cardiff transplantation 2000

British Transplantation Society 3rd annual congress

20 - 23rd March 2000, Cardiff City Hall

Image & Map Courtesy of Cardiff Marketing

Cardiff City Centre

A Guide to the City and Hotel Locations

Hotels

1. Hilton
2. Angel
3. Jurys
4. Marriot
5. Posthouse
6. Beverley
7. Sandringham
8. Holiday Inn Express
9. Goodnight Inn
10. Hayes Court

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

C53

THE FIRST YEAR OF THE NEW NATIONAL KIDNEY ALLOCATION SCHEME, JULY 1998 - JUNE 1999

Johnson RJ, Belger MA, Armstrong S, Fuggle SV, Briggs JD, Morris PJ
on behalf of the UKTSSA Users' Kidney Advisory Group

A new National Kidney Allocation Scheme was implemented on 1 July 1998 for cadaveric donor kidneys available for transplant in the UK.

1291 kidney only transplants were undertaken in the first year of the Scheme, July 1998 - June 1999: 167 (13%) had no mismatches at HLA-A, B and DR (000), 619 (48%) were favourably matched (100, 010 or 110 HLA-A, B, DR mismatches) and 505 (39%) were non-favourably matched. This represented a significant improvement ($p<0.001$) over the first six months of 1998: 7% 000, 45% favourable and 48% non-favourable. 33 highly sensitised patients received 000 mismatched grafts in the year July 1998 to June 1999 (18 in the first 6 months, 15 in the next) compared with 12 such grafts in the first six months of 1998. These results have been achieved through greater exchange of kidneys between centres/alliances (47% exchanged compared with 28% in previous 6 months).

Cold ischaemic times were available for only a subset of the transplants carried out in the first year of the new Scheme and in the previous six months. No significant change in mean cold ischaemic time was apparent despite the greater exchange of organs, 20.2 hours (s.d. 7.8) for 1 January - 30 June 1998 and 20.7 hours (s.d. 8.1) for 1 July 1998 - 30 June 1999, ($p=0.4$).

Of the 000 and favourably matched adult kidneys allocated through the National Scheme, 73% required the use of a points scoring scheme to prioritise equally matched patients. Points are allocated for recipient age, donor-recipient age difference, matchability, waiting time, sensitisation and centre Balance of Exchange. No differences were apparent in the age distribution of transplanted patients although for nationally allocated organs the mean donor-recipient age difference was significantly smaller ($p=0.008$). To date there have been no significant changes in terms of other factors.

Preliminary analysis of three months transplant outcome for transplants in the first 6 months of the new Scheme (70% follow-up) showed significant improvements compared with the outcome of transplants in three years 1994-1996 (96% follow-up).

	1 Jan 1994 - 31 Dec 1996			1 Jul 1998 - 31 Dec 1998			
HLA match	No.	% 3 month transplant survival*	95% CI	No.	% 3 month transplant survival*	95% CI	p
000	288	89	85-93	90	94	90-98	0.1
Favourable	1477	90	88-92	203	95	93-97	0.02
Non-favourable	3206	87	86-88	173	93	89-97	0.03
All	4971	88	87-89	466	94	92-96	0.0001

* transplant failures include deaths with a functioning graft

Results of the first year of the Scheme are encouraging in terms of the levels of HLA matching achieved and the preliminary analysis of transplant outcome.

C3

OUTCOMES OF PULSATILE PRESERVATION AND VIABILITY ASSESSMENT OF NHBD KIDNEYS

Balupuri S, Buckley P, Mohamed M, Mantle D, Kirby J, Soomro N, Snowden C, Manas D, TalbotD .

Dept of Surgery, University of Newcastle and Freeman Hospital, Newcastle upon Tyne, NE2 4HH

Non Heart beating Donors (NHBD) kidneys are considered as marginal organs due to variable warm ischaemic damage. The recipients of such organs suffer delayed graft function (DGF) requiring post transplant dialysis and longer hospital stay. Viability assessment of such organs reduces the incidence of DGF and PNF. This is especially important in uncontrolled (Maastricht Category II) donors. In Phase III of our NHBD programme launched in September 1998, introduction of machine perfusion enabled the assessment of these marginal donors. Since then the graft survival has been 96 % compared with the previous phase where machine perfusion or viability assessment was not done (45.5%). The parameters used are Total Glutathione S transferase (tGST) in the perfusate, the intrarenal vascular resistance (IRVR) along with flow characteristics over time on a pulsatile preservation system.

Methods: Retrieval consisted of in situ perfusion with a Double Balloon Triple Lumen cannulae in Category II donors mean age 44.9 years (range 16 to 60). Mean primary WIT was 26 min (range 15 to 44). All kidneys were machine perfused through a locally developed perfusion system. The viability was assessed by serial measurements of total GST (maximum acceptable limit of 200 units/L), Flow rates per 100 gm of kidney weight (minimal acceptable limit of 40 ml/min) and intrarenal vascular resistance (IRVR).

Results: 20 NHBD (15 Category II and 6 Category III) resulted in machine perfusion of 41 Kidneys (1 kidney imported). 27 kidneys were transplanted and 3 exported after machine perfusion and viability assessment. The remaining 11 were discarded (2 were positive for VDRL, 4 had high tGST, 4 with poor flow characteristics and 1 with high resistance).

Delayed graft function (DGF) was seen in 22 recipients (84.6%). Immediate function (IF) in 2 kidneys and 1 kidney had primary non function (PNF). There were two deaths, unrelated to transplantation.

Conclusion: pulsatile machine perfusion and assessment of NHBD kidneys has increased the donor pool by 19% in our centre.

C22

THE ROLE OF LAPAROSCOPY AND LAPAROSCOPIC ULTRASOUND IN STAGING HEPATOCELLULAR CANCER IN PATIENTS REFERRED FOR ORTHOTOPIC LIVER TRANSPLANTATION.

CP Fischer, KK Madhavan, and OJ Garden.

The Royal Infirmary of Edinburgh, The Scottish Liver Transplant Unit Lauriston Place, Edinburgh, EH3 9YW

AIMS: To determine the accuracy of laparoscopy and laparoscopic ultrasound in the diagnosis of hepatocellular carcinoma, in patients referred for orthotopic liver transplantation (OLT).

METHODS: 19 patients undergoing assessment for liver transplantation were found to have either a mass lesion on a radiological imaging study or an elevated AFP. Evaluation included abdominal ultrasound (90%), CT scanning (70%), or angiography (21%) and all patients were submitted to laparoscopy and laparoscopic ultrasound (Lap US). The accuracy of all investigations was compared with the final pathology of the explanted liver after transplant or by follow-up if OLT was not performed. The distribution of lesions (multifocal vs. unilobar), number of lesions and involvement of portal or hepatic veins was noted.

RESULTS: Laparoscopic evaluation altered the clinical decision in 50% of cases, providing crucial information not available from the other studies, (metastatic disease in 2/19 cases, lesions > 5cm in size in 2/19 cases, multifocal disease in 2/19). Two patients evaluated for OLT for suspicion of HCC were found not to have tumor by lap US - they had no further therapy and are disease free at 12 months follow up. Two additional patients were found by Lap US to have limited disease amenable to liver resection.

The accuracy of radiological imaging studies and laparoscopic evaluation was compared with the final liver specimen (in cases where transplant was performed), both for distribution of disease and total number of lesions.

Test	Accuracy for Multifocal Disease	Accuracy for Number of Lesions
Abdominal Ultrasound	50%	63%
CT scan	75%	39%
Angiography	50%	50%
Laparoscopy/Lap US	100%	100%

CONCLUSIONS: The accurate staging of hepatocellular carcinoma in pretransplant assessment is crucial to successful outcome and appropriate treatment. Laparoscopy combined with Lap US examination is the most accurate modality when compared with CT scanning, abdominal US, and angiography.

C71

OUTCOMES FOR CYSTIC FIBROSIS PATIENTS RECEIVING A HEART-LUNG OR BILATERAL LUNG TRANSPLANTATION.

C Jones, CA Rogers, AC Anyanwu, AJ Murday

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

Background: End stage pulmonary cystic fibrosis may be treated by heart-lung (HLT) or bilateral lung (BLT) transplantation. The choice of procedures must take into account not only outcomes but also the potential loss of a heart donor when heart-lung transplantation is used. This loss of donor hearts may be offset by using hearts from heart-lung recipients for domino transplant procedures.
Aims: To compare the outcome of heart-lung and bilateral lung transplantation for cystic fibrosis.
Methods: Data were taken from a multi-centre cohort study involving all pulmonary transplant centres in our country. 126 cystic fibrosis patients transplanted between April 1995 and May 1999 form the population for this analysis. Outcomes were compared using the log-rank and chi-squared tests.
Results: Heart-lung transplantation was the procedure used in 69 transplants while bilateral lung transplantation was used in 57. The choice of procedure used was largely centre dependent. The 90 day patient survival for heart-lung group was 81% (70% CI 71-92) and for the lung group 83% (70% CI 73-92) (p=0.82). There were no significant differences between the two groups in terms of rejection, infection, or airway complications in the first 90 days (rejection 58% vs. 70%, p=0.36; infection 67% vs. 52%, p=0.28; airway complications 9% vs. 6%, p>0.9; for heart-lung and lung grafts respectively). The number of patients returning to theatre (e.g. for bleeding) in the early post-transplant period (HLT 17% vs. BLT 19%), or requiring haemofiltration (HLT 10% vs. BLT 11%) or intra-aortic balloon counterpulsation (HLT 4% vs. BLT 0%), did not differ statistically between the two groups. Hearts were used from 61% of lung-block donors while 80% of the heart-lung recipients donated their heart. ***Comments:*** The similar outcome of the two groups confirms that cystic fibrosis recipients are equally well treated with either double lung or heart-lung transplantation. The large number of 'domino' hearts from the heart/lung recipients appears to correct the potential loss of donor hearts.

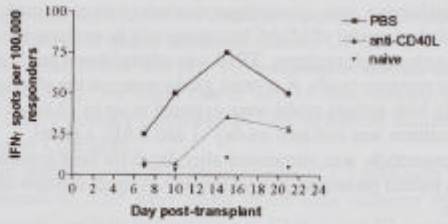
THE EFFECTS OF CD40/CD40L BLOCKADE ON THE T CELL RESPONSE TO SKIN ALLOGRAFTS

N. J. Monk, J. Marsh, R. E. G. Hargreaves, J. Pratt, S. H. Sacks and S. Jurcevic

Department of Nephrology and Transplantation, Guy's Hospital, Guy's, King's and St. Thomas' School of Medicine, King's College, London.

The engagement of the T cell receptor (TCR) by its cognate ligand, MHC-peptide, in the absence of costimulation provided by the interaction between CD28/B7 and CD40L/CD40 molecules on T cells and APCs respectively, may lead to antigen specific non-responsiveness (tolerance). Hence, there is considerable interest in the application of costimulation blockade in transplantation. Indeed, with kidney transplants in primates, anti-CD40L treatment lead to long term allograft survival and donor specific hypo-responsiveness.
In this study we have investigated the effects of CD40L/CD40 blockade on the T cell response to fully mismatched skin allografts (BALB/c to C57BL/6 mice). Mice were treated either with 250 µg of hamster anti-mouse CD40L antibody (MR1) on days 0, 2, 4, 7 and 10 post transplant (n=8) or PBS (control). In the anti-CD40L treated group, mean graft survival time was extended (MST=13 days vs 8 days in control group). On days 0, 7, 10, 15 and 21 post transplant, splenocytes from both experimental groups were stimulated with donor (H-2d) or third party (H-2k) cells and the number of IFNγ producing cells were determined (ELISPOT).

Figure 1



Transplanted mice in the control group showed an increase in the number of IFNγ producing alloreactive cells (compared with naïve) as early as 7 days post transplant peaking on day 15 (Figure 1). In contrast, the number of alloreactive IFNγ producing cells from mice treated with anti-CD40L was not greater than the naïve response until day 15. This response remained significantly lower than the control group throughout the post-transplant period studied.
In conclusion, anti-CD40L treatment leads to a delay in priming and lowers the number of alloreactive T cells. However, in this fully mismatched skin allograft model anti-CD40L treatment does not lead to the establishment of tolerance. The highly immunogenic skin allografts may need a combination of costimulation blockade and immunosuppression to achieve this goal, a concept we a currently investigating.

LABORATORY

PERI-OPERATIVE TP10 (SCR1) IMPROVES EARLY GRAFT FUNCTION AND IN COMBINATION WITH RAD AND NEORAL EXTENDS SURVIVAL IN HDAF TRANSGENIC PIG TO PRIMATE RENAL XENOTRANSPLANTATION AFTER COLD ISCHAEMIA.

Bob Soin [1,2], Conrad M Vial [3], Saqib Masroor [4], Richard A Harrison [1], Gilda Chavez [1], John R Bradley[2], Kenneth G.C. Smith [2], Sathia Thiru [2], David J White [1,2], Emanuele Cozzi [1] and Peter J Friend [5].

[1]Imutran Ltd., (A Novartis Pharma AG Co.), Cambridge, CB2 2YP U.K.; [2]University Depts. of Surgery, Nephrology & Pathology, Addenbrookes Hospital, Cambridge, CB2 2QQ, U.K.; [3]Dept. of Surgery, School of Medicine, Stanford, CA, USA; [4]Dept. of Surgery, Henry Ford Hospital, Detroit, USA; and [5]Nuffield Dept. of Surgery, John Radcliffe Hospital, Oxford University, OX3 9DU, U.K.

Introduction: Recent favourable results with living unrelated donors for renal allograft transplantation have demonstrated the importance of ischaemia/reperfusion injury on allograft early function and subsequent long-term graft survival. Complement plays a pivotal role in ischaemia-reperfusion injury after organ preservation. The central role of complement activation in hyperacute discordant xenograft rejection (HAR) is also well recognised. HAR can be overcome by use of donors transgenic for regulators of complement activity. TP10 is a soluble inhibitor of the C3 and C5 convertase enzymes. In this study the additive effect of TP10 to the human Decay Accelerating Factor (hDAF) transgene on function and survival of renal porcine xenografts transplanted, after prolonged cold ischaemia, into cynomolgus monkeys was examined.
Methods: In an established model of hDAF transgenic pig-to-primate life-supporting renal transplantation without splenectomy, TP10 was administered on days -1, 0 and +1 as part of an induction regimen (n=6). A control group received the standard induction regimen only (n=6). In both groups grafts were exposed to up to 12 hours of cold ischaemia. Neoral treatment was initiated on day -1 and RAD, a novel immunosuppressant macrolide, was introduced after day 5 for both groups. Graft rejection treated with pulsed parenteral steroid therapy. At post-mortem all grafts were assessed for rejection.
Results: Analysis of the contralateral kidneys confirmed hDAF expression after periods of ischaemia up to 12 hours. Biopsies of the control group recipients showed Ig, fibrin, C4, C3 and C9 deposition. In contrast, TP10 treated animals, were completely free from fibrin, C3 and C9. In the TP10 group, the mean day 1 post-transplantation serum creatinine level was lower than that of the controls (2.6+1.4 mg/dL vs. 4.2+0.6 mg/dL, p=0.03). The mean survival of the TP10 group was longer than that of the control group (37.5+/-12.2 days vs. 24.5+/-7.9 days; p=0.05).
Conclusions: In this model of life-supporting discordant kidney xenotransplantation without splenectomy, after up to 12hours of cold ischaemia, the expression of the hDAF transgene alone is still sufficient to abrogate hyperacute rejection. Increasing the duration of cold ischaemia from 6 to 12 hours had no detrimental effect on the early function of the hDAF transgenic renal xenograft. Improved early graft function and increased survival is obtained after prolonged cold ischaemia when early post-operative inhibition of complement activation is achieved with TP10 in combination with the action of membrane bound hDAF.

ABROGATION OF ACUTE REJECTION IN THE ABSENCE OF LOCALLY SYNTHESISED COMPLEMENT C3

Pratt J.R., Basheer S., & Sacks S.H.

Dep't of Nephrology and Transplantation, Guy's Hospital, Guy's, King's and St Thomas' School of Medicine, Kings College, London.

Synthesis of complement C3 is upregulated by renal proximal tubular epithelial cells (PTEC) in ischemia/reperfusion injury and acute rejection. Since C3 is central to the activation and terminal cascades of the complement pathway, and is implicated in direct damage to cells as well as enhancement of antigen uptake and presentation, we have investigated the role of PTEC C3 synthesis in allotransplantation using C3 gene disrupted C57BL/6 mice (BL/6).

We assessed the response of cultured wild-type BL/6 (H2b) PTEC to IFNγ, and by RT-PCR we detected a 4-fold increase in the synthesis of C3 mRNA by day 3 post stimulation, compared to unstimulated PTEC. Immunohistochemistry using a monoclonal antibody for mouse C3 detected C3 deposition on the PTEC surface. Such IFNγ treated PTEC from wild-type or C3 knockout BL/6 mice were then used as stimulators in vitro to lymphocytes taken from B10.Br (H2k) recipients of BL/6 renal allografts at day 14 post transplantation when graft histology showed signs of severe acute rejection, i.e. a florrid infiltrate, with widespread tubulitis and vasculitis.

Using an IL-2 ELISA of the supernatants from the co-culture of stimulated PTEC and primed allogeneic lymphocytes we found a reduction in allostimulation ($p<0.001$) if the PTEC were deficient in the ability to synthesise C3 (i.e. from a C3 knockout BL/6 mouse). By two-colour FACS analysis for lymphocyte subsets and the expression of complement receptors 1/2, we found that in day 14 transplant recipients 44% of spleen CD4 cells were CR1/2 positive compared to <5% in normal animals. We did not detect CR1/2 expression by CD8 cells.

