384 days (range 1-1056) in the CYA group (p=0.30). The allografts survival was longer in the TAC group (89,161 vs. 81,142 patientdays). In the TAC group 22% (n=20) of patients and in CYA group 24% (n=21) of patients experienced an acute rejection that responded to steroid treatment alone (p=0.86). The incidence of acute rejections treated by more complex therapeutic interventions was 13% (TAC) and 24% (CYA) (p=0.09). The cost calculation revealed that the main sources of the cost difference between the treatment groups were treatment medication and hospitalisation. The total cost of all immunosuppression in TAC group was higher (£916,215 vs. £602,806), but the cumulative costs associated with hospitalisation, return to dialysis and the conduct of diagnostic tests for graft dysfunction and complications was higher for the CYA patients (£1,006,748 vs. £786,611). The average cost per patient in the 1st year and the subsequent years was similar for the two treatment groups, £10,794 (CYA – 1st yr) vs. £10,695 (TAC- 1st yr) and £5,041/year vs. £4859/year respectively. All relevant clinical outcomes (patient survival rate, graft survival rate and graft rejection-free rate) were superior in the TAC group. As a result, TAC was more cost-effective than CYA in terms of cost per survivor (£20,033 vs. £20,374), cost per patient with a surviving graft (£21,555 vs. £22,670), and cost per patient with a rejection-free graft (£29,359 vs. £34,246). The sensitivity analyses proved that the results were stable.

Conclusion: The results of this analysis showed that tacrolimus is cost effective compared with cyclosporin microemulsion in kidney transplantation.

C58

COMPLEMENT ACTIVATION FOLLOWING RENAL TRANSPLANTATION: IS DEPOSITION OF C4D ATTRIBUTABLE TO RECIPIENT ANTIBODIES OR ISCHAEMIA/REPERFUSION INJURY?

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It has been proposed that activation of the complement cascade by early inflammatory events following renal transplantation contributes to the pathogenesis of acute rejection. In transplantation, complement activation is thought to occur by the antibody-mediated classical pathway but there is mounting evidence to suggest that ischaemia/reperfusion injury may also be involved. The aim of this study was to determine whether deposition of complement protein C4d in the renal vasculature was activated by antibody-mediated events or ischaemia/reperfusion injury.

Biopsies were obtained both before transplantation and after reperfusion from living related donor (LRD; n=19) and cadaveric (n=55) renal allografts. Cryosections were stained with an anti-human C4d monoclonal antibody using an indirect immunoperoxidase technique and staining of the venules, glomerular and intertubular capillaries graded semi-quantitatively. The results of the staining were analysed with respect to comprehensive antibody screening and crossmatching data, HLA mismatch analysis, cold ischaemia time, graft function and rejection.

Our results demonstrated that in normal living donor kidneys, C4d was often present in the glomerular capillaries (7/11), with moderate, occasional staining of intertubular capillaries and venules before transplantation. Similarly, C4d was present before transplantation in the glomeruli of 30/55 cadaver donor kidneys. Following reperfusion of the graft, only 1/11 LRD renal allografts demonstrated an increase in C4d deposition in the intertubular capillaries following reperfusion. In contrast, an increase in C4d deposition was detected in the intertubular capillaries (17/55; $p<0.05$) and venules (7/55) of cadaver renal allografts, but not in the glomeruli. C4d deposition following reperfusion was not associated with the level of allosensitisation, the presence of autoantibodies or crossmatch results. Moreover, the deposition of C4d post-reperfusion did not correlate with cold ischaemia time, delayed graft function nor acute rejection episodes.

The results from this study suggest that C4d deposition in the capillaries following reperfusion of cadaveric renal allografts is not activated by the antibody-mediated classical pathway, but may be manifest in part, by inflammatory events associated with ischaemia/reperfusion injury. Furthermore, the presence of C4d in transplant biopsies must be interpreted cautiously because of the high levels detected in normal kidneys.

C68

Which is the better method for control of post-renal transplantation (RTx) hyperparathyroidism (HPT) - total or subtotal parathyroidectomy (PTX)?

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HPT after RTx is uncommon but can have serious consequences both for the allograft and the patient. Which surgical PTX procedure to perform is controversial for patients on dialysis and after RTx.

Since 1967, 1780 patients have undergone RTx at this unit with long-term follow-up. In this period 21 RTx patients have undergone 26 surgical PTX. Operations performed included 14 total PTXs – with significant enlargement / hyperplasia of all glands - (9 primary total PTX and 5 effective total-PTX after removal of remnant tissue left behind after previous subtotal PTX), versus 12 (deliberate) subtotal PTX. Of the 5 patients who had undergone redo PTX for recurrent HPT, 4 had previously undergone subtotal PTX when on dialysis, and one after Tx

Mean patient age was 51 years (range 19 to 77 years), with 8 men and 13 women. Mean follow-up was 52 months (range 1 to 280 months) from PTX to death or present day. Only one patient died (four years later from cancer); all patients have functional allografts. There were no important post-operative surgical complications. Vitamin D therapy was needed long-term in the majority of patients post-PTX – 15/21 (71%) – in 11 / 12 (92%) of total-PTX compared with only 4 / 13 (31%) who had undergone subtotal-PTX ($p < 0.05$ by Chi-squared). One patient who underwent a subtotal-PTX post RTx required excision of the remnant gland for recurrent hyperparathyroidism three years later (1 / 12 or 8.25 % recurrence rate, cf. 0% for total-PTX group).

Biochemistry at the latest follow-up is tabulated:

Calcium	Mmol/l	Patients	IPTH	Nmol/l	Patients
LOW	<2.2	1 (total-PTX)	LOW	<10	12 (11 total-PTX)
NORMAL	2.2-2.6	22	NORMAL	10-65	3
HIGH	>2.6	2	HIGH	>65	6

Our results show that long-term post-PTX hypocalcaemia was rare, but that vitamin-D dependency was present in 70% of patients (and in >90% of patients after total-PTX). Long-term low-PTH (ie functional hypoparathyroidism) was present in 50% of patients, nearly all after total-PTX. This was of no clinical significance however. Total-PTX is the definitive procedure for HPT (ie no recurrences) but at the expense of long-term vitamin-D dependency.

C69

ALLOCATION OF KIDNEYS BY A BLOOD GROUP COMPATIBLE RATHER THAN IDENTICAL SYSTEM – IMPROVEMENT IN EQUITY OF ACCESS AND HLA MATCHING

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Each patient on the transplant list should have as equal a chance as possible of a cadaver kidney offer, in a manner that also provides the best chance of long term transplant survival. The current system of organ allocation optimises graft survival by HLA matching, but we believe it could be altered to improve equity of access for potential recipients.

Our recipient waiting list contains an excess of people of blood groups B and AB, who do not have equity of access to transplant offers because of their blood groups. We performed a modelling exercise using all the recipients on our current list, matching them to the last 100 donor organs transplanted in our centre. Kidneys were matched according to local policy, namely, a hierarchy of HLA DR matching; HLA A and B matching; and for matching ties, scoring based on waiting time, age match and matchability. Matching was performed by allocating kidneys on a blood group identical basis, and then again on a blood group compatible basis (except that group O kidneys were not 'offered' to group A recipients).

There were 136 recipients, 64 blood group O (47%), 37 group A (27%), 25 group B (18%), 10 group AB (8%). There were 100 donors, 51 group O, 39 group A, 6 group B, 4 group AB. The mean chance of a kidney offer to each recipient in the modelling exercise was:-

	Group O	Group A	Group B	Group AB
Group Identical	0.8	1.05	0.24	0.4
Group Compatible	0.61	1.0	0.64	0.61

In the blood group compatible system compared to the identical system, 14 kidneys were reallocated, 11 on the basis of an improved HLA DR match. Overall, 54 matches were HLA compatible by the blood group identical system, rising to 64 by the group compatible system. The chance of an offer to an Asian recipient rose from 0.39 to 0.58.

We conclude that this modelling exercise showed that allocating cadaver kidneys by a blood group compatible system could improve donor-recipient HLA matching, and also improve equity of access to transplantation for those with blood groups B and AB. We have changed our local allocation policy in light of these results. We suggest that national and other local kidney allocation systems should be examined to determine whether they offer the best possible equity of access to transplantation.

C70

GEOGRAPHICAL VARIATION IN SKIN CANCER RISK POST-TRANSPLANTATION: A MATCHED COHORT COMPARISON BETWEEN A TEMPERATE AND SUB-TROPICAL CLIMATE

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Non-melanoma skin cancer (NMSC) is an important complication of solid organ transplantation, and occurs with variable geographical prevalence according to Registry data. We conducted a prospective sequential matched cohort study to determine the actual difference in prevalence of NMSC between a Northern European and Australian Caucasian renal transplant population. 183 European and 398 Australian allograft recipients were interviewed and examined by a single dermatologist utilising a standardised pro-forma between May 1997 and July 2000. Only histologically-proven NMSC was included. 139 pairs of Caucasian recipients were matched by gender (63.3% male), age at transplantation (mean 41.3 at one centre in the UK (UK) and 41.1 in Queensland (QLD): mean difference 0.13 years) and mean follow-up (8.5 (UK) and 8.4 (QLD) years: mean difference 0.09 years). Statistical analysis was performed using conditional logistic regression Prevalence of NMSC in UK versus QLD was 17.3% vs 56.8% respectively ($p<0.001$; odds ratio (OR) = 12). NMSC prevalence (UK versus QLD) rose from 10.2% vs 28% at <5 years post-transplantation ($p=0.08$); to 15.2% vs 60% at 5-10 years ($p=0.01$); and 27.3% vs 86.4% at >10 years ($p<0.001$). Prevalence of squamous cell carcinoma >10 years post-transplantation was 20.5% (UK) vs 65.9% (QLD); $p=0.001$:OR=11: basal cell carcinoma 22.7% (UK) vs 68.2% (QLD); $p=0.003$, OR=20. The proportion of patients with actinic keratoses ($p<0.001$; OR=29.5) and warts ($p=0.002$; OR=3.5) were also increased in the Australian cohort. The distribution of skin types, eye and hair colours were not significantly different.

There is a greater variation in the prevalence of NMSC post-transplantation between a temperate and sub-tropical climate than has previously been recognised from Registry sources.

C74

PAEDIATRIC RENAL TRANSPLANTATION IN NOTTINGHAM – EXPERIENCE FROM THE FIRST HUNDRED

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The paediatric renal transplantation programme in Nottingham began in 1985. We analysed the results of the primary grafts in 100 patients between October 1985 and June 2000. The policy of the unit was to transplant infants over 2 years of age or 10 kg body weight.

The mean age at the time of transplantation was 10.4 years ± 4.5s.d. Reflux disease and posterior urethral valves were the commonest primary cause of renal failure (13% each). 33 patients received a pre-emptive transplant and 67 were on dialysis (19 haemodialysis and 48 peritoneal dialysis). The mean duration of dialysis was 9.6 months ± 10.8s.d, with patients waiting a mean of 7.9 months ± 10.25s.d before transplantation. 83% of patients received cadaveric donor kidneys (CADs) and 17% received kidneys from living related parental donors (LRDs). Immunosuppressive regime consisted of Cyclosporin, Prednisolone and azathioprine up until 1997. Tacrolimus and Mycofenolate mofetil have been introduced in our protocol over the last 3 years (part of randomised trials). Graft survival analysis was performed by the Kaplan-Meier method and the log rank test. Spearman's rank correlation coefficient (rho) was used to assess association between variables. A p-value of <0.05 was taken to be significant.

After a mean follow up of 60 months ± 47 s.d, overall patient survival was 96%. The cumulative graft survival at 1 and 5 years was 83% and 67%, respectively. There was no significant difference in graft survival between CAD and LRD transplants at 1 year (81% vs. 93%), but LRD did significantly better at 5 years (CAD 63% vs. LRD 93%, $p< 0.05$). The graft survival of pre-emptive transplant recipients < 10 years of age was significantly better than all other patients at 1 and 5 years (92% and 92%, $p<0.05$, respectively). Thrombosis (11%) and chronic allograft nephropathy (6%) were the commonest causes of graft failure. Thrombosis was more evident in the first 50 transplants (n=7) treated between 1985 and 1995 compared with the 50 transplants (n=4) between 1995 and 2000. A strong negative correlation was found between graft thrombosis and age at transplant (Spearman's rho 0.326, $p<0.01$) and time on dialysis (Spearman's rho 0.341, $p<0.01$). Time on dialysis also had a strong positive correlation with the number of acute rejection episodes (Spearman's rho 0.270, $p<0.01$). This association was not significant between 1 and 6 months.

The results are broadly consistent with those reported from other centres with a striking improvement in early graft survival in the last 5 years. The reduction in graft thrombosis has been a cumulative learning curve, including the reluctance to use small donors and those with multiple arteries and the routine use of Aspirin. The highly significant association between time on dialysis and age on the one hand and graft thrombosis on the other suggests that pre-emptive transplantation is both safe and recommended for young patients.

C75

PRE-EMPTIVE KIDNEY TRANSPLANTATION – THE BETTER OPTION IN YOUNG CHILDREN

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Pre-emptive kidney transplantation (PETs) in paediatric patients remains controversial. Some units have reported favourable effects on graft survival, while others have found a worse outcome compared to post-dialysis transplantation (PDTs). This study reports our retrospective analysis of the results of PET compared with PDTs in Nottingham.

33 primary PETs and 67 primary PDTs between 1985 and 2000 were analysed. The two groups were then split based on age at transplantation being greater or less than 10 years old. Graft survival at 1 and 5 years was analysed using the Kaplan-Meier method and the log rank test was used to test for significant differences in survival between groups. A p-value of less than 0.05 was taken as significant.

Mean age ± SD of PETs and PDTs was 11.5 ± 3.64 years and 9.8 ± 4.9 years, respectively. 12 PETs (36%) and 33 PDTs (49.3%) were less than 10 years old at transplantation. No significant difference was found between the groups with regard to immunosuppressive regimens used or the proportion of living related transplants.

Graft survival for all PETs and PDTs at 1 year was 87.9% and 80.6% and at 5 years was 71.4% and 68.8%, respectively. Graft survival of PETs less than 10 years old at 1 year and 5 years was 91.7% and 91.7%, respectively. This was significantly better than those observed in PDTs of similar age (78.5% and 63.1% respectively). In contrast, graft survival of PETs greater than 10 years old at 1 and 5 years was not different from PDTs (78.8% vs. 85.7% and 63.1% vs. 74.5%, respectively).

Our results suggest that pre-emptive paediatric kidney transplantation is best performed in children below the age of 10 years. In this age group graft survival is significantly better at 1 and 5 years. In children above the age of 10 years pre-emptive transplantation does not offer significant survival advantage for the graft at 1 year and they in fact do significantly worse than their post-dialysis transplant counterparts at 5 years.