In survival studies (n=6), B10.Br recipients of C3 sufficient BL/6 renal allografts survived for a mean of 13.5 days when blood urea nitrogen (BUN) levels reached >23mmol/l. In comparison B10.Br recipients of C3 deficient BL/6 grafts did not sucumb to acute rejection and to date have survived for >40 days (n=6) with only slightly elevated BUN (5.94mmol/l compared to normal murine BUN of 2.4mmol/l) in the absence of any immunosuppresive therapy.

These data confirm that PTEC are able to present antigen to allogeneic lymphocytes, but also show that such allostimulation is enhanced if the PTEC increase secretion of C3, such as in response to pro-inflammatory cytokines like IFNγ that are found in rejecting organs. Our data suggest such a mechanism of lymphocyte stimulation could be mediated through complement receptors on CD4 cells that subsequently provide T-cell help in allograft rejection. In the absence of the pro-inflammatory effect of local C3 synthesis, fully MHC disparate renal allografts did not sucumb to acute rejection implying that this is a previously unappreciated mechanism in transplantation that if inhibited therapeutically has the potential to further improve graft survival in the clinic.

LABORATORY

Parallel Sessions

Tuesday 21 March

15.45-17.00

Immunobiology

INDIRECT ALLORECOGNITION PLAYS A PIVOTAL ROLE IN THE PATHOGENESIS OF TRANSPLANT ARTERIOSCLEROSIS

Ensminger SM[1], Witzke O[1], Spriewald BM[1], Yacoub MH[2], Morris PJ[1], Rose ML[2], Wood KJ[1]

[1]Nuffield Department of Surgery, John Radcliffe Hospital, Oxford University, U.K.
[2]Heart Science Centre, Imperial College of Medicine, Harefield Hospital, Harefield, Middlesex U.K.

Background: Indirect allorecognition has been implicated in the initiation of chronic allograft rejection. Our aim was to develop an animal model that allowed the contribution of the direct and indirect pathway of allorecognition in the evolution of transplant arteriosclerosis to be evaluated.
Methods: Aortic allografts mismatched for a single MHC-class I antigen were transplanted into athymic *NUDE*-mice. Recipient *NUDE*-mice were reconstituted with either $CD4^+$ (indirect) or $CD8^+$ (direct) T cells in the presence or absence of depleting antibodies specific for the opposite T cell subset. Aortic grafts were analysed by performing morphometry, immunohistochemistry and quantitative RT-PCR for the detection of cytokine mRNA production. Donor specific alloantibody production was measured by FACS analysis.
Results: Reconstitution of athymic nude mice with $40x10^6$ purified $CD4^+$ T cells resulted in vascular rejection (intimal proliferation 24±8 %). Thus, T cells capable of recognising donor alloantigen presented via the indirect pathway can initiate transplant arteriosclerosis. Furthermore, indirect allorecognition by $CD4^+$ T cells was associated with the production of alloantibodies, pronounced infiltration of the graft with $CD11b/CD18^+$ and $CD40^+$ cells and the presence of intragraft mRNA for IFN-γ, TNF-α, iNOS and IL-12. Animals reconstituted with $CD8^+$ T cells also exhibited a pronounced degree of rejection (intimal proliferation: 48±12%). However, intimal proliferation was almost entirely abolished (6±6%) when $CD4^+$ T cell depleting antibody was also administered, suggesting that the presence of even a small number of $CD4^+$ T cells reactive via the indirect pathway can initiate the rejection process.
Conclusions: Indirect recognition of donor MHC-class I molecules by $CD4^+$ T cells plays a pivotal role in the process of transplant arteriosclerosis. $CD8^+$ T cells are the main effector cells for vascular rejection in this model, but their activation is dependent on $CD4^+$ T cell help following indirect allorecognition

HLA-INDEPENDENT RECIPIENT CYTOKINE POLYMORPHISMS ASSOCIATED WITH GRAFT VASCULAR DISEASE OF THORACIC ORGANS

Vamvakopoulos JE[1,2], Taylor CJ[2], McNeil K[3], Goodman R[2], Green C[4], Metcalfe SM[1].

[1]Dept of Surgery and [2]Tissue Typing Laboratory, Addenbrooke's Hospital, Cambridge, UK; [3]Transplant Unit, Papworth Hospital, Cambridge, UK; [4]Northwick Park Institute for Medical Research, Harrow, UK

Graft vascular disease (GVD) remains a major obstacle to clinical transplantation, being the leading cause of chronic allograft failure. This condition is immune-driven, although unhampered by currently used immunosuppressive agents. Studies into the pathogenesis of GVD have suggested a role for certain cytokines and growth factors.

We have asked if genetically pre-determined risk factors exist in transplant recipients who develop GVD. Eleven previously characterised, physiologically relevant polymorphisms were studied; these are located within the following genetic loci: IL1 (n=2), IL1RI, IL1RN (n=2), IL6, IL10 (n=2), TNFα, TGFβ and FcγRII. We retrospectively genotyped two cohorts of transplant recipients by SSP-PCR, one comprising individuals that had either died or been re-transplanted as a result of GVD (n=96) and the second consisting of long-term survivors with good graft function (n=83). We compared allele and genotype frequencies for each polymorphism, as well as haplotype frequencies, using the appropriate non-parametric statistical tests (χ^2 test with Yates' correction or Fisher's Exact Test).

Our findings indicated that carriage of the IL1RN*1 allele, especially in the homozygotic state, was associated with GVD-mediated cardiac graft rejection, whereas carriage of the less frequent IL1 receptor antagonist alleles (IL1RN*2-4) emerged as a significant predictor of long-term graft survival (p=0.025; odds ratio =1.91). This association was even stronger when analysis was performed across graft categories (heart, heart-lung and lung; p=0.003, OR=2.02). Similarly, carriage of the C allele of the IL1 receptor type I gene was more frequent among cardiac patients developing a more precipitous form of GVD (p=0.029; OR=3.12). Outside the IL1 gene family, the IL10/-1082GG genotype was predominant in rejecting heart-lung patients, while being absent from long-term survivors (p=0.013). Further, nearly all heart-lung patients developing early chronic rejection harboured genotypes previously shown to enhance IL10 gene transcription (p=0.044; OR=6.31).

Our findings highlight the IL1 regulatory/signalling network as a key, genetically influenced pathophysiological component of cardiac graft vascular disease. Further, while they appear to support the involvement of a T_H2-type immune response, they also suggest organ-specificity in the pathogenesis of GVD.

Abstract category: **LABORATORY**

HISTOPATHOLOGICAL CRITERIA FOR THE DIAGNOSIS OF PIG TO PRIMATE KIDNEY XENOGRAFT REJECTION. PROPOSED CLASSIFICATION FOR THE XENOTRANSPLANT COMMUNITY.

Bob Soin[1,2], Sathia Thiru[2], Emanuele Cozzi[1,2], Peter Friend[3], David White[1,2] & Gilda Pino-Chavez[1].

[1]Imutran Ltd., (A Novartis Pharma AG Co.), Cambridge, U.K.; [2]University Depts. of Surgery, Nephrology & Pathology, Addenbrookes Hospital, Cambridge, U.K.; [3]Nuffield Dept. of Surgery, John Radcliffe Hospital, Oxford University, U.K.

Introduction: Pathologists working in the field of renal allotransplantation have accepted world-wide the Banff working group classification as international standard criteria. Such agreement does not exist in discordant xenotransplantation. A grading system aimed at promoting international uniformity in reporting of the xenograft histology, would ideally have prognostic import and guide therapeutic interventions. We have undertaken the review of 235 pig-to-cynomolgus monkey renal xenotransplantation experiments correlating outcome with clinical, histological and immunopathological findings. The majority of the recipients received donor porcine organs transgenic for human Decay Accelerating Factor.

Hyperacute rejection (HAR) is characterised by haemorrhage, thrombosis and neutrophil infiltration with the temporal criterion of immediate onset with no organ function (anuria). Acute vascular and delayed xenograft rejection have been re-classified into acute humoral (AHXR) and acute cellular (ACXR) xenograft rejection . AHXR is characterised by histological features similar to hyperacute rejection but with increased amounts of mononuclear and lymphocytic infiltration. However, in contrast to HAR, the temporal course is slower and there is graft function (urine production). ACXR is characterised by lymphocytic infiltration producing direct tissue damage (vasculitis, tubulitis and glomerulonephritis) in the absence of complement, immunoglobulins and vessel thrombosis.

The vascular, glomerular and tubular lesions were graded 1-4. In addition AHXR and ACXR were graded as mild , moderate or severe based on the extent and severity of the lesions seen. It is possible for AHXR and ACXR to be seen simultaneously in different areas of the same xenograft.

Results of this study showed that 75 animals were euthanased for technical reasons and 37 euthanased without evidence of rejection within the xenograft for other welfare reasons.

There were six cases of HAR, only one from a transgenic donor. Sixty-seven animals had AHXR, 16 animals had ACXR and 34 animals showed co-existence of AHXR and ACXR. There was a tendency for animals with ACXR alone to have a lower mean creatinine as compared to those with AHXR. The fact that the tissues examined were from autopsy xenografts and not *in life* biopsies limits the prognostic value of this finding.

This work is presented to stimulate open discussion. It is hoped this will help to define a common nomenclature and lead to a grading system for renal xenograft rejection that will be accepted world-wide.

REGULATION OF NATURAL KILLER CELL INHIBITORY RECEPTORS FOLLOWING TOLERANCE INDUCTION

Joyce Popoola, M. Helena Marconell, Steve H. Sacks, Kathryn J. Wood and Wilson Wong.*

*Department of Nephrology and Transplantation, Guy's Hospital, London SE1 9RT & *Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU*

INTRODUCTION Natural Killer (NK) cells form part of the innate immune system. They do not require priming and can kill target cells independently. Their activities (which include secretion of TNFα, IFNγ and IL2) peak after 3 days of the initiation of the response, well before the T cell actions. These initial 'first steps' taken by the immune system may have important influence on the outcome by steering the adaptive T cell response towards either rejection or tolerance. Unlike T cells which use T cell receptors for alloantigen recognition, NK cells utilise inhibitory receptors (Ly49 in mice) which bind to self MHC class I molecules, sending a negative signal, inhibiting the killing process. Allogeneic cells without self MHC do not send this negative signal and are killed. Murine Ly49 receptors exhibit allelic polymorphism (A to I) which determine the allospecificity of Ly49$^+$ NK cells, allowing them to be self-tolerant. Some Ly49 molecules are 'promiscuous' (e.g. Ly49A and Ly49C binds to cells expressing $H2^{b,d,k,f,q,s,\alpha,v}$, while others are highly specific (e.g. Ly49D binds to $H2^k$ only while Ly49G2 binds to $H2^d$ only).

METHODS The role of NK cells in transplant rejection and tolerance is poorly understood. Here, we develop a tolerance induction protocol in a high responder mouse strain combination CBA ($H2^k$) to C57BL/6 ($H2^b$) and study the phenotypic changes in NK cells using 2 colour flow cytometry and following tolerance induction.

RESULTS Recipient C57BL/6 mice treated with 2 doses of anti Cd4, Cd8 monoclonal antibody (mAb) and $5x10^6$ fully allogeneic donor CBA bone marrow cells (BMC) 28 days before transplantation showed significant prolongation of donor cardiac allografts with 33% of recipients accepting their grafts indefinitely.

Treatment with mAb and donor BMC resulted in significant downregulation of Ly49A, D and C molecules that are known to bind to donor $H2^k$ BMC. Ly49G2 which does not bind was unaffected (table).

Mean percentage of splenic NK cells expressing Ly49 after tolerance induction (n=3)			
	naïve	donor CBA BMC and mAb	3rd party BALB/c BMC and mAb
Ly49A	40%	↓ 32%	↓ 29%
Ly49D	29%	↓ 26%	↑ 35%
Ly49C	68%	↔ 65%	↓ 62%
Ly49G2	30%	↔ 29%	↔ 33%

Treatment using 3rd party BALB/c BMC showed similar results. Ly49D which does not bind to BALB/c MHC was even upregulated.

CONCLUSIONS We showed that NK cells paradoxically downregulate their inhibitory Ly49 receptors following tolerance induction. This can be explained by the 'receptor calibration model' of NK tolerance: in the absence, or low levels of a particular MHC class I molecule, Ly49 receptors binding to that MHC molecule will have to be expressed at high levels in order to provide a strong enough negative signal to inhibit NK killing. In our model, we hypothesise that the introduction of allogeneic bone marrow cells under the cover of anti Cd4 and Cd8 mAb were seen by NK cells as 'self' and since these newly introduced MHC molecules were in abundance, lower levels of the corresponding Ly49 molecules were required by NK cells to generate the negative signal necessary for NK cell tolerance. This is supported by our observation that LyG2 which does not recognise CBA BMC was unchanged after CBA BMC treatment while all other Ly49 molecules were downregulated and Ly49D which does not recognise BALB/c MHC was upregulated while all the other Ly49 molecules recognising BALB/c MHC were either downregulated or unchanged.

LABORATORY

ACTIVATION OF ENDOTHELIAL CELLS BY ALLOSPECIFIC HLA ANTIBODIES - RELEVANCE TO CHRONIC REJECTION

Smith, J.D., Lawson, C., Yacoub, M.H. and Rose, M. L.

National Heart & Lung Institute, Imperial College School of Medicine, Royal Brompton and Harefield Hospitals, London.

Chronic graft rejection, characterised by a gradual occlusion of grafted vessels is the most serious complication following heart and kidney transplantation. Although often associated with chronic production of anti-HLA and anti-endothelial antibodies, the precise role of antibodies in chronic rejection remains uncertain. Here we have investigated whether MHC class I specific antibodies, either monoclonal or derived from patients, cause endothelial cell activation. Thus we investigated tyrosine phosphorlyation, NF-κB activation and cell proliferation in human umbilical vein endothelial cells (HUVEC) or microvascular endothelial cells from adult human heart (CMEC).

Ligation of monomorphic determinants of MHC class I molecules (using the mouse monoclonal antibody W6/32) on the surface of HUVEC (n=4) caused an increase in tyrosine phosphorylation of proteins of MW ~75-80KD, assessed by western blotting. Similarly, ligation of monomorphic determinants on both CMEC and HUVEC with W6/32 resulted in increased NF-kB binding compared to controls (by 74.4% and 52.5%, p=0.001), assessed by gel shift assays (n=4), and this was enhanced by addition of secondary goat anti-mouse antibody. Two HLA specific monoclonal antibodies resulted in 277% and 170% increase in NF-κB binding activity compared to controls (secondary antibody alone). Four patient IgG samples containing antibodies against MHC class I antigens were used against HLA specific HUVEC and 4 samples were incubated with HUVEC bearing irrelevant HLA antigens. Patient IgG alone enhanced NF-kB binding by 27-186%, but only when added to HUVEC bearing relevant antigens. W6/32 and allospecific IgG antibodies from patients significantly enhanced HUVEC proliferation (n=5), measured by uptake of 3H-thymidine compared to normal serum controls. The effect was antigen specific, thus patient sera containing non-HLA antibodies (n=4) has no effect on cell proliferation.

In conclusion, activation of NF-kB by human anti-HLA class I antibodies suggests their possible role in the pathogenesis of chronic vascular occlusive disease following transplantation.

Liver

C21

TRANSJUGULAR INTRAHEPATIC PORTAL-SYSTEMIC SHUNT IN ORTHOTOPIC LIVER TRANSPLANTATION

Casey JJ, Madhavan KK, Akyol M, Pleass H, Readhead D, Forsythe JLR, Garden OJ.
Scottish Liver Transplant Unit, Royal Infirmary, Edinburgh.

The use of TIPSS has increased over the past few years as an effective means of controlling variceal haemorrhage in patients with portal hypertension. As a result patients with TIPSS in situ are being increasingly considered for orthotopic liver transplantation (OLT). Controversy exists as to whether TIPSS facilitates or complicates OLT.

We therefore analysed the data from our unit to determine whether blood product usage was influenced by the presence of TIPSS at OLT.

260 consecutive liver transplants carried out between 1992 and 1999 were analysed. Patients with TIPSS in situ were identify from our database and compared to patients without TIPSS. Demographic data were collected along with aetiology of liver failure, technique of OLT, intra operative death, blood product usage and whether the TIPSS was intra hepatic at the time of transplant.

30 patients had TIPSS in situ, 230 patients did not. Of the TIPSS group 3 patients were noted to have a TIPSS extending outwith theliver. Median age in both groups was 51 years and cause of liver failure was similar in both groups with alcoholic liver disease and PBC accounting for the majority of the patients. The piggy back technique was used in the majority of patients in both groups (73% in TIPSS, 69% non TIPSS) and a temporary porto caval shunt was carried out in 18% of patients in the TIPSS group and 29% in the non TIPSS group. Intra operative mortality was 3%(1/30) in the TIPSS group and 2%(5/230) in the non TIPSS group. Blood product usage in units is shown below:

	RCC	FFP	Platelets	Cryoprecipitate
TIPSS	7(0-61)	7(0-24)	2(0-4)	0(0-10)
No TIPSS	6(0-44)	6(0-30)	2(0-18)	0(0-20)
Extra hepatic TIPSS	37(8-61)	20(4-24)	7(5-14)	2(0-3)

Data shown as median and range.

This data suggests that in comparable groups of patients the presence of a TIPSS does not increase blood product usage in patients undergoing mainly piggy back OLT. The presence of a TIPSS outwith the liver in either the extra hepatic portion of the portal vein or hepatic veins significantly increases the technical difficulty of the procedure and the need for transfusion.

C23

A PROSPECTIVE AUDIT OF 201 PIGGYBACK LIVER TRANSPLANT PROCEDURES TO DETERMINE THE OPTIMUM OPERATIVE TECHNIQUE

H.C.C. Pleass, K.G. Martin, K.K. Madhavan, M. Akyol, J.L.R. Forsythe, O.J. Garden
Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Lauriston Place, Edinburgh, EH3 9YW.

The classical technique of orthotopic liver transplantation (OLT) involves clamping the IVC and removal of the retrohepatic portion of the IVC with the diseased liver. Veno-venous bypass was developed to improve haemodynamic instability during this procedure but specific complications are associated with it's use. An alternative to the classical technique is the piggyback OLT procedure which employs caval preservation and avoids the need for veno-venous bypass. It has become the technique of choice within our unit but opinions differ as to the necessity of a temporary portocaval(PC) shunt performed as part of the piggyback procedure. A prospective database was analysed to compare results of piggyback OLT performed with or without a temporary PC shunt from October 1994 until the present day.

Results

201 piggyback OLT were performed and patient demographics are tabled below.

Indication	PC shunt	No shunt
Elective	62	84
Fulminant	15	17
Retransplants	7	16
Total	84	117

Morbidity and Mortality

	PC shunt	No shunt
Post Op renal failure	18 (21%)	29 (25%) NS
H.A.T.	3 (3.5%)	7 (6%) NS
P.N.F.	2 (2.3%)	5 (4%) NS
Biliary leak / stenosis	4 (4.7%)	9 (7.6%) NS
Venous outflow obstruction	1 (1%)	6 (5%) NS
Mortality < 30 days	6 (7%)	5 (4%) NS

Conclusion

The use of a temporary PC shunt is dependant on surgical preference and trial dissection, although is often the technique of choice in fulminants. Although performing a PC shunt adds time to the procedure, it reduces the incidence of all the major complications associated with OLT. A prospective randomised trial is required to confirm the optimum technique of piggyback OLT.

C56

LIVER GRAFT FAILURE AND RETRANSPLANTATION IN THE UK

Belger MA, Seeney FM, Hamilton CJ, McMaster P
on behalf of the UKTSSA Users' Liver Advisory Group

Background: The aim of this analysis was to investigate reasons for failure of first cadaveric liver transplants and, given the shortage of donors, to assess whether regrafts should ever be considered. Routine first cadaveric transplants, performed in adult recipients between 1 April 1994 and 31 December 1997 were analysed. 7 causes of failure were identified and those patients subsequently retransplanted were examined. Kaplan-Meier one year transplant survival estimates for the first graft and subsequent early (within 14 days) and late regrafts were calculated. The follow-up data for the one year transplant survival were 94% complete for routine first grafts and 93% for regrafts.

Results: 1613 routine adult first grafts were performed of which 433 had failed at the time of analysis. 27% were early and 73% were late failures. Of the early failures, 38% resulted from primary non-function and 14% from hepatic artery thrombosis. The majority of both these cases were retransplanted. In 92% of early failures due to technical reasons the recipient died. Of the 316 late failures, 23% were due to chronic rejection and 73% of these were retransplanted. Late failures due to recurrent disease were more likely to result in recipient death. The overall retransplant rate for the early failures was 62% and for the late failures 57%.

One year transplant survival for routine second grafts was significantly poorer than for routine first transplants, 59% (95% CI, 51%-67%) compared with 79% (95% CI, 76%-81%), (Log-rank test, $p<0.001$). Early regraft patients showed a significantly poorer one year transplant survival rate, 47% (95% CI, 33%-61%) compared with 64% (95% CI, 55%-74%) for late regrafts, (Log-rank test, $p<0.001$).

Conclusion: The majority of early failures due to technical reasons and late failures due to recurrent disease resulted in patient death. Early failure due to primary non-function or hepatic artery thrombosis and late failure due to chronic rejection generally resulted in retransplantation. The one year transplant survival rate for retransplants was significantly poorer than for first grafts and worse still when it was an early regraft, however the results suggest that it is still worthwhile retransplanting, even patients whose first graft failed early.

C66

LIVER TRANSPLANTATION IN PATIENTS OVER SIXTY YEARS OF AGE

Garcia C.E., Garcia R., Gunson B., Neuberger J., Mayer D.

Liver Unit – Queen Elizabeth Hospital – Birmingham – UK

The European Liver Transplant Registry Database has shown a rise in the percentage of patients aged over 60 years from 9% in 1990 to 16% in 1998. However, the European data also suggest that outcome is inferior in the over sixties compared with younger patients. UNOS data show a similar disparity in outcome, suggesting that elderly patients should be more rigorously selected than younger patients.

Patients and Methods : Between January 1990 and September 1999, a total of 875 adults underwent liver transplantation for chronic liver disease. Outcome and risk factors were compared for patients <60 and ≥ 60 years old.

Results : 702 patients were <60 years (48.4% females) and 173 were ≥60 years old (66.9% females). The main liver disease diagnoses were PBC (30.2%), PSC (15.4%), ALD (12%) in the <60 group, and PBC (53.8%), ALD (8.7%), PSC (7.5%) in the ≥60 group. There were similar numbers of Child-Pugh A patients in the two groups (8.3 vs 8.9%) but a significantly higher proportion of Child-Pugh C in the younger patients (55.9 vs 42.9%; $p<0.05$). The incidence of pre-transplant complications (encephalopathy, ascites, gastrointestinal bleeding) as well pre-transplant biochemical tests (albumin, bilirubin, PT) were similar in patients aged under and over 60. The median time of the duration of stay in intensive care unit after transplantation was 3 days for both groups. The 7 year actuarial survival for the over 60s was similar to that for younger patients ($p=0.2$). However, for Child-Pugh C patients, there was a trend towards worse survival in the over sixties ($p=0.08$).

Conclusions. There were no significant differences in overall morbidity and mortality between groups of <60 and ≥60 years old. Therefore the advanced age by itself is not a contraindication to liver transplantation. However, we advise transplanting these patients at an earlier stage before their disease has progressed to Child-Pugh C.

ACTIVE TGFβ$_1$ EXPRESSION IN PROTOCOL LIVER BIOPSIES: COMPARATIVE STUDY BETWEEN CYCLOSPORIN A AND TACROLIMUS.

Mohamed M A S, Balupuri S, Gerstenkorn C, Robertson H, Kirby JA, Talbot D.

Transplant Immunobiology Unit, Department of Surgery, University of Newcastle, Newcastle upon Tyne NE2 4HH, UK +191 222 8514.

Chronic rejection remains a major cause of graft dysfunction following solid organ transplantation. It has been proposed that this fibroproliferative disease may be promoted by overproduction of the growth factor TGFβ. In this respect it is significant that Cyclosporin A has been associated with upregulation of TGFβ by some human tissues as renal tubular epithelial and interstitial cells. The current study was designed to measure the active TGFβ expression in protocol liver biopsies performed at day six post-transplantation.

The day six protocol liver biopsies were divided into two groups on the basis of immunosuppression with cyclosporin A (19) and tacrolimus (18). The sections were first dewaxed and then incubated with primary chicken antihuman active anti-TGFβ$_1$ antibody. After washing and treating with secondary rabbit anti-chicken antibody conjugated with FITC, the sections were analyzed by semi-quantitative scanning confocal fluorescence microscopy. Data was expressed as the ratio of the mean fluorescence of the experimentally stained tissues to the corresponding value from control sections.

The day six protocol liver biopsies from the patients treated with Cyclosporin A expressed significantly more active TGFβ$_1$ mainly around the central veins of the hepatic lobules (range 1-6.3, median ratio of 1.8) than sections from patients receiving Tacrolimus (range 0.9-1.2, median ratio of 1.1) ($P=0.00048$, student t test).

These results suggest that there is a greater expression of active TGFβ$_1$ in liver biopsies from patients receiving cyclosporin A than in biopsies from tacrolimus-treated patients. This may have implications for the development of chronic graft rejection though long-term follow up is not yet available.

Abstract category: Laboratory.

Short Clinical Presentations

C60

SANGCYA USE IN ADULT RENAL, HEART AND LIVER TRANSPLANTATION.

First MR, Gaston RS, Pan S-H, Renlund D. [Introduced by: Pascal Diesel]

University of Cincinnati Medical Center
231 Bethesda Avenue, MSB5502
Cincinnati, OH 45267-0585 USA

SangCya is a novel, oil free formulation of cyclosporine (CyA) and is bioequivalent to Neoral in healthy volunteers. Additional study of bioequivalence (BE) via a comparison of intra-subject variability (ISV) between products, and clinical inter-changablity in renal, hepatic, and cardiac transplant recipients has been performed.

Determining ISV: ISV comparison was tested using a single dose, randomized, open label, 4 period, 2 sequence, crossover design in 20 healthy subjects. Equivalence is established for a particular log metric if the upper 95% confidence interval (CI) for the BE criterion is = theta (a standard suggested by the US Food and Drug Administration for evaluating ISV). CIs were determined using the bootstrap nonparametric percentile method (N=2000 bootstrap samples), with stratification for sequence. Results of ISV: log AUC0-24, log AUC0-infinity and log Cmax were comparable for ISV between SangCya and Neoral (p=0.835, p=0.431 and p=0.503, respectively). For each PK parameter, the upper 95% CI for theta was below the acceptable limit for bioequivalence (theta < 2.495).

Determining BE in Txp recipients: Blinded randomized studies compared SangCya to Neoral have been performed. PK studies were completed in: 42 stable renal Txp recipients (39 have >3m f/u); 26 stable liver Txp recipients (18 have >6m f/u); and 26 stable cardiac Txp recipients (all have >6 m f/u). Results of bioequivalence testing in Txp recipients: Example PK parameters are given below for each transplant group (AUC values are ng*h/ml.)

Txp Type	$AUC_{(0\text{-}12)}$ SangCya	$AUC_{(0\text{-}12)}$ Neoral	p-valu
Renal	3319±1263	3267±1197	0.900
Liver	3397±957	3572±1448	0.846
Cardiac	3658±1236	3682±1551	0.854

All PK parameters, efficacy (defined by graft function, episodes of acute rejection and graft survival), and incidence of side-effects were equivalent between SangCya and Neoral. PK parameters of CyA and its major metabolites are identical (data not shown).

Determining long-term safety: An open label study of 41 stable renal Txp recipients was performed; results after 2 years of follow-up indicate that serum Cr has remained stable throughout the study period (1.4 ± 0.4 mg/dl, p=0.33 from baseline). Two patients had AR within 3 months of starting therapy.

Conclusion: SangCya and Neoral are bioequivalent in normal volunteers and adult renal, heart, and liver Txp recipients. These data demonstrate that SangCya is inter-changeable with Neoral.

C67

CHRONIC ALLOGRAFT NEPHROPATHY : A SINGLE CENTRE RANDOMISED TRIAL OF CYCLOSPORIN WITHDRAWAL AND MYCOPHENOLATE MOFETIL OR TACROLIMUS SUBSTITUTION.

McGrath, JS and Shehata, M.

The Nottingham Transplant Unit, Nottingham City Hospital, Nottingham, U.K.

Background: Chronic allograft nephropathy (CAN) represents the leading cause of late graft loss and, although its pathogenesis is unclear, evidence suggests a contributory effect for cyclosporin (CyA). The advent of alternative immunosuppressants has directed attention towards 'CyA-sparing' treatment regimes. This study assessed the impact of complete CyA withdrawal and conversion to mycophenolate mofetil (MMF) or tacrolimus (FK) treatment on graft loss, change in isotope GFR (0,6 and 12 months) and change in slope of serum creatinine. Proteinuria, blood glucouse, lipid profile, blood pressure control and anti-hypertensive requirements were also studied.
Method: Patients with biopsy-proven chronic allograft nephropathy were randomised to receive either MMF (n=15) or FK (n=15) with complete withdrawal of CyA therapy. All patients were on CyA and prednisolone prior to conversion. FK was commenced at 0.15 mg/kg/day and then adjusted according to TAC-II serum levels. In this group azathioprine dose was unchanged (1.2 mg/kg). In the MMF group azathioprine was stopped and MMF was commenced at 2g / day. CyA dose was unchanged for 4 weeks and then reduced in a step-wise fashion to complete withdrawal at 12 weeks. Patients receiving less than 10mg prednisolone per day had their dose increased to 10mg / day in both groups.
Results: At submission, mean follow-up was 7.1 months (range 3-12 months). There were no episodes of acute rejection in either group. Two grafts were lost in FK group. Average pre-conversion creatinine and GFR were similar in both groups. In the MMF group there was a 38% improvement in serum creatinine at 6 months compared to 9% in FK group. GFR findings were similar. No significant changes were observed in proteinuria. Lipid profile and blood pressure control improved in both groups.
Conclusions: Complete substitution of cyclosporin in patients with CAN results in:

1. Initial stabilization of serum creatinine in both groups.
2. FK does not appear to result in further improvement in serum creatinine or GFR.
3. MMF is associated with a significant improvement in graft function as evidenced by GFR and slope of serum creatinine, but only after complete withdrawal of CyA.
4. The results of this study suggest that the improvement in graft function is not solely attributable to CyA withdrawal.

C41

ASSESSMENT OF POTENTIAL RENAL DONORS WITH SPIRAL CT

Jan W[1], Koffman G[2], Chuie S[3], Rankin S[3].

1.RADIOLOGICAL SCIENCES, GUY'S, KINGS AND ST. THOMAS' SCHOOL OF MEDICINE
2.Renal Transplant Unit, Guy's and St. Thomas' Hospital.
3.CLINICAL RADIOLOGY DEPARTMENT, GUY'S AND ST. THOMAS' HOSPITAL

Purpose: To assess spiral CT as the primary imaging technique in the evaluation of potential live renal donors.

Materials and Methods: Forty-nine potential renal donors underwent spiral CT examination before and after contrast enhancement. An abdominal X-ray for the assessment of the renal collecting system and ureters was performed immediately after the CT. The axial scans were reviewed and were reconstructed and displayed in a three-dimensional format. The radiological reports were compared with the results of surgery (thirty-one donors).

Results: CT and surgical findings agreed in 90% of donors (28/31). In three cases small accessory arteries (all < 2mm in diameter) were missed. Early branching arteries were diagnosed correctly in all cases. The sensitivity and specificity for identifying the main renal veins and arteries was 99.6%, and for the accessory renal arteries 91% and 96.5%, respectively. Of the forty-nine donors, four (8%) had findings on CT that precluded further consideration for donation.

Conclusion: Technical advances in CT helical technology as well as rapid data processing have combined to allow improved non invasive imaging of arterial and venous anatomy. CT angiography is rapidly replacing IVU and conventional angiography as the primary imaging modality for evaluating potential renal donors. This technique reduces the number of examinations as well as the risk and cost of imaging in these subjects.

C46

LOW DOSE ASPIRIN AS PROPHYLAXIS AGAINST RENAL VEIN THROMBOSIS IN RENAL TRANSPLANT RECIPIENTS.

Gray, D.W.R., Robertson A.J., Nargund, V., Morris P.J.

Nuffield Department of Surgery, Oxford Transplant Centre, Churchill Hospital, Oxford.

Background:

Renal vein thrombosis (RVT) is an infrequent event that accounts for a high proportion of early renal allograft losses, since graft failure secondary to acute irreversible rejection is now relatively rare. The cause of renal vein thrombosis may be related to technical problems, clotting disorders, diabetes or cyclosporine, but is often difficult to define.

Methods:

A retrospective study was performed to examine the influence of aspirin on the incidence of RVT in cadaveric and living related renal transplant recipients receiving cyclosporine based triple immunosuppresssion. The Oxford Transplant Centre database was used to identify all early (<30day) non-immunological graft failures and case histories were examined for clinical and pathological evidence of RVT. In July 1991, aspirin (75mg od starting immediately before and continuing for one month post transplant) was introduced as routine prophylaxis against RVT. Prior to this, aspirin prophylaxis was not used.

Results:

In the six year period from July 1985 to June 1991, there were 27 cases of RVT in 475 transplants (5.6%). In the subsequent 6 year period, there were 6 cases of RVT in 480 transplants (1.2%) ($p < 0.01$).

Conclusion:

Although not abolished, this indicates a significant reduction in the incidence of RVT associated with the addition of low dose aspirin. In the absence of a prospective randomized trial, this study provides the best evidence to date to support the continued use of low dose aspirin as prophylaxis against renal vein thrombosis following renal transplantation.

C8 CHRONIC ALLOGRAFT NEPHROPATHY: A PROSPECTIVE RANDOMISED TRIAL OF CYCLOSPORIN REDUCTION WITH OR WITHOUT RAPAMYCIN

RN Saunders, S Carr, M L Nicholson

Department of Transplant Surgery, Leicester General Hospital, Leicester UK

Introduction: Both immune and non-immune factors such as Cyclosporin (CyA) toxicity are important in the pathogenesis of chronic allograft nephropathy (CAN). Rapamycin (Rapa) has immunosuppressive properties but in addition inhibits the cytokine driven proliferation of smooth muscle cells and fibroblasts that may play an important role in the generation of this condition. Rapa has been proposed as a possible treatment for CAN with potential benefits over CyA reduction alone. The aim of this trial was to investigate this hypothesis.

Methods: Thirty renal transplant recipients with biopsy confirmed CAN immunosuppessed with CyA were recruited. They were randomised to receive either a 40% dose reduction in CyA to maintain trough levels at 50-75ng/ml (control) or a 40% dose reduction in CyA with the addition of Rapa 2mg/day. Patients were assessed at 1,2,4,6,8,12 and 24 weeks.