C76

ACUTE GASTROINTESTINAL BLEEDING FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION IN THE SETTING OF LOW DOSE STEROID IMMUNOSUPRESSION

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INTRODUCTION: Gastrointestinal bleeding (GIB) following orthotopic liver transplantation (OLT) has a reported incidence of 2.3 - 8.9% in series which use conventional steroid regimen for immunosupression. In cases where the bilary reconstruction was performed with a Roux-en-Y loop an incidence of GIB as high as 19% has been reported.

AIM: To analyse the incidence and clinical presentation of GIB following OLT in a setting of an immunosupression regimen of low dose steroids, calcineurin inhibitors and azathiaprine.

METHODS: Analysis of prospectively collected information on all 1143 adult patients (1251 OLT) who underwent OLT at our institution between Jan 1990 and Oct 2000. GIB was characterised as clinically apparent acute blood loss from the gastrointestinal tract requiring blood transfusion. All patients received acid suppression with either ranitidine or a proton pump inhibitor. Immunosupression consisted of a combination of a calcineurin inhibitor (cyclosphorine or tacrolimus), azathioprine and prednisolone; the latter was started at 20mg postoperatively and tapered to zero within 3 months.

RESULTS: GIB was observed in 42 patients (incidence 3.7%) with a median time of clinical presentation following OLT of 44 days (range 2 - 2236). In 26 patients (62%) GIB occurred within the first three postoperative months. Identified causes of GIB were 20 (48%) peptic upper gastrointestinal ulcers; 11 (26%) bleedings from gastric or oesophageal varicies; 3 (7%) oesophagitis/gastritis; 3 (7%) rupture of a hepatic artery pseudoanneurysm into a Roux loop. In 5 patients (12%) the cause could not be specified. All patients were resuscitated with intravenous fluid and a median of 6 units of blood (range 2-45) was transfused. Endoscopy was performed in 38(90%) patients. Endoscopic therapeutic intervention was successful in 9(21%) instances to control bleeding, surgery was required in 15(36%) patients and 18(43%) patients were managed conservatively. The 30-day mortality following GIB was 33% and one-year patient and graft survival was 43% and 29% respectively.

CONCLUSION: GIB following OLT occurred at a relatively low overall incidence (3.7%) in our series and was seen predominantly (62%) during the first 3 postoperative months. It is associated with a high rate of death and graft failure emphasising the importance of optimised prevention and early detection.

C79

Rapamycin and reduced dose Cyclosporin produce effective immunosuppression for Denovo renal transplant recipients.

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Introduction: Cyclosporin (CyA) causes both acute and chronic nephrotoxicity and thus attempts are underway to limit CyA exposure in renal transplantation. Rapamycin (Rapa) is a non-nephrotoxic drug with synergistic immunosuppressive properties when combined with CyA. The aim of this study was to determine the safety and efficacy of a reduced dose CyA / Rapa regimen compared to conventional full dose CyA and FK506 regimens in denovo renal transplant recipients.
Methods: Forty-five consecutive cadaveric renal transplant recipients were studied. Fifteen patients initially received CyA 5 or 7 mg/kg/day with Rapa 2mg/day, 15 received CyA 15 mg/kg/day and 15 received FK506 0.2 mg/kg/day. All patients were given Prednisolone 20 mg/day. Full dose CyA was reduced by 2mg/kg/wk aiming to achieve trough levels of 300 ng/ml at 1 month. Reduced dose CyA and FK506 were adjusted to maintain trough levels at 150-200 ng/ml and 5-15 ng/ml after 1 month respectively. Data was analysed on an intention to treat basis.
Results: Patient characteristics, HLA mismatch and ischaemic times were similar in each group. One patient taking full dose CyA and one taking Rapa died. Allograft survival was 14/15 (93%), 11/15 (73.3%) and 14/15 (93%) in each group respectively. Acute rejection occurred in 4/15 (27%) Rapa, 4/15 (27%) full dose CyA and 5/15 (33%) full dose FK506 patients. Delayed graft function was seen in 2 patients in both full dose regimens and 3 of those taking Rapa. After 3 months follow-up, mean serum creatinine (±sd) was 149 (64) vs 179 (89) vs 141 (61) µmol/L and mean (±sd) estimated GFR was 67.9 (26.7) vs 62.3 (33.2) vs 64.3 (21.8) ml/min for each group respectively. Rapa caused hypertriglyceridaemia that was persistent at 3 months (3.24 (1.49) vs 1.83 (0.60) vs 1.91 (0.85) mmol/L, $p<0.01$). There were no significant differences in serum cholesterol between groups but 7/15 Rapa patients were started on HMG CoA reductase inhibitors over this period compared to 7/15 and 2/15 and 1/15 in the full dose CyA and FK506 groups ($p<0.05$). Rapa did not cause clinically relevant haematological abnormalities.
Conclusion: The use of a combination of Rapa and reduced dose CyA in denovo renal transplant recipients is safe and effective, yielding results comparable to conventional immunosuppressive regimens. Although hyperlipidaemia was not dramatic, a significant proportion of the Rapa patients were taking lipid-lowering medication.

C81

BASILIXIMAB FOR GVHD AFTER LIVER TRANSPLANTATION?

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Graft-versus-host-disease (GVHD) after liver transplantation (OLT) is a rare but well documented complication. Treatment usually consists of supplementing standard immunosuppression with ATG or OKT3, but in most the disease progresses with pancytopenia, sepsis, and multi-organ failure, resulting in mortality rates over 80%. Recently two patients in our unit with established GVHD after OLT have been treated with Basiliximab, a chimeric antibody against the alpha-chain of interleukin-2 receptor (IL-2R). Our rationale was based on the hypothesis that inhibition of activated donor derived lymphocytes might ameliorate the disease.

Both patients were male, one 45 years old with alcohol related liver disease and alpha-1-antitrypsin deficiency, and the other 61 year old with hepatitis B virus cirrhosis and hepatocellular carcinoma. The clinical presentation is detailed below.

Patient	Onset of Skin rash	Diarrhoea	Peak Chimerism (Flow cytometry)	Peripheral Blood	Biopsy confirmation
1	Trunk, palms, soles appearing on day 24	Present from day 30	5%	Lympho-penia	Skin, rectum
2	Back, palms, legs appearing on day 26	Profuse starting day 8	40%	Pancytope nia	Marrow, skin

Treatment comprised 3 doses of 1 gm methyl prednisolone followed by 2 doses of 20 mg of Basiliximab with an interval of 4 days between the two doses. This resulted in depletion of CD25 positive lymphocytes. Patient 1 responded slowly to therapy with reduction of skin rash by day 7. Diarrhoea persisted and a white cell scan showed increased uptake in the terminal ileum. Right hemicolectomy was performed, removing 40 cm of small bowel which had multiple ulcerated, mucosal areas in Peyer's patches. The patient is alive and well, with normal liver functions 4 months after OLT. Patient 2 had complete resolution of skin rash within 5 days but remains pancytopenic. No clinical adverse side effects attributable to Basiliximab occurred in either patient. This is, to our knowledge, the first report of the use of monoclonal antibodies against IL-2R, in the treatment of GVHD after OLT.

C85

INFLUENCE OF PRE-TRANSPLANT PROCEDURES ON LIVER TRANPLANTATION (LT) FOR PRIMARY SCLEROSING CHOLANGITIS (PSC)

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Introduction:, A significant number of patients undergoing LT for PSC have had previous surgical or endoscopic interventions. We wished to assess whether these procedures influenced the outcome of LT for PSC.

Methods: We reviewed 152 PSC patients transplanted between 1986 and May 2000 who underwent a total of 173 LT. (11% of a total 1336).

Results: 108 (71%) were male. Pre LT biliary interventions were performed in 44 (29%) patients - cholecystectomy and common bile duct exploration (CBDE) 18(12%), bilioenteric anastomosis (BA) 12(8%), radiological procedures (RP) (sphincterotomy, transhepatic drainage, stent) 11(7.2%). In addition, 13 (13%) patients had a colectomy, and of these 1 had a BA and 2 had biliary endoscopic procedures. Median follow-up was 4.4 years. Overall actuarial survival was 85%, 70%, and 60% at 1, 5 and 10 yrs respectively. Graft survival was 75%, 61% and 45% at 1, 5 and 10 yrs respectively. Graft loss was due to hepatic artery thrombosis (HAT) 10, recurrent PSC 4, chronic rejection 4, massive hemorrhagic necrosis 4 and PGNF 1. 60% (6/10) of patients with HAT had had intervention pre LT (1 CBDE, 2 BA, 1 RA, 2 colectomy).

	Patients	Age	ITU (days)	Hosp (days)	Blood loss(U)	Op.time (hrs)	Child (A,B,C) %
non intervention	98	44(7-67)	5(1-99)	19(1-130)	6(0-29)	5.7(3-10)	1,49,69
CBDE	18	48(35-66)	9(1-27)	23(2-53)	11(0-30)*	6.4(5-10) †	0,33,67
BA	12	48(40-64)	5(1-12)	17(1-29)	20(0-80)*	7(5-9) †	0,33,67
RP	11	42(18-57)	4(1-10)	17(10-27)	9(0-50)	5.8(4-8)	0,36,64
colectomy	13	45(34-56)	8(1-24)	23(6-58)	4(1-10)	6.3 (3-8)	20,40,40

*p<0.001, † P<0.06

Survival was better in patients with no previous intervention –87%, 80%, 70% at 1, 5 and 10 yrs respectively compared with 73%, 60% and 30% in biliary surgical intervention groups (CBDE, BA) ($p<0.05$). Colectomy, however, led to an improvement in survival up to 90% after 10 yrs ($p<0.05$).

Discussion: One third of PSC transplanted patients have had a pre LT biliary intervention. 8.5% of patients have had a colectomy pre LT. Surgical intervention increases blood loss and operation time and can be associated with a graft loss secondary to HAT. Survival is better in patients without pre LT biliary or radiological intervention.

C87

RENAL TRANSPLANTATION IN PATIENTS WITH URINARY DIVERSIONS.

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AIMS: This retrospective study looks at renal transplantation and outcome in patients with urinary diversions in two large transplant units. The cause of kidney failure, type of diversion, co-morbid conditions, surgical problems, complications, patient and graft survival are evaluated.

MATERIALS AND METHODS: Patients with urinary diversions form 2% of patients transplanted. Since 1980, 60 patients have had 64 kidney transplants into urinary diversions, the majority (57) into ileal conduits, one into a colonic conduit and two into ileocaecocystoplasties. Ten of these transplants have been live-related while 12 (20%) have been in children In about 50% the primary etiology was spina bifida while the other half consisted of a variety of primary pathology, including posterior urethral valves, ectopia vesicae, cystectomy for tumour, tuberculous cystitis etc. The operation itself was technically more demanding due to different factors, including kyphoscoliosis, retroperitoneal fibrosis, hypoplastic iliac vessels and variable intraperitoneal positions of the conduit. Most of the kidneys were placed upside down so that the ureter took the shortest and most direct route to the bladder. Post operative management was unremarkable with all patients receiving standard immunotherapy.

RESULTS: The 1 year patient survival is 95% whereas the graft survival is 90% and at 3 years the patient survival 88% and graft survival 82%Complications included, urinary leaks, bowel obstruction and infarction, volvulous of an intraperitoneal kidney, calculous obstruction and IVC thrombosis among others.. There was a rejection rate of 44% treated in the standard way with two patients requiring ATG. Over 90% of recipients had one or more urinary tract infections which were easily treated, but recurrent infections suggested obstruction of the ureter.

CONCLUSION: Patients with urinary diversions form a small but significant group of those transplanted. In spite of multiple co-morbid problems and technical difficulties along with increased peri-operative complications, the eventual patient and graft survival is not inferior when compared to the rest of the transplant population. A successful transplant in this particularly unfortunate renal failure group contributes significantly to their quality of life.

CLINICAL

C89

THE ROLE OF ADJUVANT CHEMOTHERAPY IN LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is the commonest malignancy in men worldwide. For patients developing HCC without cirrhosis or with minimal disease (Childs A) liver resection provides the most appropriate form of therapy. The majority of cases of HCC however arise in the presence of more severe liver disease, in whom orthotopic liver transplant (OLT) provides the only form of curative treatment. In early series unselective use of OLT alone in HCC led to a high rate of tumour recurrence, prompting the use of adjuvant chemotherapy administered either pre-operatively by an intra-arterial route or post-operatively. We have performed OLT in a series of 18 patients with HCC, which developed as a complication of cirrhosis in 17 cases. An adjuvant regime of doxorubicin is used where 10mg/m^2 is given via the hepatic artery pre-operatively, and 10mg/m^2 is given peripherally during the anhepatic phase of OLT and at weekly intervals post-operatively to a maximum dose of 200 mg/m^2. Of the 18 patients treated with this regime 12 are alive and tumour free (follow up 1 to 52 months, median 15 months). Four patients died of recurrent HCC within the first year post-operatively and two patients died in the early post-operative period due to complications of the transplant procedure. Known risk factors for recurrence of HCC were compared in patients who died of tumour recurrence and those surviving free of tumour. A clear pattern of risk factors was not identified, in that both groups contained patients with multifocal tumours, tumours greater than 5cm diameter and microscopic vascular invasion by tumour. However the serum alpha-foetoprotein measured pre-operatively provides an indicator of tumour recurrence, as levels >1000 were noted in three of the four patients who died of recurrent HCC whereas levels <250 were noted in patients surviving free of tumour. In conclusion one-year survival rates of >50% are obtainable in patients with HCC treated with OLT and adjuvant chemotherapy, and serum alpha-foetoprotein estimation may be a useful indicator of patients at high risk of recurrence.
CLINICAL

C93

MEASUREMENT OF 2 HOUR POST-DOSE CYCLOSPORIN LEVEL (C2) AND ITS ASSOCIATION WITH EARLY ACUTE RENAL ALLOGRAFT REJECTION

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It is reported that the incidence of early acute renal allograft rejection (AR) may be reduced if 2 hour post-cyclosporin (CsA) dose blood levels of 1700 ± 350 ng/ml are achieved within 5 days of transplantation.

Over the past few months (June 2000 – present), we have measured both trough (C0) and 2 hour (C2) whole blood CsA levels on a thrice-weekly basis unit in newly transplanted patients. During this period, CsA dose adjustments have been made solely on the basis of C0 values. We have then examined retrospectively the relationship between C2 levels at or around the fifth post-operative day and the occurrence within the first 20 days following transplantation of AR. It was decided to take a C2 measurement of 1500 ng/ml as a target minimum.

Thirty-three patients were transplanted during the period of study. The standard immunosuppressive regimen consisted of prednisolone, azathioprine (AZA) and CsA, but some patients received Simulect and/or mycophenolate mofetil (MMF). C2 data was available in all but 3 cases. Eleven patients had C2 values of above 1500 ng/ml (group A). None of these developed AR. Three of the 11 had received Simulect, 2 of them in combination with MMF instead of AZA. The remaining 19 patients had C2 values of below 1500 ng/ml (group B). Five of these developed AR. Of the 14 in group B who did not develop AR, 2 had received Simulect and 2 others were taking MMF instead of AZA (AR did not occur in these patients).