Results: Both groups were well matched in terms of patient characteristics, mean (±SD) time after transplantation (Rapa 84±40 vs Control 69±30 months, p=0.33) and mean (±SD) CyA trough levels after dose reduction (Rapa 68±21 vs Control 56±19 ng/ml, p=0.10). Rapa trough levels had stabilised by 4 weeks with a mean of 7.7±3.4 ng/ml.

	Control		Rapamycin	
	Pre	Post	Pre	Post
Serum Cr (µmol/L)	204 (44)	193 (42) **	206 (45)	204 (59)
Cr Clearance[a] (ml/min)	34.9 (10.0)	36.8 (9.3) *	40.1 (9.5)	41.7 (9.8)

Values expressed as mean (sd). [a]Cockroft and Gault formula. (*p<0.05, **p<0.025 Paired two-tailed t-test).
Side effects from Rapa were common with the majority of patients reporting minor symptoms and demonstrating haematological or lipid abnormalities.

Conclusion: These preliminary results suggest that Rapa at a dose of 2mg/day has no significant benefits over CyA reduction on the renal function of patients with CAN.

C33

BLADDER RECONSTRUCTION AND TRANSPLANTATION OVER AN 18 YEAR PERIOD

Appanna T, Derby C, Fenn N, Steinberg J and Stephenson TP

Department of Urology, University Hospital of Wales, Heath Park, Cardiff

Introduction

Follow up was undertaken of patients who had undergone bladder reconstruction and transplantation for end stage renal failure.

Patients and Methods

28 patients were reviewed, 23 had neuropathic bladders, and all but 5 were walkers. 15 had substitution cystoplasties and 8 had clam enterocystoplasties. 4 had required an artificial urinary sphincter (AUS). 4 of the 5 non-walkers had continent diversions. In 11, the reconstruction involved undiversion. Live donor transplants were always preferred where possible (18).

Results

22 patients are well with stable renal function, though 5 have had second grafts. 2 deaths have occurred, one with normal renal function and one post-reconstruction. 4 returned to long term dialysis.

Conclusion

No patient should be transplanted into a surface diversion. Ideally patients should be transplanted before the end stage renal failure is reached, and this and the preferred use of live donor grafts avoids the potential hazards of the "dry" cystoplasty. Concerns about the AUS and bacteriuria in suppressed patients on self catheterisation have proved unfounded.

C12

PROTEINURIA, RENAL TUBULAR CATABOLISM AND INJURY IN CHRONIC VASCULAR REJECTION

Rustom R, Sells RA, Grime S, Maltby P, Shenkin A, Smith L, Brown M, Hammad A & Bone JM.

Sir Peter Medawar Unit, Royal Liverpool University Hospital, Liverpool, UK.

Purpose of Study After the first year of transplantation, chronic vascular rejection (CR) is the commonest cause of graft loss. There are few clues to aetiology but proteinuria is a common feature. In diseased native kidneys proteinuria and progression to failure are linked. We proposed a pathogenic role for this excess protein in dissimilar disease, at the tubular level. We demonstrated in both nephrotic patients with normal function and in those with failing kidneys increased renal tubular catabolism and turnover of a peptide marker, Aprotinin (Apr) and linked this to increased ammonia excretion and tubular injury. These injurious steps were suppressed by reducing proteinuria with Lisinopril. Do similar mechanisms and such a linkage also occur in proteinuric transplanted patients with CR? Does Lisinopril have a similar benefit?

Methods 11 patients with biopsy proven CR were studied. Lisinopril (10-40 mg) was given daily for 2 months in 7 patients. 4 others were given oral sodium bicarbonate ($NaHCO_3$, 0.03-0.12 g/Kg) for 2 months before adding Lisinopril. Renal tubular catabolism of ^{99m}Tc-Apr, was measured before and after Lisinopril by gamma-ray imaging and urinary radioactivity. Urine was assessed fortnightly for N-acetyl-ß-glucosaminidase (NAG) and ammonia before and after treatment.

Results (Lisinopril alone) (Means ± SEM)	Before Lisinopril	After Lisinopril
Proteinuria (g/24 h)	7.8 ± 2.2	3.4 ± 1.9*
^{51} CrEDTA clearance (ml/min/1.73 m^2)	28.4 ± 4.2	24.5 ± 6.9
Kidney uptake of ^{99m}Tc-Apr* (% of dose)	21.5 ± 4.2	18.8 ± 3.9
Metabolism of ^{99m}Tc-Apr* (% of dose/h)	0.5 ± 0.05	0.3 ± 0.005**
Fractional degradation of ^{99m}Tc-Apr* (/h)	0.04 ± 0.009	0.02 ± 0.005 **
Plasma bicarbonate (mmol/l)	19.1 ± 0.7	17.4 ± 0.8**
Plasma potassium, K (mmol/l)	4.6 ± 0.7	5.3 ± 0.2**
24 h urinary K (mmol/24 h)	76.4 ± 8.3	58.0 ± 6.2***
Urinary ammonia (mmol/l)	1.1 ± 0.2	0.75 ± 0.2
Urinary NAG (µmol/24 h)	2108 (1044-3816)	1008 (76-2147)*

p value * <0.05, **< 0.02, ***<0.0001

In the 4 patients who were also given $NaHCO_3$, the mean plasma HCO_3 increased to 26.6 ± 1.5 mmol/l ($p<0.02$). Acidosis did not recur after Lisinopril (24.3 ± 0.3), and neither were there significant changes in K (plasma & urine).

Conclusion In CR, Lisinopril led to a reduction in proteinuria, renal tubular catabolism of Apr* and NAG, albeit at the cost of increased acidosis (not previously described). Co-administration of $NaHCO_3$ prevented the acidosis and hyperkalaemia. Longer term studies are in progress using Lisinopril (and $NaHCO_3$) to see if progression of CR can be mitigated.

Posters

Tuesday 21 March

C2

CORRELATION OF α AND TOTAL GLUTATHIONE S TRANSFERASE (GST) ESTIMATION IN MACHINE PERFUSED NON HEART-BEATING DONOR KIDNEYS AS A VIABILITY PARAMETER.

Balupuri S, Mantle D, Cornell C, Mohamed MAS, Buckley P, Manas DM, Kirby J, Talbot D.

Dept.of Surgery and Freeman Hospital, University of Newcastle. Newcastle upon Tyne. NE2 4HH

Shortage of donor kidneys has led to a renewed interest in non-heart beating donor (NHBD) organs. Since organs suffer ischaemic damage, viability parameters have been proposed to evaluate such damage before transplantation. Machine preservation allows enzymatic evaluation. αGST, a cytosolic enzyme, is released into the perfusate by the proximal tubular cells in response to ischaemia. Measurement of αGST is done by an ELISA system which is time consuming and expensive. We analysed total GST, a simpler and cheaper biochemical test and correlated it with alpha GST measurements.

METHODS. **αGST** was determined by using NEPHKIT –ALPHA immunoassay. (Biotrin® International, Dublin, Ireland) Diluting the sample 1:500 times was followed by a standard assay procedure.

Total GST activity was measured by a spectroscopic assay based upon the rate of change in absorbance occurring at 340nm when the substrate 1- chloro-2,4-dinitrobenzene (DNB) is conjugated with glutathione. The reaction was carried out at 25°C in phosphate buffer, 0.1M pH 6.5; glutathione 1.55mM; and substrate, 4.2mM; using Cobas Mira® analyser. The assay was duplicated. Samples with GST activity >300 U/l were diluted 1:5 with phosphate buffer and re assayed. Inter-assay imprecision was< 6%.

RESULTS 30 NHBD kidneys were machine perfused for 8 hours. In 74 samples analysed for alpha and total GST we found that in 10, the value of alpha GST was too high and was off the scale of calibration curve requiring a higher dilution. This was consistent with the very high tGST observed in those cases. In another 8 samples, where tGST was very low, alpha GST too could not be detected on the standard calibration curve. Again we find this consistent with our observations. In the remaining 56, there was direct significant correlation between total GST and α GST ($r = 0.771$ Pearson Correlation).

CONCLUSION Total GST correlates well with α GST estimation and can replace it as a quicker method of ischaemic damage determination.

C25

CONVERSION TO GENERIC ORAL CYCLOSPORINE SOLUTION IN PEDIATRIC HEART TRANSPLANT RECIPIENTS

Chinnock R, Johnston J, Thomas M

Loma Linda University Children's Hospital
11175 Campus Street, Loma Linda, CA 92354 USA

Purpose: Prior studies have established bioequivalence between two modified cyclosporine (CyA) oral solutions (SangCya and Neoral) in adult healthy volunteers and transplant patients. Less is known of the equivalency of these two preparations in the pediatric transplant population. This study was undertaken to compare CyA dose, CyA trough level and renal function in pediatric heart transplant recipients converted from Neoral to SangCya.
Methods: Substitution of Neoral oral solution with SangCya was initiated at our center after insurance companies changed their formulary because of financial reasons. We did not change our routine follow-up patient monitoring other than to obtain a follow-up CyA trough level soon after conversion. From 1/99 through 9/99, 22 stable patients were converted on a mg/mg basis, with appropriate adjustments to maintain CyA target levels. Patient weight, CyA dosage requirements, CyA levels, BUN and Creatinine levels were compared before and after conversion. Results were evaluated using paired samples T-test.
Results: Median (range) age at transplantation was 80 days (9 – 4040 days) with a median (range) time of conversion post-transplant of 5.1 years (0.67 to 12.8 years). Pre- and post-conversion values are as noted in the table. Normalized CyA levels are expressed as median (range), all others are mean ± SD.

	Neoral (pre-conversion)	SangCya (post-conversion)	p-value
CyA daily dose (mg)	114 ± 43	115 ± 42	0.15
CyA daily dose (mg/kg)	5.6 ± 2.4	5.7 ± 2.4	0.20
Trough CyA level (ng/ml)	145 ± 48	153 ± 58	0.55
CyA level (normalized to mg dose)	1.2 (0.6 to 5.0)	1.4 0.6 to 5.0)	0.85
CyA level (normalized to mg/kg dose)	21.8 (12 to 105)	25.5 (13 to 107)	0.74
Blood urea nitrogen (mmol/L)	9.3 ± 2.1	8.6 ± 2.1	0.58
Serum Creatinine (µmol/L)	53 ± 18	62 ± 18	0.64

There were no significant changes in CyA trough blood level normalized to daily dose, nor in renal function pre- and post-conversion. Only 1 acute rejection has occurred, 9 months after conversion.
Conclusion: Pediatric heart transplant recipients can be safely converted from Neoral to SangCya on a mg/mg basis. Rejection rates and renal function, at least in the short term, are equivalent. CyA medication cost savings could be realized.

C35

THE RELATIVE EFFECT OF ACUTE REJECTION ON DELAYED GRAFT FUNCTION IN RECIPIENTS OF ASYSTOLIC OR CADAVERIC DONOR KIDNEYS

M Metcalfe, SA White, G Murphy, S Jain, T Horsburgh, P S Veitch, M L Nicholson

Departments of Surgery, University of Leicester & Leicester General Hospital,

The combined effect of acute rejection (AR) and delayed graft function (DGF) are detrimental to renal allograft graft survival. The aim of this study was to assess the impact of acute rejection episodes in recipients of an asystolic or cadaveric (CAD) donor kidney with DGF.

All recipients of a NHBD kidney (n=69) or cadaveric kidney (n=66) with DGF were retrospectively identified over a 10 year period. Delayed graft function was defined as the need for dialysis within the first 7 days after transplantation with the exception of those required for fluid overload and hyperkalaemia. Primary immunosuppression comprised either cyclosporin or Tacrolimus. All patients with DGF underwent weekly needle core biopsies using ultrasound guidance to exclude AR. Histologically confirmed AR was graded according to severity and treated with intravenous steroid. AR episodes resistant to steroid were treated with ATG. All patients with primary non function were excluded from analysis.

Of those patients receiving a NHBD kidney (22F:47M median age 49 yrs), 94% experienced DGF. Conversely of those receiving a CAD kidney (n=440) the incidence of DGF was 15%. Both groups were well matched and the results are compared in the table below.

	NHBD (n=69)		CAD (n=66)	
	AR	No AR	AR	No AR
Recipient age (yrs)	50	49	42	46
Donor Age (yrs)	47	49	42	45
HLA-DR mismatch	13 (80)	26(49)	13(65)	17(37)
Anastomosis time (mins)	29	30	36	31
Cold ischaemic time (hrs)	16	17	22	16
DGF (Days) *	24	16	11	10
12 month Creatinine	201	141	176	155
1 Year graft survival (%)	88	91	86	92

All median values unless stated (percentages). * ANOVA P<0.05

The presence of acute rejection episodes in kidneys retrieved from a NHBD prolongs the duration of DGF but does not appear to influence graft function or graft survival at 1 year.

C40

ASSESSMENT OF POTENTIAL LIVING KIDNEY DONORS.

Dudley C, Hamilton K, Harper S, Mathieson P, Feest T, Tomson C, and Lear PA

Richard Bright Renal Unit, Southmead Hospital, Westbury-on-Trym, Bristol BS10 5NB

Living donor assessment in our unit is performed according to a defined protocol. From 1995-1998 148 potential living kidney donors were initially identified. Eighty-eight subjects were excluded for a variety of reasons after blood tests initiated by the transplant co-ordinator. These reasons included blood group incompatibility, positive cross match etc. Sixty blood group compatible potential donors were medically assessed. Thirty-one donors (52%) have undergone nephrectomy (26 LRDs, 5 spousal). Donor, recipient and graft survival is 100%. Twenty-nine (48%) patients have not progressed to donor nephrectomy for a variety of reasons:

Reason for exclusion from donor nephrectomy	n
Recipient received cadaveric transplant during donor work up	7
Positive cross match	6
Glomerulonephritis on renal biopsy	4
Renal impairment	3
Severe obesity (BMI>35 kg/m^2)	3
Other	6

Six (10%) potential donors had renal biopsies.

Reason for biopsy	Histology	Donor outcome
Microscopic haematuria	IgA	Excluded (also partial Factor XI deficiency)
Microscopic haematuria	IgA	Excluded
Microscopic haematuria	IgA	Excluded
Albuminuria and possible hereditary renal disease	Normal	Nephrectomy
Microscopic haematuria	Normal	Excluded (multiple renal vessels)
Microscopic haematuria	IgM and C1q on immunohistology	Excluded

Over this period, live donor transplantation accounted for 12% of the total transplant programme. Nearly half of all potential blood group compatible living kidney donors did not progress to organ donation after medical assessment. Previously unrecognised renal disease in donors was surprisingly common (12%). Donor assessment is time consuming, labour intensive and costly. The costs in excluded donors totalled £31 000. Based on our experience, we would recommend that: 1) Cross matching is performed immediately after blood group compatibility is established 2) If the initial donor assessment is satisfactory, removal of the recipient from the cadaveric waiting list should be considered 3) Extra resources should be made available if living donation is to increase in the UK.

C44

A PROSPECTIVE RANDOMISED TRIAL COMPARING MYCOPHENOLATE MOFETIL AND AZATHIOPRINE AS AGENTS TO ALLOW CYCLOSPORIN DOSE REDUCTION IN CHRONIC RENAL ALLOGRAFT NEPHROPATHY

S Jain, M Metcalfe, SA White, PN Furness and ML Nicholson
Department of Transplant Surgery, Leicester General Hospital, Leicester, UK.

Cyclosporin nephrotoxicity is felt to be important in the development of chronic allograft nephropathy (CAN) and dose reduction is a recognised therapeutic option in this condition. This study compared two agents that can be used as additional immunosuppression to allow cyclosporin dose reduction, azathioprine (AZA) and mycophenolate mofetil (MMF).

Twenty-one renal transplant patients on cyclosporin based immunosuppression with clinical and biopsy evidence of CAN were enrolled in this study. Patients all had 40% reduction in cyclosporin dose and were then randomised to receive either AZA (n=11) or MMF (n=10) as additional immunosuppression. Renal function was assessed using the Cockroft and Gault estimate of GFR. This was plotted against time to calculate rate of change of renal function for six months before and after entering the trial.

In the AZA group 3 patients had a worsening in the rate of decline of renal function. Two of these suffered graft failure and returned to dialysis during the course of the trial at 8 weeks and 12 weeks respectively. The other 8 patients on AZA, and all those on MMF benefited from cyclosporin reduction with either a retarded rate of decline (n=5 for AZA, n=6 for MMF), or an actual improvement (n=3 for AZA, n=4 for MMF) in renal function. Comparison of the mean rate of change of GFR before and after entering the trial confirmed a significant improvement for those patients on MMF. In the AZA group there was a continued decline in the mean slope overall, but this was mainly because of the two patients who had failed. When these were left out there was an overall improvement in the other patients though this did not quite reach significance.

		MMF (n=10)	AZA (n=11)	AZA excluding failures (n=9)
Rate of change of GFR (ml/min/month)	Pre-trial	-0.37	-0.31	-0.24
	Post-trial	0.85*	-0.45	0.41†

*p<0.01 vs pre-trial slope, †p=0.09 vs pre-trial slope

Gastrointestinal side effects were common in the MMF group occurring in five patients, and requiring cessation of MMF in two. There was one acute rejection episode during the course of the study in a patient on azathioprine and this was treated successfully.

In conclusion while cyclosporin reduction using azathioprine did lead to an improvement in renal function in most patients with CAN, the effect was greater when MMF was used as additional immunosuppression. Acute rejection episodes and graft loss due to CAN were also prevented. This may be due to an additional effect of MMF on continuing immune damage.