The difference in outcome between groups A and B did not reach statistical significance ($p = 0.06$). The median value of C0 at or around day 5 post-transplant in patients who developed AR was 350 (322-379) ng/ml, compared to 365 (278-515) ng/ml in those who did not develop AR ($p = 0.3$).

In summary, this analysis of C2 data collected at or around day 5 post-transplant appears to support the notion that higher peak blood levels of CsA are associated with a lower incidence of early AR. We have not rigorously examined the effect of the many potential confounding factors which may influence graft outcome. Our findings also suggest that early CsA dose alterations should be guided by C2 rather than C0. Success in achieving target C2 levels may require the construction of dosing algorithms based on CsA pharmacokinetic data.

C97

THE EFFECT OF WEIGHT DIFFERENTIAL ON THE OUTCOME OF ORTHOTOPIC LIVER TRANSPLANTATION IN ADULTS

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Introduction - The process of matching donors and recipients by body weight is an important element of successful orthotopic liver transplantation (OLT).

Aim - The aim of this study was to evaluate the effect of weight differential (W.D) between donor and recipient in OLT in adults.

Methodology - 102 patients whose donor:recipient weight ratio was > 1 and who received their first OLT in our unit between 1st June 1995 and 31st May 2000 were studied. For each donor-recipient pair, the donor:recipient weight differential (WR) was calculated using the formula

$$W.D = \frac{\text{donor wt - recipient wt}}{\text{recipient wt}} \times 100$$

Recipients were thereafter divided into 3 groups - Group I - W.D = < 10%; Group II - W.D = 10-20%; Group III - W.D = > 20%. Data on the results of OLT were collected from a prospectively maintained database and the incidence of wound complications, vascular complications, septic complications and ascites were studied.

Results - Patients from Group III spent more time in hospital post transplant (36.8 days) compared to Group I (31.6 days) and Group II (31.3 days). They also spent longer time in ITU (8.7 days) compared to 6.3 days in Group I and 5.2 days in Group II. The mean number of days spent in hospital in the first 90 days post transplant was also different - (Group I - 32.7; Group II 33.0; Group III 37.1).

COMPLICATION	Grp I (n = 47)	Grp II (n = 35)	Grp III (n = 19)
Wound Failure	1 (2.1%)	1 (2.8%)	3 (15.8%)
Vascular Complications	7 (14.9%)	4 (11.1%)	3 (15.8%)
Graft Failure	8 (17%)	3 (8.3%)	6 (31.6%)
Re Transplant	6 (12.8%)	2 (5.6%)	4 (21.1%)
Death	10 (21.3%)	6 (16.7%)	5 (26.3%)

Complications and mortality are shown in table above.

Conclusion:- Although complications pertaining to wound failure, graft failure and vascular complications seem to be more when the W.D is > 20%, these do not seem to influence the ultimate outcome of OLT. There is however, slight increase in hospital stay and stay in ITU in these patients.

C100

Two year outcome of renal allograft recipients comparing Cyclosporin A (Neoral)-led with Tacrolimus-led therapy.

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Abstract

Background. There is a lack of long term follow up data comparing the Neoral formulation of cyclosporin with tacrolimus in kidney allograft patients. Despite this tacrolimus has replaced cyclosporin as the calcineurin phosphatase inhibitor of choice in many centres. This single-centre retrospective, observational cohort study aimed to investigate outcome in terms of graft survival, acute rejection rates and renal function over a two year follow up period.

Methods and Results. Of 109 consecutive patients transplanted between June 1995 and June 1998, 62 were commenced on cyclosporin Neoral-led and 47 on tacrolimus-led immunosuppressive therapy. The demographics of these groups and their supplementary immunosuppression were not significantly different. The groups were found to have identical graft survival at 24 months (86.8% in both groups) with comparable patient survival at 97.9% for cyclosporin and 100% for tacrolimus-treated patients. The incidence of acute rejection was significantly less with the use of tacrolimus (29.8% vs.51.6%). Glomerular filtration rate (GFR) was estimated using the Jelliffe equation; a statistically significant decline in GFR being observed only in the cyclosporin-treated patients (0.17ml/min/month).

An increased incidence of diabetes mellitus in those treated with tacrolimus documented in other studies was not found in this study but systolic blood pressure at 12 months was lower in tacrolimus-treated (133±17mmHg) compared with cyclosporin-treated (144±17mmHg) patients.

Conclusion. The results indicate a satisfactory outcome with tacrolimus-led immunosuppressive therapy. Although not a head to head study these are superior to that experienced by comparable patients recieving Neoral-led therapy in this institution.

C103

A SINGLE CENTRE ANALYSIS OF CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANT RECIPIENTS IN THE CMV PCR ERA

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In renal transplantation CMV infection is one of the most common and potentially serious complication. Although many centres in the UK use prophylaxis treatment, this strategy must depend on local variations such as the intensity of immunosuppression protocols and incidence of CMV infection. Our local immunosuppressive practice comprise cyclosporin (Neoral) based immmunosuppression protocols and no antibody induction or salvage treatment. We use no CMV prophylaxis. With routine use of CMV PCR we have analysed 212 consecutive patients transplanted between June 1997 and May 1999 and followed up for at least 6 months at our centre for disease incidence and clinical outcomes.

The serological status of our recipients were R-/D+, 16.9%; R+/D+, 32.7%; R+/D-, 24.2%, and R-/D- 26.4%. For clinical suspicion of disease PCR analyses were performed on 54% of patients. The total incidence of confirmed CMV disease was 4.3%. On subset analysis 8 of these patients were R-/D+, representing 22.3% of this subset. The remaining patient was R+/D-. Five (5) patients received treatment for acute rejection before infection occurred. Four (4) patients were live donor recipients, 2 of these had been treated for acute rejection. All infections occurred between 5 and 8 weeks post transplant and clinical features were fever (100%), leukopenia (67%), graft dysfunction (56%) and pneumonitis (22%). The median creatinine 1 week before infection was 176 (range 95-280) µmol/l and 3 months after infection 150 (109-284) µmol/l respectively. There was successful treatment of reactivation of infection in one patient by a further course of Gancyclovir. One (1) graft was lost because of chronic rejection 18 months post transplantation.

Our data shows a low overall incidence of CMV infection in our centre with no impact on clinical outcome. We believe the cost/benefit ratio of CMV prophylaxis and/or monitoring at each centre should be assessed in context of local disease patterns. Clinical strategies may change as immunosuppressive treatments evolve and if the incidence of CMV disease increases.

CLINICAL

C104 COMBINED LIVER & RENAL VS RENAL TRANSPLANTATION ALONE IN PRIMARY HYPEROXALURIA TYPE 2

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Primary Hyperoxaluria type 2 (PH2) is a rare autosomal recessive disorder caused by deficiency of glyoxylate reductase/hydroxypyruvate reductase (GRHPR). GRHPR is predominantly expressed in the liver and lack of activity leads to conversion of glyoxylate to oxalate by alternative pathways. Oxalate excretion is entirely renal. Additionally, hydroxypyruvate is reduced to L-glycerate in the absence of GRHPR activity. The resultant clinical hallmarks of PH2 are L-glyceric aciduria and hyperoxaluria which can lead to calcium oxalate stone formation. Ultimately, this may cause end stage renal failure (ESRF). A net negative oxalate balance cannot be achieved by dialysis and without further intervention, fatal systemic oxalosis is unavoidable. In primary hyperoxaluria type 1 (PH1), a more common disorder of glyoxylate metabolism caused by a different enzyme defect, combined liver and kidney transplantation has become the preferred option. Hyperoxaluria is more severe in PH1 and recurrent oxalate deposition occurs if renal transplantation alone is undertaken. The optimal treatment of patients with ESRF due to PH2 is uncertain although combined transplantation where renal failure has occurred provides the opportunity to correct the underlying defect in addition to restoring effective oxalate excretion.

We report our experience in two unrelated families suffering from PH2 including the first report of combined hepato-renal transplantation for management of ESRF. In both families, the diagnosis was made in the index case by enzyme assay on liver biopsy material. In the first family, a 31 year old male received a cadaveric renal allograft in isolation within 3 months of reaching ESRF. After five years, graft function remains well preserved and there is no radiographic evidence of oxalate lithiasis or systemic oxalosis despite continued elevated oxalate production and excretion. The patient's brother, 5 years older, similarly developed nephrolithiasis in childhood but has since retained near normal renal function. In the second family, combined transplantation was undertaken in a 41 year old male who had been on haemodialysis for 7 years. Bone biopsy confirmed systemic oxalosis but extensive work up revealed no functional evidence of cardiac or peripheral vascular oxalate deposition and he was deemed fit for transplantation. The post operative course was complicated. Urgent re-transplantation of the liver was necessitated on day 4 due to hepatic artery thrombosis. At the same time, the renal graft failed. Although the second liver transplant was successful, repeat renal transplantation became impossible as a result of extensive venous thrombosis. The patient remained on haemodialysis and even though excess oxalate production had stopped, he died 13 months later from systemic oxalosis. This patient also has an affected brother (aged 28 at present) who has had nephrolithiasis but retains normal renal function.

Our experience indicates that renal transplantation alone may be an effective option in at least some individuals with PH2 and that the criteria for selecting candidates for combined transplantation requires further consideration. Furthermore, whilst it is still not established what determines the severity of disease in PH2, the affected siblings with independent renal function indicate that clinical management targeted at preventing the progression of stone disease is likely to be crucial.

C107
Anaemia in transplant patients, data from the UK Renal Registry

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In 1998, there were 4,853 transplant patients being followed by the Registry with quarterly data collected on haemoglobin, creatinine, BP in addition to other biochemical parameters. There are no recommended Hb standards for renal transplant patients.

	No. of patients	No patients died	Death rate (95% CI)	K-M 1 yr survival (95% CI)
Transplant	4853	121	2.6	97.4%
Censored at dialysis			(2.1- 3.1)	(97.0% - 97.9%)
Transplant	4853	141	3.0	97.1%
Incl dialysis return			(2.5 –3.5)	(96.6% - 97.5%)

After excluding all patients within the first 6 months post transplant, 6% (95% CI 5.4-6.9%) of all transplant patients were anaemic with a haemoglobin < 10g/dl and 2% (95% CI 1.5 –2.4%) had an Hb < 9g/dl.

The variation between centres was unexplained (1-10% of transplant patients with Hb <10g/dL depending on unit) but possible reasons include quality of graft function, type of immunosuppression and use of erythropoietin in where there are failing grafts.

Centre	% HB <10	HB > 10 95% CI Lower CI	Upper CI	% Hb <9	% missing data in qtr
A	5.2	2.8	8.6	0.0	25
B	9.0	5.8	13.1	0.0	30
C	6.3	3.8	9.6	0.0	11
D	5.3	3.2	8.0	0.6	15
E	5.6	2.1	11.7	0.6	34
F	3.4	0.2	14.3	1.0	41
G	8.4	6.1	11.2	1.2	25
H	3.1	0.5	9.3	1.3	37
I	4.5	1.2	11.4	1.4	18
J	6.8	3.5	11.7	1.5	27
K	4.0	2.2	6.6	1.6	26
L	3.8	0.9	9.4	2.1	26
M	6.9	5.1	9.2	2.2	14
O	4.3	2.0	7.9	2.5	21
P	9.1	3.4	18.5	3.0	27
Q	6.3	3.8	9.4	3.0	9
R	5.1	1.9	10.6	3.4	22
T	3.2	1.2	6.8	4.2	14
E&W	6.1	5.4	6.9	1.9	24

As expected Hb was lower in women and those with failing transplants creatinine.

		Haemoglobin							
Gender	Creatinine	Mean Hb	Std dev	5th centile	Lower quartile	Median Hb	Upper quartile	95th centile	No. with data
Male	<250	13.5	1.6	10.8	12.3	13.5	14.6	16.1	1913
Male	250+	11.4	1.9	8.7	10.0	11.2	12.6	14.8	284
Female	<250	12.4	1.6	9.9	11.2	12.4	13.4	15.1	1235
Female	250+	10.6	1.7	7.5	9.4	10.8	11.7	13.3	142

C110

Adult Multivisceral Abdominal Transplantation – U.K. Experience

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The development of increasingly powerful immunosuppressive agents in the last decade has allowed the transplantation of multi-visceral abdominal organ clusters as a therapy for diseases that were previously untreatable. Such operations provide an elegant anatomical solution and also offer the *theoretical* possibility that the transplantation of a large volume graft may help the development of a tolerant state in the recipient. The number of patients that have undergone such procedures worldwide is small and it is difficult to define evidence-based criteria for the selection of such patients. The key factor determining whether the patient receives a graft, once listed for transplantation, is the availability of a size-matched, multi-organ donor. The magnitude of the procedure is increased by the previous multiple laparotomies and resections that these patients have undergone. The authors present the outcome of four patients transplanted in the 1990's in the United Kingdom.

Patient	Centre	Year of transplantation	Organs removed	Organs implanted	Outcome / follow-up
1	Leeds	1994	L/Pr/SB/Sp/ Co	L/Pr/SB	Alive / 6yrs
2	Leeds	1994	L/Pr/SB/Sp/ Co	L/Pr/SB	Died at 2 yr. - spinal met.
3	Camb	1994	St/L/D/Pr/ SB/K	St/D/L/Pr/ SB/K	Died 5yrs- fall at home
4	Camb	1998	St/L/D/Pr/ SB/ Sp/ K	St/D/L/Pr/ SB/K	Alive / 2yrs

St- Stomach; D- Duodenum; L- Liver; SB- Small Bowel; Pr- Pancreas; Sp- Spleen; K- Kidney; Co – Colon.

Patient 1 was transplanted for widespread abdominal carcinoid and continues to have good graft function. The other procedures in this series have been performed for both benign and malignant disease. In some cases massive intra-operative transfusions were required. All patients required substantial post-operative care on ITU. Prolonged hospital rehabilitation and stay after operation was also often necessary.

Multi-visceral abdominal transplantation is technically possible but has considerable resource implications and requires the commitment of surgical, anaesthetic, nursing and support services. This series proves it to be a feasible treatment for a small number of selected patients with life-threatening disease.

C113

POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD) OF DONOR ORIGIN IN LIVER TRANSPLANT RECIPIENTS

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The majority of PTLD are associated with Epstein-Barr virus infection and uncontrolled proliferation of the lymphocytes in the presence of immunosuppression. Traditionally, it was believed that the affected lymphocytes were of recipient origin but recently there have been isolated reports of donor derived PTLD in liver transplant recipients.