C50

THE PREPARATION FOR AND PROGRESS OF AN ACCIDENT AND EMERGENCY DEPARTMENT BASED NON HEART BEATING KIDNEY DONATION PROGRAMME - A REPORT AT 16 MONTHS

Buckley P.E.[1], Balupuri S.[1], Russell K.[1], Rose P.[1], Robson L.[1], Sen B[2] and Talbot D.[1]

Freeman Hospital[1] and the Royal Victoria Infirmary[2], Newcastle upon Tyne.

This paper reports on the progress of an Accident and Emergency Department (A&E) Non heart beating donation (NHBD) programme which was relaunched in August 1998. Preparation for this programme included:- (i) agreement of support for such a programme from the medical and nursing staff of the A&E department of the selected hospital and subsequent education of these staff, (ii) agreement from H.M. Coroner for the insertion of the double balloon triple lumen (DBTL) cannula and in situ perfusion of the kidneys in the potential donor prior to the arrival of relatives, and therefore without the consent of the relatives to this procedure, (iii) agreement of the Coroner's Pathologist to allow nephrectomy prior to the autopsy, (iv) lack of objection from the local public following a media campaign to inform them of the programme, (vi) a visit to the Maastricht to observe their machine perfusion and viability testing techniques and subsequently to set up our own system and (vii) enlist the support of staff in the Transplant Unit to assist the original team of three

The preparation for the programme was completed and the programme commenced in August 1998. The success of the programme in terms of referrals of potential donors through to the numbers of kidneys actually transplanted in the 16 months period from August 1998 is shown in Figure 1.

Figure 1 Outcome of NHBD referred from the A&E dept. Aug. 1998- Nov 1999

No. of referrals to transplant team	=35		
No. of attendances at the dept.	=20		
Potential donors not used	=23		
		Team unable to perform viability testing	= 8
		Deceased medically unsuitable	= 7
		Relatives refused donation	= 3
		Deceased high risk for viruses	= 2
		Coroner refused	= 1
		Failed cannulation	= 1
		Relatives unavailable	= 1
Actual donors	= 12		
Kidneys transplanted	= 16		
Kidneys unsuitable	= 8		

The use of a single person to perform the viability testing led to the loss of 8 potential donors.

Twenty attendances at the A&E dept. resulted in 16 cannulation and in situ perfusion procedures being performed, thus there was some wastage of equipment and fluids. Sixteen kidneys were used for transplantation. We feel that this programme is a useful source of kidneys and plan to continue. We have increased the number of transplant unit staff involved and will closely monitor all outcomes.

C54

HOMOZYGOSITY ON THE NATIONAL KIDNEY TRANSPLANT WAITING LIST

Johnson RJ, Neubert AR, Fuggle SV, Ray TC, Belger MA, Briggs JD
on behalf of the UKTSSA Users' Kidney Advisory Group

Background: Homozygous patients are over-represented on the national kidney transplant waiting list. 22% of the current waiting list were HLA-DR homozygous compared with 15% of donors in 1997. This problem stems from the use of kidneys from homozygous donors for heterozygous recipients over a number of years. The UK kidney allocation scheme identifies a larger pool of recipients who are well matched for a homozygous than a heterozygous donor. Whilst investigating the scale of the problem, two definitions of homozygosity were considered: complete homozygosity at the HLA- A, B and DR loci and homozygosity at the HLA-DR locus irrespective of the homozygosity state at the other two loci.

Methods: First, a recent waiting list was analysed and waiting times were compared for homozygous and heterozygous patients; secondly, recipient characteristics were investigated to identify who had received kidneys from homozygous donors in the year between 1 July 1998 and 30 June 1999.

Results: Homozygous patients waited significantly longer for a transplant than their heterozygous counterparts (both definitions). For instance, the median waiting time for HLA-DR homozygous blood group O patients was 887 (IQ range 378-1498) days compared with 545 (IQ range 202-1191) days for HLA-DR heterozygous patients ($p=0.0001$). Logistic regression showed that homozygosity strongly influenced whether waiting time was in excess of 2 years even when allowing for other relevant factors.

Analysis of 1169 transplants of nationally allocated adult kidneys showed a significantly higher proportion of HLA-DR homozygous organs transplanted into 000 HLA-A, B, DR mismatched recipients, highly sensitised patients (HSPs), children and HLA-DR homozygous recipients. Only 25% of all kidneys from adult HLA-DR homozygous donors were transplanted into HLA-DR homozygous recipients.

Conclusions: Because the current UK National Kidney Allocation Scheme prioritises children, HSPs and 000 HLA-A, B, DR mismatched recipients, nationally allocated homozygous organs are frequently transplanted in heterozygous recipients. Homozygous recipients, particularly those homozygous at the HLA- A, B and DR loci, often receive locally allocated, non-favourably matched grafts. The allocation of homozygous organs to prioritised groups of recipients contributed to the excess of homozygous patients on the waiting list.

C57

SMALL ADULTS - ARE THEY DISADVANTAGED IN LIVER TRANSPLANTATION?

Seeney FM, Belger MA, Hamilton CJ, Mayer AD, Rolles K, McMaster P
on behalf of the UKTSSA Users' Liver Advisory Group

Background: The aim of the study was to determine if 'small' adults were disadvantaged under the national liver allocation scheme in the UK, because of their requirement for a small donor's organ. This was investigated by assessing whether 'small' adults were disadvantaged compared with other adults and paediatric patients when waiting for a liver transplant and in subsequent transplant survival. Two definitions of small adult were considered: adults weighing ≤45 kg and ≤60 kg.

Results: Only 3% (28%) of 1713 registrations during the period 1 January 1995 to 31 December 1997 were adult patients ≤45 kg (≤60 kg). Kaplan-Meier estimates of median waiting time to transplant indicated significant differences between the 3 patient categories; ≤45 kg definition (log-rank test, p = 0.0128), ≤60 kg definition (log-rank test, p = 0.0001). Small adults weighing ≤45 kg waited longer than paediatric patients, a median of 90 days (95% CI 62-136) compared with 75 days (95% CI 57-91). Heavier adults had the shortest waiting time; median 50 days (95% CI 45-54). The median waiting time for small adults weighing ≤60 kg, 67 days (95% CI 57-79), was comparable with that for paediatric patients, but heavier adults waited considerably shorter for transplant, 45 days (95% 39-50). Small adults ≤45 kg had a higher proportion of deaths on the waiting list than both paediatric patients and 60 kg or less small adults, 11% compared with 7% and 8%, respectively.

The one year Kaplan-Meier transplant survival rates for both definitions of small adult were poorer than paediatric patients and heavier adults, although not significantly so. The rates for transplants between 1 January 1994 and 31 December 1995 were; small adults ≤45 kg 74% (95% CI 56%-92%), small adults ≤60 kg 73% (68%-78%), adults >45 kg 78% (75%-81%), adults >60 kg 80% (76%-83%) and paediatric patients 79% (71%-87%).

Conclusion: Overall small adults ≤45 kg had a longer median waiting time than paediatric patients. Small adults, using either classification, had the highest proportion of patient deaths compared with paediatric patients and heavier adults. For both definitions of small adults, one year transplant survival rates were lower than for other adult or paediatric patients, although not significantly. It was concluded that small adults were disadvantaged when waiting for a liver transplant. This resulted in the introduction of a small adult registration scheme, giving patients ≤45 kg the same priority status as children.

C63

ARE DIFFERENTIAL HOMOCYSTEINE LEVELS AN IMPORTANT RISK FACTOR FOR THE TRANSPLANT RECIPIENT TREATED WITH NEORAL AND PROGRAF?

Quiroga I, Morris-Stiff G, Darby C, Lord R, Jurewicz A.

Welsh Transplant Research Group, Cardiff
C/o Ward B5, University Hospital of Wales, CF4 4XW

Hyperhomocysteinaemia is widely recognised as an independent risk factor for the development of atherosclerotic cardiovascular disease. The aim of this study was to document plasma homocysteine levels in end-stage renal failure and sequentially following renal transplantation. We have compared the data between the calcineurin inhibitors Neoral and Prograf in a prospective randomised trial. Post transplant differential homocysteine levels between the two treatment groups may have implications for the development of cardiovascular disease and chronic rejection.

As a part of this study, homocysteine levels were measured in the plasma of cadaveric renal transplant recipients at baseline, three, six and twelve months. The samples were analysed by high performance liquid chromatography and a level of 16 micromoles per litre was taken as the upper limit of normal. At each time point serum creatinine, albumin and drug trough levels were also assessed and an ultrasound guided renal biopsy was performed and histological changes classified according to the Banff criteria.

Assessment point	Median Homocysteine level Neoral n= 45	Median Homocysteine level Prograf n= 46	Neoral versus Prograf	% outside normal range Neoral	% outside normal range Prograf
Baseline	24.7	24.0	P= 0.19	82.2	93.5
3 months	17.7	15.6	P= 0.09	67.4	45.6
6 months	16.3	14.3	P= 0.08	51.3	32.5
12 months	16.3	12.8	P= 0.006	61.5	17.4

There was a significant correlation between homocysteine levels and creatinine at 3, 6 and 12 months (Pearson coefficient 0.511, 0.37, 0.616). There was a trend towards lower homocysteine levels in the Prograf treated patients versus Neoral, at 3 and 6 months: this difference had become significant at 12 months. By 12 months many more patients in the Prograf treated group had normalized their homocysteine levels compared to the Neoral treated patients. There was no correlation between homocysteine and either age, sex, serum albumin and immunosuppressant trough levels.

At the relatively early time point of 12 months post transplantation, there is no significant difference in the development of cardiovascular events or histologically diagnosed chronic graft nephropathy between Prograf and Neoral treated patients. We will continue to monitor these data over at least the next five years.

Plasma and urinary electrolyte regulation after pig-to-primate renal xenotransplantation.

B.Soin[1,2], A.Zaidi[3], E. Cozzi[1,2], D.Ostlie[4], A. Lockhart[1], K.G.C.Smith[5], J.R.Bradley[5], D.J.White[1,2] & P.J.Friend[6]

[1]Imutran Ltd (A Novartis Pharma AG Company), P.O Box 399,Cambridge CB2 2YP, UK. [2] University dept. of Surgery, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK. [3]Dept of Cardiothoracic Surgery,Heath Hospital, Cardiff, U.K.[4] Dept. of Surgery, Mayo Clinic,AZ 85259 USA [5] Dept. of Nephrology, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK. [6]Nuffield Dept. of Surgery, John Radcliffe Hospital, Oxford OX3 9DU, UK.

Introduction: The pathological consequences of discordant xenotransplantation have dominated xenotransplantation research. The question of physiological compatibilty between species has been previously raised and speculations made based on *in vitro* and small animal *in vivo* studies. There are definite reported failures of hormones to function between species. The use of human Decay Accelerating Factor (hDAF) transgenic porcine organs, in combination with novel immunosuppressive regimes, has produced prolonged survival of over two months in life supporting models.This has allowed study of the other metabolic and hormonal actions of a renal xenograft.

Methods: The hDAF transgenic pig-to-primate renal xenotransplantation model with native nephrectomies is well established in our unit. The recipients receive an induction regime based on cyclophosphamide, cyclosporin A and corticosteroids in combination with other immunosuppressives. In some studies recipient splenectomy was also included in the immunosuppressive protocol. Serum electrolytes were measured on a daily basis and are reported for twenty-two animals surviving at least 20 days. Analysis of urine collected from cynomolgus recipients (n=7) were performed three to four times a week post-transplantation.

Results: The animals were active and healthy in the post-operative period, before termination of the experiments usually because of rejection causing renal failure or complications related to immunosuppression. Plasma creatinine, sodium and potassium remained within normal limits until terminal renal failure developed. Fluid balance and bodyweight were well maintained throughout the period the animal remained well. Urinary electrolytes were well preserved but a significant increase in proteinuria (mainly globulins and albumin) was detected on electrophoresis. The plasma calcium was maintained at near normal levels after transplantation. Plasma phosphate levels fell continuously after renal xenotransplantation in all animals studied.

Conclusion: Transgenic pig to primate renal xenografts maintain plasma electrolyte homeostasis (apart from a previously reported hypophosphataemia) and show a urinary electrolyte excretion pattern comparable to the primates native kidney function. The increased proteinuria detected may be physiological, due to differences in protein handling by the pig kidney. More probably, it is a pathological consequence of complement and immune complex damage to the kidney. Our experience, in this life-supporting model with up to 78 days survival, demonstrates good regulation of electrolytes by a pig to non-human primate renal xenograft.

THE EXPRESSION OF ENDOTHELIN AND INDUCIBLE NITRIC OXIDE SYNTHASE IN HUMAN RENAL ALLOGRAFTS

NJ Evans, SA White, GR Bicknell, PN Furness, ML Nicholson

University Department of Surgery, Leicester General Hospital, Leicester

Endothelin and inducible nitric oxide (iNOS) have potent vasoactive properties. The aim of this study was to determine the role of endothelin-1,-2, and ñ3, and iNOS in the development of chronic renal allograft nephropathy (CRAN).Renal cortical and glomerular tissue from 41 consecutive patients was analysed. Donor categories were cadaveric (n = 17), living related (n = 9), and asystolic (n = 15). Transplant recipients were randomised to either Neoral or Tacrolimus immunosuppression. All patients underwent a needle core percutaneous renal transplant biopsy at 1 week and 6 months. Individual glomeruli were plucked and placed in a lysis binding buffer. Total mRNA extraction was performed with paramagnetic Dynabeads. Endothelin and iNOS transcripts were amplified by reverse transcriptase PCR and quantified in an ELISA. The results were corrected for cellularity by a housekeeping gene (GAPDH) and expressed as arbirary units. Renal cortical collagen III protein deposition was used as a marker for fibrosis pertaining to CRAN by immunohistochemistry and computerised histomorphometric assessment. Statistical comparison was made using the Mann Whitney U Test and the Kruskall-Wallis one way analysis of variance test.Patients were well matched in terms of age, sex, acute rejection episodes and organ ischaemia. There was no detectable endothelin expression in any biopsies at either time point. The results of iNOS expression are summarised in the table below

	Cortical		Glomerular	
	1 week	6 months	1 week	6 months
Tacrolimus	0.57	0.57	6.59	0.57
Cyclosporin	0.72	0.20	9.44	2.41
Asystolic	0.71	0.44	4.78	2.37
Cadveric	0.46	0.37	11.04	0.75
Living-related	1.13	0.52	7.37	0.73

No correlation was found between cortical iNOS expression at 6 months and collagen III, but a negative correlation between iNOS expression at 1 week and collagen III deposition reached marginal significance ($p = 0.07$).This study demonstrates that the expression of inducible nitric oxide synthase was not influenced by immunosuppressive agents or source of donor organ. Furthermore, increased expression in the early post-operative period may have a protective effect against CRAN in the long-term.

REVERSAL OF COSTIMULATION BY CELL SURFACE EXPRESSION OF AN ANTI-CD152 SINGLE CHAIN ANTIBODY.

Malde, P. Dorling, A. George, A.T. and Lechler, R.I.

Dept. of Immunology, Imperial College School of Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN.

The balance between signalling through CD28 AND CD152 is known to determine the outcome of T cell receptor occupancy. Both, CD28 and CD152 share sequence homology and bind to the same ligands, CD80(B7-1) and CD86(B7-2). Unlike CD28, CD152 is up regulated on the surface of T cells following an activation stimulus provided through the TCR and CD28. Although it is expressed at low levels on the cell surface, it has 10- to 20- fold higher affinity for CD80 and CD86 than CD28. Also, CD152 and CD28 have antagonistic functions. CD28 enhances T cell responses and T cell survival by enhancing IL-2 mRNA transcription, high affinity IL-2 receptor expression and induction of cell survival factors. In contrast, CD152 down regulates T cell activation. The regulatory role of CD152 is emphasised by massive lymphoproliferation observed in CD152 knockout mice. More recent data implicates the role of CD152 in the induction of T cell tolerance. We have isolated CD152-specific single chain antibodies (sFv) from a"phage display library" using soluble human and mouse CD152-Ig fusion protein. Membrane bound and soluble forms of the sFvs were generated. The soluble sFv bound to human CD152 in a comparable manner to that of a commercial anti-human monoclonal antibody prepared by conventional methods. These sFvs were expressed as cell surface molecules on a B7-expressing, MHC class II positive transfected cell line. Co-expression of the sFv with B7 led to 90% inhibition of proliferation and reduced responsiveness on subsequent rechallenge in mixed lymphocyte responses when the transfectants were cultured with allogeneic CD4+ T cells. Experiments using these transfectants will shed light on the influence of CD152 ligation on different T cell populations. In addition, introduction of these sFvs into donor cells may prove useful in the induction of transplantation tolerance.

IN VITRO 'ACCOMMODATED' ENDOTHELIAL CELLS INDUCE A NITRIC OXIDE-DEPENDENT TH-2 PATTERN OF CYTOKINE PRODUCTION FROM HUMAN CD4+ T CELLS.

Dorling, A. Brookes, P. Jordan, W. Delikouras, A. and Lechler, R.I.

Dept. of Immunology, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN

Transplanted xenografts, protected from hyperacute rejection and delayed xenograft rejection by xenoreactive natural antibody (XNA) depletion or inhibition of complement can sometimes continue to function despite the return of anti-donor antibody and complement back to pre-transplant levels, a phenomenon termed graft 'accommodation'. Although the precise mechanisms underlying accommodation are still not clear, recent observations from a hamster-heart-to-rat model have allowed the definition of an accommodated phenotype. This is characterised by endothelial cell (EC) expression of 'survival gene' products, such as Bcl-2, Bcl-xl and A20 and anti-oxidant proteins such as HO-1, a skewing of the antigraft antibody response towards production of non-complement fixing subtypes and a predominantly Th-2-like pattern of cytokine secretion by infiltrating leukocytes (compared to the Th-1-like pattern found in rejecting grafts).