Since 1982 , 16/1388 (1.2%) of liver transplant recipients at this institution have developed PTLD and are divided into Gp 1 (n=3) and Gp 2 (n=13). At time of diagnosis the mean age(range) of Gp 1 patients was 50yrs(45-55) and Gp 2 was 51 (28-73). Gp1 patients have been identified as having a donor derived origin of PTLD by chromosomal analysis or HLA typing and one patient is currently under evaluation. The median time (months) from transplant to diagnosis of lymphoma in Gp1 was 5 (5-11) and in Gp2 was35 (21-140). Gp1 patients had disease confined to the liver allograft/hilum whereas Gp 2 patients had predominantly extrahepatic disease. There was no significant difference in number of treated rejection episodes in each group. All patients were treated by reduction of immunosuppression which was complicated by development of acute rejection in 4/14 patients. Additional treatments were tailored to each case and included chemotherapy, radiotherapy, and surgery. One patient in Gp 1 is being considered for retransplantation. Seven (54%) patients in Gp 2 have died from the disease at median time 9 months (0-36) from time of diagnosis. One patient in Gp 1 died at 1 month post lymphoma diagnosis.

Patients with donor derived PTLD appear to form a definable subgroup which is probably currently under – diagnosed. Identification of these patients is required in order to further define the clinical syndrome (disease localised to allograft and short interval from time of transplantation to PTLD) such that we can formulate effective management strategies and consider the role of retransplantation in this cohort.

C117

Azathioprine toxicity and Thiopurine Methyltransferase (TPMT) genotype in renal transplant patients.

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Introduction

Azathioprine toxicity is an important issue in renal transplantation. Thiopurine methyltransferase (TPMT) is an enzyme that catalyses the methylation of thiopurine drugs, including azathioprine. About 10% of people have intermediate TPMT activity and 1 in 300 inherit TPMT deficiency as an autosomal recessive trait. The gene for TPMT is located on chromosome 6p22.3 and is known to be polymorphic. TPMT genotype and enzyme activity phenotype are strongly correlated. The most common mutation in Caucasians is TPMT*3A which results from point mutations at codon 154 in exon 7 (G469A) and codon 240 in exon 10 (A719G). TPMT*3B has only the exon 7 mutation and TPMT*3C has only the exon 10 mutation. Other less common mutations also exist. A number of previous studies have suggested that azathioprine toxicity may correlate with TPMT genotype, although this has not been studied in renal transplant patients.

Objective

The aim of this study was to establish whether azathioprine toxicity in renal transplant patients could be correlated with TPMT mutations.

Methods

Using contemporaneous notes, 245 patients were found to have been treated with azathioprine following renal transplantation in the ten years between 1989-1999. Of these, adequate clinical data and DNA was available for 88 patients. A white cell count of less than $3.5x10^9$/cmm was considered as azathioprine toxicity.

TPMT genotyping was perfomed using Polymerase Chain Reaction (PCR) – Restriction Fragment Length Polymorphism (RFLP) analysis. Alleles were assigned as either wild type (TPMT*1) or mutants (TPMT*3A, 3B, 3C).

Results

Azathioprine toxicity leading to discontinuation of treatment in less than 2 months was seen in 11 patients. A further 8 patients had a low white cell count which settled on reduction of the azathioprine dose. 36.8% of individuals who developed toxicity possessed a TPMT mutation at codon 154 or codon 240, compared with only 7.2% in individuals who did not develop toxicity.

In total 12 of the 88 (13.6%) individuals had a mutant TPMT allele, 7 of these (58.3%) developed toxicity. Of particular interest is that of those individuals possessing a mutation at codon 154 (N=9) ie TPMT*3A or 3B, 7 (77.8%) developed toxicity.

Discussion

There is a correlation between TPMT genotype and azathioprine toxicity. Toxicity not accounted for by one of the polymorphisms described here may be accounted for by other mutations within the TPMT gene. TPMTgenotyping may be a valuable aid to clinical decision making in patients starting azathioprine.

C118

RANDOMIZED CLINICAL STUDY COMPARING UNIVERSITY OF WISCONSIN AND CELSIOR SOLUTION IN LIVER PRESERVATION FOR TRANSPLANTATION

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Introduction. Although University of Wisconsin (UW) solution remains the most commonly used for all intrabdominal organs, Celsior solution (CS) widely utilized in clinical setting for the preservation of intrathoracic organs, has been recently proposed as a cold storage solution for abdominal organs **Aim.** This study aimed to compare the effectiveness of UW and Celsior (CS) solution in the preservation of the liver in a single transplantation center. **Methods.** From sept. 1998 to sept. 1999 we randomized 56 multiorgan donors (> 15 years) and performed 55 OLT. All recipients were > 15 years. Cold ischemic time was always < 13 hrs. In 49 pts we performed the Piggy-Back technique; in 6 pts we used a veno-venous by-pass. The pts were divided in 2 groups: CS group (n=25 pts) and UW group (n=30 pts). A standard immunosuppressive therapy was used. **Results.** There were not statistical differences in donor characteristics (age, death cause, ICU stay, blood pressure, hypotension episodes, μg dopamin , AST, ALT, Tot. Bil., % steatosis) in CS and UW groups. There were also not statistical differences in recipient characteristics (age, UNOS status). Cold and warm ischemia time were comparable as well. 1st p.o. day bile production (mean±SD) was 149.1 cc ±106.7 in UW group *vs* 158.9 ± 106.5 in CS group. (p=n.s.). 3rd p.o. day bile production was 177.1 ± 95.7 in UW group *vs* 168.2 ± 94.0 in CS group. (p=n.s.). 1st p.o. day PT (%) was 45.7±14.3 in UW group and 48.7±15.8 in CS group (p=n.s.). 3 rd p.o. day PT (%) was 64.5±16.5 in UW group and 66.6±18.5 in CS group (p=n.s.). Intensive care unit p.o. stay was 7.5±14.2 days in UW group and 4.2±2.8 in CS group (p=n.s.). Rejection episodes (<15d.) were 11 in UW group and 4 in CS group (p=n.s.). 30-day patient survival was 86.7 % in UW group and 84.7 % in CS group (p=n.s.) AST levels at 1, 3, 5, 7, 15 p.o. day in UW group were respectively: 1073±920.3; 595±735.5; 105.6±84.6; 61.3±36.6; 48.7±45.7. AST levels at 1, 3, 5, 7, 15 p.o. day in CS group were respectively: 965±1446.9; 493.2±878.7; 112.4±137.0; 60.9±53.4; 34.5±27.4 (p=n.s.). ALT levels 1st, 3rd, 5th, 7th, 15th, p.o. day in UW group were respectively: 785.6±587.8; 867.7±871.3; 387.9±275.4; 232.4±146.7; 132.1±144.2. ALT levels at 1, 3, 5, 7, 15 p.o. day in CS group were respectively: 724.4±1005.9; 665.8±828.7; 325.2±283.8; 205.8±158.9; 107.3±104.3 (p=ns). Total bilirubin levels at 1, 3, 5, 7, 15 p.o. day in UW group were respectively: 4.7±2.2; 3.2±2.0; 5.2±2.8; 7.0±5.2; 5.7±5.0. Total bilirubin levels at 1, 3, 5, 7,15 p.o. day in CS group were respectively: 6.6±5.8; 4.5±5.8; 6.7±4.8; 6.7±5.0; 6.8±9.1 (p=ns). Kaplan-Meier analysis showed no statistical significance in graft or patient survival between the groups at 3 or 12 months **Conclusion.** CS and UW are both effective in cold storage of the liver for transplantation. Contrary to UW, Celsior could be used as preservation solution for both thoracic and abdominal organs.