However, the physiological stimuli that initiate accommodation in vivo are not clear. An attractive hypothesis is that accommodation might only arise if endothelium is initially exposed to a low concentration of antibody. We have established an in vitro model of porcine EC accommodation to investigate this possibility. In previous in vitro studies, we have reported that low concentrations of human normal globulin (HNG), which is a potent source of human anti-pig XNA, induced a change in the phenotype of porcine EC consistent with the development of accommodation. A prominent feature was the neo-expression of inducible nitric oxide synthase (iNOS).

In this study, we have shown that MHC class II - expressing porcine EC, incubated with low concentrations of HNG to induce 'accommodation', caused activated human T cells to produce significantly less IFN-gamma compared to control EC, whereas IL-5 and IL-10 production was maintained. This distinctive EC phenotype was accompanied by iNOS expression and by increased nitric oxide production. Two observations suggested that this nitric oxide was actively involved in the process of influencing T cell cytokine production. First, DETA-NO, a nitric oxide donor, induced similar changes in porcine EC to those induced by HNG. Second, a nitric oxide synthase inhibitor, L-NMMA, specifically inhibited the HNG-mediated changes in cytokine profile. Experiments to dissect the mechanisms underlying this phenomena suggested that nitric oxide was acting in at least two ways, via a direct action on the human T cells and indirectly through an as yet undefined action on the EC.

These results imply that pre-treatment of EC with low concentrations of XNA can significantly influence any subsequent T cell-mediated immune responses. our observations further support the hypothesis that low concentrations of XNA may provide the physiological stimulus leading to xenograft accommodation.

LABORATORY

TACROLIMUS, BUT NOT CYCLOSPORIN REDUCES EXPRESSION OF FIBROSIS ASSOCIATED GENES IN A MODEL OF RENAL ISCHAEMIA REPERFUSION INJURY

S Jain, GR Bicknell and ML Nicholson

Department of Transplant Surgery, Leicester General hospital, Leicester, UK

Cyclosporin based immunosuppressive regimens are associated with significant chronic nephrotoxicity, manifest in the long-term mainly as graft fibrosis. The cytokine TGFβ is thought to have a significant role in this. There have been claims that tacrolimus is a less fibrotic drug than cyclosporin, and this study was designed to determine if there were differences in the expression of fibrosis associated genes between the two drugs, in a model of renal fibrosis caused by ischaemia reperfusion (IR) injury.

Male Wistar rats underwent clamping of the right renal pedicle for 45 minutes together with left nephrectomy. This model has previously been shown to be associated with upregulation of fibrosis associated genes. Experimental groups (n=6 per group) were: CONTROL (IR injury only), CYCLOSPORIN (IR injury followed by cyclosporin A 10mg/kg/day orally) and TACROLIMUS (IR injury followed by tacrolimus 0.2mg/kg/day orally). Animals were culled at 4 months and renal tissue snap frozen. Messenger RNA was extracted and quantitative reverse transcriptase polymerase chain reaction used to amplify genes of interest. Expression of all genes was as a ratio to the housekeeping gene GAPDH.

Results in the table are expressed as mean (standard error of the mean)

	CONTROL	CYCLOSPORIN	TACROLIMUS
TGFβ	1.46 (0.12)	1.55 (0.21)	1.13 (0.06)*
Collagen III	1.15 (0.07)	1.45 (0.22)	1.05 (0.14)
MMP2	1.02 (0.22)	0.65 (0.13)	0.94 (0.11)
MMP9	0.55 (0.14)	0.35 (0.20)	0.58 (0.10)
TIMP1	0.64 (0.02)	0.71 (0.30)	0.41 (0.05)*
TIMP2	0.48 (0.04)	0.45 (0.05)	0.40 (0.04)

*p<0.05 vs CONTROL (students t-test)

Cyclosporin did not have any significant effect on the expression of genes associated with fibrosis compared to the control group, although levels of MMP2 and MMP9, two collagen degrading enzymes, did fall. In the tacrolimus treated group there was reduced expression of TGFβ, and TIMP1 an inhibitor of extracellular matrix breakdown. This suggests tacrolimus is less fibrogenic than cyclosporin and may have implications for immunosuppressive use in renal transplant fibrosis.

LABORATORY

SOLUBLE IL-15 RECEPTOR (sIL-15Rα) PROLONGS MOUSE CARDIAC ALLOGRAFT SURVIVAL.

Smith XG, Ruchatz H*, Bolton EM, Frick TW, Liew FY*, Bradley JA.

University of Cambridge Department of Surgery, Box 202, Addenbrooke's Hospital, Cambridge CB2 2QQ; *University of Glasgow Department of Immunology, Western Infirmary, Glasgow G11 6NT.

IL-15 is produced by several cell types (not T cells) and shares biological activities with IL-2. It uses the IL-2Rβ and the γ_c chains but has a unique IL-15Rα chain, through which it mediates T cell JAK-STAT signalling and proliferation. In this study we targeted the IL-15/IL15R pathway in mouse recipients of cardiac allografts, by administering a soluble fragment of IL-15Rα. CBA/Ca (H-2^k) recipients of a vascularised heterotopic heart graft from B10.BR (H-2^k) or BALB/c (H-2^d) mice were treated with 60μg sIL-15Rα daily for 10 days. sIL-15Rα induced indefinite survival of cardiac grafts mismatched for multiple minor histocompatibility complex (mHC) antigens (B10.BR to CBA/Ca, MST >100 days, n=10); grafts in control mice survived 10 days (MST, n=15). Long-surviving recipients subsequently accepted donor strain, but rejected third party, skin grafts. Cardiac grafts in Major HC-mismatched recipients (CBA/Ca to BALB/c) treated with sIL-15Rα alone were rejected but treatment combined with a single dose of anti-CD4 mAb caused prolonged allograft survival (MST 60 vs 14 days [controls], n=7). MHC-mismatched graft survival was associated with persistence of anti-CD4 and profound CD4 T cell depletion, together with diminished in vitro proliferation and IFN-γ production on challenge with donor antigen.
These findings demonstrate involvement of IL-15 in the immune response to allografts and suggest that antagonists of IL-15 may have potential therapeutic value in transplantation.

LABORATORY

ANALYISIS OF THE INDIRECT PATHWAY IN THE DONOR-SPECIFIC ALLORESPONSE IN A SERIES OF LONGSTANDING KIDNEY TRANSPLANTS.

Hernandez-Fuentes, MP; Baker, RJ; Brookes, PA; Chaudhry, AN; Lechler, RI.

Department of Immunology, ICSTM, Hammersmith Hospital.
Du Cane Rd, London W12 ONN, UK.

Acknowledgements: MRC, MEC from Spain

Despite the success attained in reducing acute rejection rates, graft loss due to chronic allograft nephropathy (CAN) remains one of the major obstacles to clinical transplantation. $CD4^+$ T cells can be primed to respond to alloantigens by the indirect pathway, whereby donor-derived MHC peptides are processed and presented to them by recipient APCs. The presence of donor-specific IgG alloantibodies has been related to the presentation of alloantigens through this pathway. Since the direct alloresponse diminishes with time after transplantation, the question remains as to what role the indirect pathway plays in the onset of CAN.

To assess the immunological contribution of the indirect pathway in this process we have studied a series of 22 patients, all of whom have received a live-related kidney allograft (median time since transplantation 13 years). Nine of these patients have developed CAN (8/9 confirmed by biopsy) while thirteen have maintained good graft function. We have measured their responses to a cell membrane preparation from donor PBMC as a source of donor-alloantigens and we have screened for the presence of donor specific alloantibodies by flow cytometry crossmatching and ELISA.

Frequencies to donor-specific peptide preparations were measurable and they were significantly higher in the CAN group than in the group with good function ($p<0.05$). Anti-HLA Abs, detected by ELISA, were present at a higher rate, though not significant, in the CAN group. So far, donor-specific IgG Ab detected by flow cytometry seemed to be absent in the CAN group.

There is evidence supporting the presence of indirect allorecognition in long standing renal transplanted patients but the relationship with CAN remains to be defined.

Category: LABORATORY

PREDICTION OF EARLY POST-TRANSPLANT FUNCTION OF NON-HEART-BEATING DONOR LUNGS.

Aitchison J.D., Orr H.E., Flecknell P.A., Kirby J.A., Dark J.H.

Department of Surgery, Medical School, Newcastle upon Tyne, NE2 4HH

Introduction. Use of lungs from non-heart-beating donors (NHBD) is a promising way of increasing numbers of organs for transplantation. However, the warm ischaemic interval prior to retrieval may lead to significant reperfusion injury post transplantation, which develops in up to twenty percent of transplanted lungs from current brain-stem-dead donors. New techniques are therefore be required to identify those lungs least affected by this injury. We have developed an isolated ventilation and perfusion technique to assess oxygenating capacity (pulmonary venous O_2), pulmonary vascular resistance (PVR) and airway compliance of donor lungs retrieved after hypoxic cardiac arrest. This technique uses our established porcine lung transplant model and is readily applicable to clinical use.

Methods. All animals were anaesthetised throughout and euthanased without regaining consciousness. Lungs were retrieved at intervals of 1 hour (NHBD1) or 2 hours (NHBD2) after hypoxic death from Landrace cross pigs (approx. 50kg). Control organs were retrieved from matched animals immediately after cross-clamping. Standard techniques and modified Eurocollins pulmoplegia were used. A single lung from each animal was ventilated with 100% oxygen and perfused for 6 minutes with deoxygenated and neutrophil depleted blood. For the NHBD2 group, the contralateral lung was transplanted with post transplant function assessed.

Results. All expressed as mean (sd), † differences v control $p<0.05$, student's t test. After one hour's warm ischaemia, oxygenation did not differ significantly between NHBD1 (n=4): 59 (13); 60 (15); 57 (16) kPa and Controls (n=5): 65 (5); 65 (3); 61 (5) at 2;4;6 minutes of assessment respectively. However, after two hours' warm ischaemia, oxygenation deteriorated significantly during assessment, NHBD2 (n=6): 66 (4); 54 (8)†; 45 (15)† and Controls (n=8): 62 (5); 63 (5); 62 (3). The assessment technique showed no significant differences in PVR between NHBD1: 32 (8); 36 (10); 29 (9) Wood units, and Controls: 34 (7); 31 (6); 30 (5), or between NHBD2: 42 (14); 42 (11); 52 (29), and Controls: 43 (24); 38 (20); 34 (19), at the same intervals.

In the two hours' warm ischaemic group, the contralateral lungs also showed significant deterioration in oxygenation after transplantation, NHBD2 (n=6): 56 (15); 55 (23); 32 (20)† and Controls (n=6): 54 (17); 63 (14); 67 (14) at 30;60;300 minutes post transplantation respectively. Differences in PVR between NHBD2: 47 (47); 29 (16); 77 (47) and Controls: 40 (38); 28 (14); 64 (50) were not significant.

Conclusions. The assessment technique showed no significant differences in performance between lungs subjected to one hour's warm ischaemia and control lungs, whereas after two hours' warm ischaemia the oxygenating performance deteriorated significantly. A similar deterioration in oxygenating capacity was seen when the matched contralateral lungs were transplanted after two hours' warm ischaemia. This method may therefore detect occult warm ischaemic damage.

LABORATORY

SIGNIFICANCE OF CYTOKINE GENE POLYMORPHISM IN RENAL TRANSPLANTATION

S.George[1], D.Turner[2], M.Reynard[2], G.Turakhia[1], C.Navarrete[2], P.Sweny[1], O.N.Fernando[1], S.H.Powis[1], Z.Varghese[1].

[1]Renal Transplant Unit, Centre For Nephrology, Royal Free and University College Medical School, Rowland Street, London. NW3 2PF.
[2]National Blood Service, North London Centre, Colindale Avenue, London. NW9 5BG.

Pro-inflammatory and anti-inflammatory cytokines are believed to influence the outcome of transplantation. Polymorphisms in the promoter region are known to influence the amount of cytokine produced. The aim of this study was to examine whether cytokine gene polymorphism had any predictive value in assessing the risk of acute allograft rejection and the eventual transplant outcome.

One hundred and three renal transplant patients were genotyped for IL-10, TNF-α, and IL-2 promoter region polymorphisms. Also IFN-γ, TNF-a, and TNF-d microsatellite polymorphisms were analysed. Patients were grouped on the basis of biopsy proven acute rejection episodes (more than one episode in the first year after transplantation) and creatinine levels. Frequency of IL-10 (-1082) AA, low producer genotype was high in the rejection group when compared to rejection free group ($p \leq 0.02$). TNF-α and IL-2 did not show any significant difference between the two groups. The frequency of the TNF-a9 allele of the microsatellite polymorphism was high in the rejection group when compared to the rejection free group ($p \leq 0.005$). This mirrors previous findings in Japanese renal transplant patients (H.Asano et al.,Tissue Antigens 1997: 50: 484-488). IFN-γ and TNF-d microsatellites did not show any significant difference between the two groups.

Recipient IL-10 promoter and TNF-a microsatellite polymorphisms may predict the risk of acute rejection following renal transplantation.

"LABORATORY"

HIGH IL-10 SECRETION IN MIXED LYMPHOCYTE REACTION TOGETHER WITH HLA MISMATCHING PREDICTS ACUTE REJECTION OF RENAL ALLOGRAFTS

N Cartwright[1], A Demaine[2], R McGonigle[3], P Rowe[3], J Shaw[3], R Szydlo[4], E Kaminski[1]

[1]Immunology Section, Derriford Combined Laboratories, Derriford Hospital, Plymouth, [2]Department of Molecular Medicine, Plymouth Postgraduate Medical School, [3]Department of Renal Medicine, Derriford Hospital, Plymouth, [4]Biostatistics Section, LRF Centre for Adult Leukaemia, Imperial College School of Medicine, London

Graft rejection following renal transplantation remains a major clinical problem despite improvements in tissue typing methodology. It is now clear that cytokines are major regulators of both cellular and humoral immune responses, and play an impotant role in allograft rejection. In this study, we measured cytokine secretion in MLR between patient-donor pairs pre and post renal allografting to determine whether particular cytokines would predict acute rejection. Pre- and post-transplant one way MLRs were set up in the patient-versus-donor direction between fifty seven renal allograft pairs and secretion of cytokine protein (IL-2, IL-4, IL-6, IL-10 and IFN-γ) into the supernatant was quantified using ELISA.

Significant inter-individual variations in protein secretion in MLR were observed for all cytokines studied. Univariate analyses demonstrated that high levels of IFN-γ and IL-10 in MLR and baseline IL-4, together with female donor sex and a high degree of HLA and HLA-DR mismatching were significantly associated with rejection. However, multivariate analysis revealed the greatest risk of rejection (RR=25.5, p=0.003) was associated with a combination of high IL-10 secretion in MLR and mismatching for at least 4 HLA antigens (HLA-A, -B and –DR). Post-transplant MLRs revealed a global reduction in cytokine secretion.

These findings suggest that a combination of high cytokine secretion in MLR, in particular IL-10, and HLA mismatching are associated with an increased incidence of renal allograft rejection. We are currently investigating whether cytokine secretion in MLR is determined by cytokine gene polymorphisms. In future, assays for measuring either cytokine secretion or gene polymorphisms may prove to be useful in aiding recipient or live donor selection and tailoring immunosuppressive therapy.

Flow Cytometric Crossmatching: Antibody Detection, Sensitisation and Clinical Significance

Andrea W Harmer, B Sean Carey, Robert W Collins, Alasdair J Heads, Gareth Page, Robert W Vaughan on behalf of the South Thames Renal Transplant Group

The flow cytometric crossmatch (FCXM) is a more sensitive technique for the detection of donor specific antibodies than the standard cytotoxic crossmatch. Many centres consider a positive FCXM a contra-indication to transplantation in sensitised recipients. Fewer centres use the FCXM for unsensitised patients, mainly due to the perception that the test is overly sensitive for this group.

In this prospective trial of FCXMs for unsensitised patients we performed FCXMs at the same time as the standard crossmatch but did not use the results for organ allocation. FCXM results are expressed as relative median fluorescence (RMF) which is the median fluorescence of the test sample divided by that of the negative control. The RMFs have been analysed with respect to graft outcome.

A total of 498 FCXMs were performed in 'unsensitised' recipients (Panel Reactivity<40%, no previous transplant) between April 1998 and September 1999. The results are shown in the table below.

	RMF<1	1.0-1.99	2.0-2.29	2.3-3.99	4.0+
No. FCXMs	235	229	11	16	7
Transplanted	84	84	2	3	2
Functioning	78	80	2	3	2

The definition of positivity which is used for sensitised recipients is RMF of 2.3 or greater (based on a retrospective study of sensitised recipients). Over 95% of 'unsensitised' recipients would be considered as negative by this criteria. Five patients with an RMF greater than 2.3 have been transplanted all are functioning well 7-18 months post-transplant. Two recipients had RMFs greater than 4.0 but both were shown to have positive auto-FCXMs with RMFs greater than 4.0. The remaining 5 FCXMs with RMF>4.0 were in individuals with detectable antibody but a PR<40%.

These results suggest that a higher cut-off for FCXM positivity is appropriate in our 'unsensitised' recipients than that which we have previously shown to be of clinical significance in the sensitised patients. We believe that a FCXM RMF up to 3.99 should not contra-indicate transplantation in our 'unsensitised' patients.