CLINICAL

C119

KIDNEY PRESERVATION WITH UNIVERSITY OF WISCONSIN AND CELSIOR SOLUTION: A PROSPECTIVE MULTICENTER RANDOMIZED STUDY

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Background. Although University of Wisconsin (U.W.) solution continues to be the most commonly used for intrabdominal organs a new solution, Celsior (CS), already utilized for heart and lungs, have been proposed for kidney and liver preservation. **Aim.** Aim of this study was to compare the effectiveness of UW and CS solutions in the preservation of the kidney in a multicenter trial of 4 transplantation centers. **Methods.** Up to September 2000 we randomized 100 donors (>15 years) and performed 187 transplants. All recipients were > 15 years. Hypothermic preservation time was always < 32 hrs. We used a standard surgical technique and a standard immunosuppressive therapy. The pts were divided in 2 groups: CS (n=99 pts) and UW (n=88 pts). Univariate analyses employed Mann-Whitney test and chi-square tests to assess differences between study groups. **Results.** There were not statistical differences in donor characteristics (age, death cause, ICU stay, systolic blood pressure, hypotension episodes, μg dopamin, serum creatinine, blood nitrogen, urine output) in CS and UW groups. There were also not statistical differences in recipient characteristics (age, HLA matching and cross-matching). Cold and warm ischemia time were comparable as well. 1st p.o. urine output (mean±SD) was 1663.8cc ± 1418.3 in UW group *vs* 1637.5 ± 1301.7 in CS group. (p=ns). 3rd p.o. day urine output was 1234.9±1107.5 in UW group *vs* 1637.9 ± 1455.5 in CS group. (p=ns). 5th p.o. day urine output was 1137.9±1078.5 in UW group and 1694.5±1282.4 in CS group (p=ns). 7th p.o. day urine output was 1285.5±1109.5 in UW group and 1810.2±1432.3 in CS group (p=n.s.). Mean p.o. dialysis treatments were 1.9±3.5 in UW group and 1.0±3.3 in CS group (p=n.s.). Rejection episodes (<30d.) were 13.1% in UW group and 12.1% in CS group. Delayed graft function was 33.3 % in UW group and 31.3 % in CS group (p=n.s.) S-cr levels (mg/dL) at 1, 3, 5, 7, 15 p.o. day in UW group were respectively: 7.3±2.6; 6.6±3.6; 5.9±4.02; 5.1±3.8; 3.5±2.4. S-cr.levels at 1, 3, 5, 7, 15 p.o. day in CS group were respectively: 6.2±2.2; 4.9±3.4; 4.2±3.2; 3.8±3.0; 2.6±2.4 (p=n.s.). S-cr. value in the discharge day in UW group was 2.1±0.8 and 1.9±1.6 in CS group. The 2-year graft survival in kidneys preserved with Celsior was 84% as compared to 75% for U.W. preserved kidneys without significant statistical difference. **Conclusion.** CS has the same efficacy as compared to UW solution in the kidney cold storage for transplantation.

C120

OUTCOME OF ABO INCOMPATIBLE LIVER GRAFTS IN CIRRHOTIC PATIENTS.

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BACKGROUND: ABO-incompatible grafts comprise 2% of European Liver Transplant Register (ELTR) since 1988. Most units have abandoned these due to high rejection rates and poor outcome. However in severely decompensated cirrhotics, terminal disease or the presence of neoplasia result in a high risk of death or tumor progression on the waiting list. AIM/PATIENTS: Evaluation of ABO-incompatible grafts in cirrhotics expected to die within weeks; n=14: ALD=4; HCV=3; PBC=2;1 each PSC, A1AT, Budd-Chiari, HBV/HDV, AIH; median bilirubin 236 umol/l(25-986 umol/l), median creatinine 117 umol/l,(69-513 umol/l); or malignancy (n=4). Standard doses of calcineurin inhibitor with steroids and azathioprine were given. OUTCOME: HAT in 2 (both retransplanted, 1 alive). Protocol biopsies showed no rejection in 4, mild 7, moderate 4, severe 3(10 treated with steroids). Other deaths 1 year, 4:2 multi-organ failure,1 cerebrovascular accident,1 splenic artery rupture. Only 1 had chronic ductopenia which resolved (alive at 39 m). Survival at 1 year was 72.2% (median 2 years,range 5 d-5 yr) compared to an expected median survival of 79% (41-82) (derived from ELTR normalized mortality ratio - Lancet 2000;356:621). CONCLUSION: ABO incompatible liver grafting results in acceptable cellular rejection and a sufficiently good survival, in severely decompensated cirrhotics very likely to die before a compatible donor becomes available. These patients should be made eligible for ABO incompatible grafts in current allocation schemes.

C121

ASSOCIATION OF CYTOKINE SINGLE NUCLEOTIDE POLYMORPHISMS WITH B7 COSTIMULATORY MOLECULE EXPRESSION IN KIDNEY ALLOGRAFT RECIPIENTS

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African-American (AA) race continues to be associated with increased risk for allograft loss, suggesting that AA patients may form an immunologically higher risk group. Previously, we have shown that peripheral immune cell costimulatory molecule expression is significantly higher in AA, compared to Caucasians (CS). Polymorphic variations in the genes for cytokines have been associated with a number of immunological conditions, and with transplant rejection. This study was performed to determine the distribution of single nucleotide polymorphisms (SNPs) in cytokine genes in AA (n=52) and CS (n=53) renal transplant patients. Whole blood was obtained from patients seen in the Post-transplant clinics. Cytokine protein production was determined after *in vitro* stimulation with PHA, IL-1β, or LPS using ELISA, and cell surface B7 (CD80, CD86) expression was measured using FACS analysis. Following DNA extraction, cytokine SNPs were typed using ARMS-PCR. There was a significant association between IL-10 genotype and acute rejection episodes, but only in AA patients ($p<0.01$). Also, AA patients were significantly more likely to carry the IL-6 G allele ($p<0.0001$) associated with high IL-6 protein production, but less likely to have the IFN-γ T allele ($p<0.05$) associated with high IFN-γ production. Incubation of peripheral blood cells with recombinant IL-6 resulted in increased surface CD80 and CD86 expression, while recombinant IL-10 decreased CD80 expression. This study demonstrated a clear correlation of patients with IL-6 G "high" allele with increased CD80 expression, and IL-10 G "high" allele with decreased CD80 expression. These data raise the possibility that one of the mechanisms involved in racial variation in disease outcome may be local cytokine regulation of costimulatory molecule expression.

CLINICAL

C125

RENAL TRANSPLANTATION AND THE WWW: WHERE IS IT @

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BACKGROUND

Most families in the United Kingdom are now said to have access to the Internet and as such the World Wide Web is becoming an increasingly important media for information dissemination. The aim of this study was to determine Internet usage by our transplant community and to assess the quality and sources of the information currently accessible to the surfer.

METHODS

100 transplant recipients were surveyed to determine: computer access, Internet availability and performance of transplant-related searches. In addition, an Internet search was performed using 5 of the most popular search engines and the search phrase 'kidney transplant'. The top 10 best-matched web pages were accessed to determine their: country of origin, information source and quality.

RESULTS

Half of the patients surveyed possessed or had regular access to a computer. However only 20% had Internet access and only half of these individuals had performed a transplant-related search. Of the top 50 web pages reviewed, 49 were American based. Suprisingly, there were only 2 duplicate hits amongst the combined top 50 hits of the 5 search engines. Of the 48 different sites visited, the information sources were; Hospitals (11), Associations (7), News articles (7), Journal articles (5), Personal accounts (4), Industry (4), Link sites (3) and Mailboxes (2) leaving 5 inaccessible sites. The quality of information was either good or excellent on all the Hospital sites whilst the majority of the remaining sites contained little information from the patient perspective.

CONCLUSIONS

Whilst current Internet use amongst our transplantation population is limited, it will undoubtedly be an invaluable source of patient information in the near future. Whilst the US market is well catered for, there is a desperate need for more United Kingdom based sites, containing generic information, preferably produced by an unbiased third party.

CLINICAL