The question of clinical significance rather than antibody detection is clearly important in patients awaiting transplantation. We are able to detect HLA specific antibodies with RMFs lower than 2.3 in dilutions of sera. For example during the last UK NEQAS cycle we successfully identified antibodies in 10 cases with an RMF of 1.5 – 2.3 using our standard FCXM technique. However our data indicate that a FCXM of this strength is not related to deleterious graft outcome as during our study period 15 unsensitised patients and 8 sensitised patients have been successfully transplanted with RMFs falling in that range.

In conclusion the results of this study indicate that it may be advantageous to use different definitions of positivity for FCXMs in sensitised and 'unsensitised' recipients. We have also shown that less than 2% of 'unsensitised' potential recipients are likely to have a positive FCXM if we use an RMF of 4.0 to define positivity, indicating that the adoption of the FCXM for unsensitised patients is unlikely to lead to significant numbers of patients being denied transplants.

MOUSE ISLETS OF LANGERHANS ARE NOT DESTROYED BY HUMAN SERUM DESPITE BINDING OF HUMAN XENOANTIBODY AND COMPLEMENT ACTIVATION

Titus, T., Badet L., Chang L-W, McShane P., Gray, D.W.R.

The Nuffield Department of Surgery, John Radcliffe Hospital, Headington,Oxford, United Kingdom

BACKGROUND:
Mouse pancreatic islets transplanted beneath the kidney capsule of non-human primates are rapidly destroyed and histological evaluation shows a process of necrosis and neutrophil infiltration. The mechanism of this early destruction remains unclear, but humoral xeno-recognition by natural antibodies could potentially affect the islet viability by human complement activation, leading to cell lysis. We investigated humoral xeno-recognition in a mouse to human combination and the effect of human serum on mouse islet viability.

METHODS:
Freshly isolated islets were analysed by immunohistochemistery for expression of Gal(α1,3)Gal and Von Willebrand factor and for binding of human IgG and IgM and complement components. The alternative pathway was not assessed. Complement mediated lysis was evaluated by ^{51}Cr release assays after incubation of islets for 4 hours in human serum, with and without added neutrophils, and compared with lysis of sheep red cells as a positive control.

RESULTS:
Freshly isolated islets showed significant expression of Gal(α1,3)Gal, surprisingly not correlated with Von Willebrand expression, but the bulk of the cells, presumably the endocrine cells, were negative. Following islet incubation in 100% human serum before frozen section human IgG and IgM was found to have bound with a pattern similar to that of Gal(α1,3)Gal expression .Islets incubated in 100% human serum showed C3 and C4 complement deposition surrounding the islets with weaker C5b-9 deposition but with the same pattern of as observed for IgG and IgM binding. Interestingly, islet tissue exposed to human serum after frozen section bound much more IgG and IgM.
Four hours incubation in 100% human serum then measurement of ^{51}Cr release from labelled fresh islets, showed 16% of specific lysis (abolished by using heat inactivated serum). In contrast, more than 65% of sheep red cells were destroyed in the same condition, indicating that the complement system was functional. The addition of neutrophils did not change the percentage of specific islet lysis.

CONCLUSION
These results indicate that despite xeno-recognition by human natural xeno-antibodies and consequent complement deposition, human serum is not able to induce more than 15% islet lysis, and addition of neutrophils makes no difference, whereas islets are badly damaged in vivo. Humoral xeno-recognition with neutrophil activation is either not the main event in early islet destruction, or else the in vitro systems tested here do not provided the correct additional factors.

Medawar Medal

Wednesday 22 March

11.00-13.00

Delegates are reminded that the eligibility criteria for the Medawar Medal are as follows:

Candidates must be a member of the Society and aged 35 years or under on the first day of the Annual Congress. In exceptional circumstances people outside the age range but still in training will be considered with an appropriate signature from their Head of Department.

The work must be original and innovative and have been performed largely or entirely in the UK.

BONE MARROW CELLS TRANSDUCED TO EXPRESS AN ALLOGENEIC MHC CLASS I GENE USING RECOMBINANT ADENOVIRUS CAN PROMOTE SURVIVAL OF A FULLY ALLOGENEIC CARDIAC GRAFT.

Fry, J.W., Morris, P.J., Wood, K.J.

Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford. OX3 9DU

Introduction Replication-defective recombinant adenovirus vectors are highly effective at transducing many cell types at different developmental stages, but the ability of adenovirus to transduce bone marrow is controversial. In this study, we investigated (i) whether adenovirus was a suitable vector for the transduction of bone marrow cells, and (ii) whether delivery of adenovirus encoding a single donor alloantigen to recipient bone marrow cell (BMC) lineages, could induce experimental tolerance to a fully allogeneic allograft.

Methods The mouse MHC class I gene, H2-K^b, was sub-cloned into a modified pXCX adenovirus transfer vector under the control of the SV40 promoter. AdSV40K^b was produced by co-transfection with the adenovirus genome shuttle plasmid pJM17, in the complementary cell line 293, and purified by caesium chloride ultracentrifugation to obtain titres of 7×10^9 pfu/ml. K^b expression in a transduced fibroblast target cell line (3T3) was confirmed by FACS .

Results Co-culture of CBA/Ca ($H2^k$) BMCs with AdSV40K^b resulted in $CD3^+$ and $GR1^+$ cells being transduced the most efficiently, with a 20% and 18% increase respectively in K^b mean fluorescent intensity (MFI) as compared to untransduced, cultured CBA BMCs. $M1/70^+$ cells increased their K^b expression by 9% following transduction by the virus, and $CD45R/B220^+$ cells by 6%.
Administration of 5×10^6 AdSV40K^b-transduced CBA BMCs on day -27 with respect to transplantation in combination with 50µg doses of a depleting anti-CD4 monoclonal antibody given on days -28 and -27 resulted in significant prolongation of graft survival compared to mice treated with anti-CD4 alone (MST>100 days; n=10; 60% long-term survival; $p<0.05$). Increasing the period of transduction at the same MOI, from 4 to 24 hours, still resulted in significant graft survival but with a reduced number of long-term survivors (MST=71 days; n=5; 40% long-term survival; $p<0.05$).

Conclusions Adenovirus can be used for bone marrow cell transduction, particularly of lineages that are involved in antigen presentation and recognition. In addition, pretreatment of recipient mice with anti-CD4 antibody and recipient BM transduced with a single allogeneic MHC class I gene was sufficient to prolong the survival of fully allogeneic cardiac grafts.

This work was supported by the United Kingdom Medical Research Council.

C13

EFFECTS OF ANGIOTENSIN-CONVERTING-ENZYME INHIBITORS (ACE-i) ON PROGRESSION TO END-STAGE RENAL FAILURE

Rustom R, Sells RA, Brown M, Hammad A, Bakran A, Owens G, Ahmad RA, Bell GM & Bone JM.

Sir Peter Medawar Unit, Royal Liverpool University Hospital, Liverpool, UK.

Purpose of Study After the first year of transplantation chronic vascular rejection (CR) is the commonest cause of renal transplant (Tx) loss with few clues to aetiology, but proteinuria is a common feature. In diseased native kidneys proteinuria and progression to failure are linked, and ACE-i are increasingly used to reduce proteinuria and delay progression. Does treatment with ACE-i also reduce the rate of decline of graft function in CR. There are no available data.

Methods We selected 27 recepients with biopsy proven CR receiving cyclospoine-based immunosuppression (without tacrolimus or MMF). In Group 1 (n=12), no ACE-i was given. In Group II (n=15) maintenance ACE-i therapy was commenced at various times after Tx in patients developing proteinuria or for blood pressure control. Time from Tx to CR diagnosis was comparable (5.0 y ± 1.1 in Group I vs 5.5 y ± 1.3 in Group II), as was the follow-up time from CR diagnosis (1.7 y ± 0.5 vs 1.4 y ± 0.5). The mean period of ACE-i therapy in Group II was 3.9 ± 0.7y. Data were analysed in terms of mean creatinine clearance (Cr Cl), proteinuria, and progression from Tx to CR, and from CR to last follow-up (FU).

Results (Means ± SEM)	Group 1 n=12	GroupII n=15
Proteinuria at CR (g/24 h)	2.2 ± 0.4	1.5 ± 0.4
Progression from Tx to CR (ml/min/y)	-26.2 ± 8.5	-11.6 ± 2.8
Cr Cl at last FU (y)	7.6 ± 1.2	26.8 ± 3.6 **
Proteinuria at last FU (g/24 h)	4.2 ± 0.8	1.2 ± 0.3*
Progression from CR to last FU (ml/min/y)	-14.4 ± 2.4	-2.7 ± 1.9 *
Graft survival	1/12 (8%)	15/15 (100%)

p value *< 0.001, ***<0.0001

Mean arterial blood pressure was comparable in both Groups throughout Tx, CR and at last FU.

Conclusion All patients in Group II fared better in terms of renal function, less proteinuria and overall progression than those in Group 1. Thus ACE-i may protect graft function in CR. Controlled trials are urgently needed and are in progress.

OPERATIONAL TOLERANCE TO MOUSE HEART ALLOGRAFTS: VESSEL HISTOLOGY FOLLOWING CD4/CD8 BLOCKADE ALONE OR IN COMBINATON WITH IMMUNOSUPPRESSIVE DRUGS.

Moffatt, SD and Metcalfe, SM.
Department of Surgery, University of Cambridge, Addenbrooke's Hospital , CB2 2QQ, UK.

CBA ($H2^k$) mice were rendered tolerant to fully mismatched BALB/c ($H2^d$) hearts using monoclonal antibodies (mabs) to block the CD4 and CD8 co-receptors of the TCR. After 360 days, all surviving grafts were excised and their histological status recorded. Other groups of recipient mice received dual therapy of CD4/CD8 blockade plus either cyclosporin A, FK506, or prednisolone. This allowed us to ask if graft integrity was altered in drug+mab groups compared to those receiving mabs alone.

Each experimental group consisted of 6 recipients. Treatments were as follows: **(i)** no therapy; **(ii)** *tacrolimus* (1mg/kg, i.p daily, 0d to 14d); **(iii)** *liposomal FK506* (1mg/kg ip daily, 0d to 14d) **(iv)** *cyclosporine* (25mg/kg, i.p, daily, 0d to 14d); **(v)** *prednisolone* (5mg/kg i.p. daily, 0d to 14d) **(vi)** blocking *mabs* YTS105 and YTS177 to CD4 and CD8 respectively (2mg total, i.p, starting day 0 and on alternating days thereafter for a total of 6 doses). The remaining groups combined the above protocols to give **(vii)** *tacrolimus plus mabs;* **(viii)** *liposomal-FK506 plus mabs;* **(ix)** *cyclosporine plus mabs;* and **(x)** *prednisolone plus mabs.*

Any longterm surviving heart allografts were removed at 360 days for histological analysis: the numbers were as follows for the respective groups:- **(i)** 0/6; **(ii)** 2/6; **(iii)** 1/6; **(iv)** 0/6; **(v)** 0/6; **(vi)** 6/6; **(vii)** 5/6; **(viii)** 4/6; **(ix)** 2/6; and **(x)** 4/6. There were marked differences between recipients receiving CD4/CD8 blockade alone compared to drug-mab combination therapies. Of particular note, in the tacrolimus plus mabs group, 5/6 recipients showed operational tolerance with minimal graft histopathology: this was similar to the mab alone group where all recipients retained their grafts at 360d. In marked contrast, combination of cyclosporin A with mabs resulted in surviving grafts showing marked features of vessel intimal thickening characteristic of chronic graft rejection.

Overall, although all recipients studied showed operational tolerance to their grafts at 360d, specific drug-mab combinations resulted in discrete tissue pathologies. This contrasted to the excellent graft preservation where CD4 and CD8 blockade was the sole therapy.

LABORATORY

L14

CD40 / CD154 BLOCKADE: THE PERSISTENCE OF CHRONIC REJECTION HIGHLIGHTED BY A COMBINED CARDIAC AND AORTIC TRANSPLANT MODEL

J.Stephen Billing, Stephan M.Ensminger, Peter J.Morris and Kathryn J.Wood

Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU

Objective: Blockade of CD40/CD154 costimulation has been shown to induce long-term allograft acceptance in a murine cardiac transplant model, either as monotherapy or in combination with donor alloantigen treatment. It remains uncertain whether such treatment prevents chronic rejection, and assessment of chronic vascular rejection in the cardiac model is subjective and difficult. Aortic allografts have recently been used to study chronic graft vasculopathy, but it is currently unclear whether the vascular changes seen represent those in solid organ transplants. To address this problem we have developed a model of combined cardiac and aortic transplantation to allow precise quantification of transplant arteriosclerosis.

Methods: CBA ($H2^k$) recipient mice were treated with 250μg of anti-CD154 mAb (MR1) on days 0, 2 and 4, either alone or in combination with an infusion of $5x10^6$ CBK bone marrow (BM) cells on the day of transplant. CBK is a transgenic strain that expresses the donor MHC class I molecule K^b on a recipient-type $H2^k$ background. There were three transplant groups: (a) C57BL/10 ($H2^b$) cardiac allograft (b) C57BL/10 aortic interposition graft (c) successive cardiac (cervical position) and aortic transplants days 0 and 1. In animals receiving aortic transplants, the grafts were harvested at 30 days, the optimal time point to assess graft vasculopathy in the aortic allograft model. The degree of arteriosclerosis was assessed using digital image analysis and was expressed as the percentage reduction in luminal cross-sectional area.

Results: Following MR1 ± BM treatment, cardiac allografts continued to beat for more than 100 days and histology showed well preserved myocardial structure. However, the coronaries showed varying degrees of transplant arteriosclerosis. To quantitate the severity of vasculopathy aortic allografts from the combined cardiac/aortic transplant group were examined. These revealed a pronounced intimal proliferation (58±12% reduction in lumen) after MR1 treatment alone, not significantly different from that observed in untreated controls (62±10%). Following treatment with MR1 and BM, chronic graft vasculopathy was still present, with luminal reduction of 48±9% in the combined transplant group. The degree of vascular disease in the single aortic transplant group was not significantly different (55±8% after MR1 alone and 56±7% after MR1 + BM). Syngeneic aortic grafts did not develop any intimal proliferation.

Conclusions: This combined cardiac and aortic transplant model permitted quantitative assessment of chronic graft vasculopathy, while monitoring graft survival by cardiac palpation. Although there was a correlation between vascular changes in aortic and cardiac allografts, only the aorta allowed accurate quantification of the lesions. This novel model demonstrated that CD154 blockade, with or without alloantigen delivery, could not prevent chronic vascular rejection even though acute rejection was overcome.

LABORATORY

SIGNALLING THROUGH CD31 PROTECTS ENDOTHELIAL CELLS FROM APOPTOSIS

Evans PC, Taylor ER and Kilshaw PJ.

Molecular Immunology Programme, Babraham Institute, Cambridge, UK.

Introduction

Endothelial cells form a critical barrier to immune cells and exert control over the inflammatory process. Long-term graft survival is associated with expression of protective genes (including A20, bclxl and hemoxygenase-1 (HO-1)) by endothelial cells. Overexpression of A20 in cultured endothelial cells has been shown to confer protection from apoptosis and prevent inflammatory molecule expression through inactivation of NF-κB. A20 is itself induced by NF-κB and therefore completes a negative feedback loop.

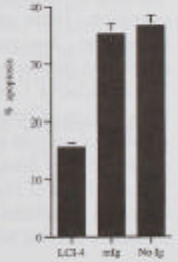
CD31 (PECAM-1) expressed at endothelial cell junctions is ligated by leukocytes during transendothelial migration. Our laboratory has recently shown that cross-linking of CD31 using a monoclonal antibody triggers signalling events in endothelial cells including tyrosine phosphorylation of this molecule and subsequent complexing with SHP2 and other unidentified phosphoproteins. In this study we examined the effects of CD31 cross-linking on endothelial cell apoptosis, activation of NF-κB and expression of A20, bcl-xl and HO-1.

Methods

Cultured human umbilical vein endothelial cells (HUVEC) were used. Sub-confluent cells were subjected to serum starvation for 48 hours in the presence or absence of mouse monoclonal antibody to CD31 (LCI-4) or whole mouse IgG as an additional control (mIg). Apoptotic cells were then detected by staining with FITC-annexinV followed by flow cytometry. The presence of activated NF-κB in nuclear extracts was determined by electrophoretic mobility shift assay (EMSA). A20, bclxl and HO-1 transcripts were detected by RT-PCR.

Results

Cross-linking of CD31 with LCI-4 conferred significant protection from apoptosis compared to controls ($p<0.01$, see figure); the results shown are based on triplicate wells and are representative of two independent experiments. CD31 cross-linking also led to detection of activated NF-κB in nuclear extracts and elevation of A20 mRNA levels whereas bclxl and HO-1 mRNA levels were unaltered.



Conclusions

Signalling through CD31 on endothelial cells leads to protection from apoptosis in association with NF-κB activation and expression of the protective gene A20.

C27

COMBINED TRANSPLANTATION OF THE HEART-LUNG AND -LIVER

Praseedom RK, McNeil KD[1] Wallwork J[1] Watson CJE Jamieson NV Friend PJ

The University Department of Surgery, Addenbrooke's Hospital and Transplant Unit Papworth Hospital, Cambridge, United Kingdom.

R.K.Praseedom, Box 210, Addenbrooke's Hospital, Cambridge CB22QQ

Background - Combined transplantation of the heart, lung and liver may be indicated in patients suffering from either end stage respiratory failure complicated by advanced liver disease or end stage liver failure complicated by advanced lung disease. In either case, disease in the second organ precludes safe isolated transplantation of the organ in failure. The largest group of patients in this category are those with cystic fibrosis, in 3% of whom, terminal lung disease is complicated by cirrhosis of the liver.

Methods- A retrospective review of the nine patients who have undergone combined heart-lung-liver transplantation in Cambridge (1986 - 1999) has been carried out. An en bloc technique of harvesting and implanting the organs was developed and the recipient heart was used in a second patient when appropriate. Details of the pre-operative status, operative procedures and post-operative course of each patient were collected from the databases held at Addenbrooke's and Papworth Hospitals. Survival was calculated using the Kaplan - Meier curve.

Results - Five male and 4 female patients underwent combined heart-lung-liver transplantation between 1986 and 1999. The underlying pathology was cystic fibrosis in seven patients, primary biliary cirrhosis with severe plexogenic pulmonary hypertension in one patient and alpha 1-antitrypsin deficiency in one patient. There were three peri-operative deaths. The mean percentage of predicted Forced Expiratory Volumes in the first second (FEV1) were 29.5% and 76.8%, before and after the operation. There were 3 episodes of biopsy proven acute rejection in the lungs and one in the liver. Two patients developed obliterative bronchiolitis, of whom one also developed chronic rejection of the liver. Chronic renal failure occurred in 2 patients. The cumulative 1-year and 5-year survival was 56% and 40% respectively.

Conclusions - Combined heart-lung-liver transplantation is a feasible option for a small number of patients particularly those suffering from end stage respiratory failure and advanced liver disease, in whom isolated lung transplantation would be contraindicated. The incidence of rejection in this small number of patients is lower than would be expected in isolated lung transplantation, possibly as a consequence of a protective influence exerted by the liver.

EOSINOPHILS ARE MAJOR EFFECTOR CELLS FOR THE DEVELOPMENT OF TRANSPLANT ARTERIOSCLEROSIS AND DEPEND ON THE PRESENCE OF IL-4

Ensminger SM[1], Witzke O[1], Spriewald BM[1], Sorensen H[2], Morris PJ[1], Rahemtulla A[2], Rose ML[3], Wood KJ[1]

[1]Nuffield Department of Surgery and [2]Medicine, John Radcliffe Hospital, Oxford University, U.K.

[3]Heart Science Centre, Imperial College of Medicine, Harefield Hospital, Harefield, Middlesex U.K.

Introduction: We have previously shown that transplant arteriosclerosis develops in CD40 knockout ($CD40^{-/-}$) mice in the absence of alloantibodies. The aim of this study was to dissect further the effector mechanisms of transplant arteriosclerosis in $CD40^{-/-}$ recipients.

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 mRNA production was analysed by competitive RT-PCR on day 14 after transplantation.

Results: Transplant arteriosclerosis was very pronounced in both $CD40^{-/-}$ and $CD40^{+/+}$ mice [intimal proliferation 59±5% ($CD40^{-/-}$) vs. 58±4% ($CD40^{+/+}$)], whether or not $CD8^+$ T cells were present [intimal proliferation 46±7% ($CD40^{-/-}$+anti-CD8) vs. 50±10% ($CD40^{+/+}$+ anti-CD8)] indicating that CD8+ T cells are not the principal effector cells in this situation. In $CD40^{-/-}$ recipients depleted of $CD8^+$ T cells the number of eosinophils infiltrating the graft was markedly increased [eosinophils/grid 21±8 ($CD40^{-/-}$+anti-CD8) vs. 7±4 ($CD40^{-/-}$+ anti-CD8)] even though the number of $CD4^+$ T cells and $CD11b^+$ leukocytes was reduced compared to CD8 depleted $CD40^{+/+}$ controls. The increased presence of eosinophils correlated with augmented production of Th2 cytokines within the graft: the IL-4 : IFN-γ ratio was increased 8-fold. To test the hypothesis that IL-4 production and the subsequent recruitment of eosinophils into the graft was responsible for the intimal proliferation, $CD40^{-/-}$ CD8 depleted recipients were treated with anti-IL4 mAb. This significantly reduced the level of intimal proliferation (18±5% compared to 46±7%) and eosinophil infiltration into the graft [eosinophils/grid 21±8 vs. 6±4].

Conclusion: Eosinophils are a major effector population for the development of transplant arteriosclerosis in $CD40^{-/-}$ recipients in the absence of $CD8^+$ T cells.

APPLYING A NOVEL TARGETED COMPLEMENT REGULATOR TO MODULATE ISCHAEMIA / REPERFUSION INJURY LEADS TO IMPROVEMENTS IN GRAFT FUNCTION

[1]Pratt J.R., [2]Smith R.A.G., & [1]Sacks S.H.

[1]Dep't of Nephrology and Transplantation, Guy's Hospital, Guy's, King's and St Thomas' School of Medicine, Kings College, London.
[2]AdProTech Plc, Royston, Herts, SG8 5HD

Complement activation has been implicated as a mechanism of tissue injury in ischaemia/reperfusion damage that is inherent in organ transplantation. We have developed a novel method to target complement inhibition in donor organs.

Our approach utilises a linear arrangement of membrane-binding units, termed Sequential Membrane Addressins (SMAs). Each SMA exhibits a different binding specificity that, when combined, confer strong association of a biopharmaceutical to a target cell membrane. An SMA targeting tag was added to a recombinant form of short consensus repeats 1-3 of human complement receptor type 1 (CD35; a known regulator of complement activation). Analysis of this construct (APT070) in vitro showed binding to cell membranes, and >100-fold increases in potency as an inhibitor of antibody-mediated haemolysis compared to constructs with no SMA.

We used APT070 in DA to DA rat renal isografts as a model of complement mediated transplant ischaemia/reperfusion injury. Syngeneic donor rat kidneys were perfused either with 5mls of Marshalls kidney preservation solution containing 200µg APT070 (n=16), or 5mls of Marshalls alone (n=16), and then exposed to 30 minutes of cold ischaemia followed by 60 minutes of warm ischaemia. Transplant recipients were analysed over a period of 7 days. Post transplant we found reduced deposition of complement components by immunohistochemistry for C3 or C5b-9 membrane attack complex in the group that received APT070 perfused kidneys. This was coincident to reduced histopathological signs of acute tubular necrosis, vascular damage and neutrophil infiltration - which can be indicative of complement activation. By analysis of blood urea nitrogen and serum creatinine we also detected significant improvements in renal function over the first 7 days compared to controls ($p=0.0004$).

In a subsequent experiment, analysis over 20 weeks showed improved renal function in the treated group, compared to controls ($p=<0.0001$), throughout the time course. At 20 weeks the treated isografts exhibited markedly reduced tubular atrophy and transplant arteriosclerosis compared to controls.

Our data reveal a significant short and long-term improvement in graft function can be achieved from reducing damage at the very outset of transplantation by inhibiting the complement cascade in the donor organ. This has been achieved with a single bolus of a derivative of a naturally occurring complement inhibitor with no known toxicity. By protecting the graft at the very outset of transplantation, our approach produced a significant long term benefit in graft function that could translate to improved clinical transplant survival if applied to human donor organs.

LABORATORY

Parallel Sessions

Wednesday 22 March

15.45-17.00

Clinical

C15

DIRECT VISUALISATION OF CYTOMEGALOVIRUS-SPECIFIC CD8$^+$ T CELLS IN RENAL TRANSPLANT RECIPIENTS

Hilton RM, Hargreaves REG, Vaughan RW[1], Sacks SH and O'Callaghan CA[2].

Department of Nephrology and Transplantation, Tissue Typing[1] Guy's Hospital, London, SE1 9RT, Institute of Molecular Medicine[2], John Radcliffe Hospital, Oxford OX3 9DS.

Cytomegalovirus (CMV) is the single most important pathogen affecting the clinical course of organ transplant recipients. The virus directly mediates tissue injury and clinical disease and has in addition a variety of indirect effects, including immunosuppression and a putative role in acute and chronic allograft rejection. Once infected the virus is harboured for life, activation from latency being induced by many of the factors to which transplant recipients are exposed, including immunosuppressive agents, allogeneic reactions and systemic infection.

Replicating CMV is highly cell-associated, the key host defence being MHC-restricted cytotoxic T lymphocytes (CTL). Serological tests play a key role in defining the clinical risk from CMV at the time of transplantation, seronegative recipients of organs from seropositive donors having a greater than 50% risk of symptomatic disease, but are of little diagnostic value thereafter. Prophylaxis against CMV infection in high-risk individuals has been shown to be safe and effective, and, in addition, to significantly decrease the rate of acute cellular rejection during the first six months after transplantation.

We have studied CMV-specific CD8$^+$ T cell frequencies in renal transplant recipients in the first three months following transplantation using fluorescent tetrameric MHC class I-peptide complexes. Using HLA-A*0201 tetramers containing peptide epitopes from the immunodominant matrix protein pp65 we have found that the presence of a high frequency of CMV-specific CTL in patients who are seropositive for CMV at the time of transplantation correlates with resistance to CMV disease. Conversely, CMV-seronegative recipients have no detectable CMV-specific CTL and show no expansion of such T cells at the time of acute CMV infection.

These data imply that maintenance of CMV latency in immunosuppressed individuals is dependent upon the presence of sufficiently high numbers of virus-specific CD8$^+$ T cells, and that the ability to recruit such cells plays an important role in the control of clinical disease. Measuring the frequency of CMV-specific CTL in transplant recipients more accurately predicts the risk of CMV disease than serological tests, and may be of use in targeting high-risk individuals who would benefit from CMV prophylaxis.

NON-COMPLIANCE IN RENAL TRANSPLANT RECIPIENTS WITH FUNCTIONING GRAFTS

SD Charlett, A Asderakis, A Valentine, P Dyer, RWG Johnson

Renal Transplant Unit, Manchester Royal Infirmary Manchester

Background: Non-compliance has been described as the third leading cause of renal allograft failure. This study assesses the magnitude of non-compliance in recipients of functioning renal transplants, identifying demographic and clinical risk factors, and evaluating its impact on late acute graft rejection.

Patients and Methods: 156 adult transplant recipients with functioning kidneys, who received consecutive renal allografts from January 1990 to December 1994, having a minimum follow-up of 4.5 years. For the purpose of this study we designed a Compliance Self-Declare Questionnaire that included data on living arrangements, employment status, medical regime, knowledge of medication, perceived medication side effects, and attitudes and behaviour towards medication side effects (including intentional or not tablet omission).

Non-compliance was defined objectively as more than three serum cyclosporine levels less than 80ng/ml or FK506 less than 5ng/ml (that were not medically directed or justified), and by the average number of missed appointments per year.

Non-compliance was also defined as an admission of deliberately omitting prescribed medication or failing to remember medication on, at least, one or more occasions per month. Questions regarding non-compliance were asked in more than one format.

We used as outcomes the declared and objective levels of compliance, episodes of clinical and biopsy proven acute rejection, and late rejections.

Results: 129 patients (82.6%) responded to the questionnaire. The overall self declared non-compliance was 18.5%. In univariant analysis, declared non-compliance was associated with age at transplant [33.3% of those ≤ 30 years declared non-compliance compared to only 9.7% of patients from the ages of 30 to 55 years ($p = 0.014$) and 18.5% of those over 55 years]. Younger age was also associated with repeatedly low serum cyclosporine levels (44.2 % of patients ≤ 30 years old vs. 40.2% in the > 30 years age group). 12.7% of females compared with 21.7% of males admitted failing to take medication as prescribed (OR=0.6), while 37.9% of females and 43.8% of males were recorded with low serum drug levels. Present or previous side effects were correlated with 'unintentional' non-compliance, and 'feeling upset' as a result of side effects was correlated with self-declared non-compliance. Non-attendance at clinic was correlated with male sex ($p=0.08$) and the number of tablets prescribed ($p=0.02$). Acute rejection and biopsy proven rejection were associated with DR mismatch ($p = 0.0001$) as expected, whereas late rejections were not. Late rejections were associated with male sex ($p=0.052$) and living without support ($p=0.10$). In addition we identified 6 cases where not taking immunosuppressive drugs directly caused late acute rejection.

Conclusion: Non-compliance is a significant problem in kidney transplant patients. Young age and male sex were associated with both objective and subjective measurements of non-compliance. Late acute rejection can be directly caused by non-compliance. Previous side effects and feeling upset as a result of side effects were correlated with unintentional and self-declared non-compliance, respectively. The number of tablets and the kind of immunosuppression were not shown to affect compliance.

CLINICAL

C5

A COMPARISON OF OPEN AND LAPAROSCOPIC LIVE DONOR NEPHRECTOMY

ML Nicholson, PS Veitch

Division of Transplant Surgery, Leicester General Hospital

Introduction: Traditional open donor nephrectomy (ODN) leaves a significant wound and this raises concerns about postoperative pain and the need for a prolonged recovery period. Some of these problems may be lessened by the use of laparoscopic live donor nephrectomy (LDN) which has recently been introduced into surgical practice. This study compares the efficacy, morbidity and donor recovery following ODN and LDN.

Patients and methods: An initial series of LDN (n=12) were compared to a historical control group of ODN (n=34). LDN was performed via a transperitoneal approach. The renal artery was double liga-clipped flush with the aorta and the renal vein was divided using an endovascular stapler. The kidney was removed in an endocatch bag introduced through a 6 cm Pfannensteil incision. Donors were managed with a patient controlled analgesia system (PCAS). Post-operative pain scores, return to normal activities and complications were compared in the donors. Graft function and technical complication rates were compared in the transplant recipients.

Results: (mean ± sem)

		ODN (n=34)	LDN (n=12)	P value
Donor:	Length of stay (days)	46.0±1.8	3.9±1.1	<0.0001
	PCAS use (hrs)	64± 18	39±12	<0.001
	Return to work (wks)	12±6	5±3	<0.02
Recipient	Serum creatinine (μmol/l)	139±35	142±43	N.S
	Technical complications	2 (6%)	2 (17%)	N.S
	Graft survival	32 (94%)	12 (100%)	N.S.

Conclusions: Laparoscopic donor nephrectomy is technically feasible and is likely to remove many of the disincentives to living kidney donation. The initial results of kidney transplants from LDN are good but the potential for higher rates of technical complications is of some concern and much more experience will be required before the place of this new technique can be defined.

C19

AUDIT OF LABORATORY PRACTICE IN ANTIBODY SCREENING AND CROSSMATCHING FOR RENAL TRANSPLANTATION

SV Fuggle, CJ Taylor on behalf of the UKTSSA Users' Kidney Advisory Group and Histocompatability and Immunogenetics (H&I) Laboratories providing Services for Renal Transplantation

An audit of Laboratory Practice was conducted at the request of members of the UKTSSA Users' Kidney Advisory Group in those laboratories providing H & I services for renal transplantation. The aim was to assess the degree of variation in policy between laboratories that may affect the national allocation of kidneys. A questionnaire was formulated to examine Antibody Screening Strategy, Crossmatch Policy and Interpretation of the Crossmatch and was distributed to the 24 H & I Laboratories providing these services. A 100% response rate was achieved.

Antibody screening strategy there is variation in the strategies adopted by different laboratories for antibody screening but all appear to be designed to meet the requirements for organ exchange. In all centres samples for antibody screening are obtained on at least a 6 monthly basis, but 19/24 centres obtain samples every 2-3 months. Many laboratories reported difficulties in obtaining regular samples from all patients on their waiting list. Only 16/24 centres obtain samples following transplantation, to be archived and screened if a patient returns to the waiting list. The strategies and techniques used for antibody screening vary but 18/24 laboratories now use sensitive flow cytometry (FC) or elisa techniques to screen for the presence of antibody and all use complement dependent cytotoxicity (CDC) to assist in specificity definition.

Crossmatch Policy The techniques used for crossmatching are of particular interest as varying levels of sensitivity may be achieved. Crossmatching is routinely performed by CDC in all laboratories, but 12 laboratories additionally use FC at the time a cadaver donor is being considered. In a further 7 laboratories a FC crossmatch is performed retrospectively or used in the preparation for living donor transplants.

Interpretation of the Crossmatch Sets of crossmatch results were given, covering scenarios that may lead to differences in opinion and laboratories were asked whether they would recommend transplantation. The results showed that for sensitised patients 61% of centres would consider a positive result from an IgM anti-HLA antibody present in current serum a contraindication to transplantation, while only 26% of centres would consider an IgM antibody in historic serum, current serum negative, a contraindication. A positive result from a FC crossmatch in the absence of antibody detected by CDC was considered a contraindication if present only in historic sera in 87% of laboratories and in current sera in 91% of laboratories. Historic serum IgG positive, current serum negative crossmatches were considered a contraindication in 91% of laboratories. In this analysis it is recognised that any variation in approach must be taken in the context of factors specific to the donor and recipient and in particular of the clinical practice of the particular Transplant Unit.

This audit provides an overview of current policy and practice in H & I Laboratories and represents a snapshot of an evolving area of clinical laboratory practice.

C20

INFORMED CONSENT IN RENAL TRANSPLANTATION – DOES IT REALLY EXIST ?

McLaren AJ, Morris-Stiff G, Casey J on behalf of the Carrel Club

Informed consent prior to a surgical procedure should be relevant to individual patients, describe the risks involved and the possible complications (where either have an incidence of 1% or more) and be sought by a doctor suitably trained in the procedure. The aim of this study was to examine how closely transplant surgeons in the UK approach this ideal.

A questionnaire survey was sent to 62 Consultant Transplant Surgeons identified from the UKTSSA Users Directory. This sought details of current practice regarding information provided to patients of the risks involved in transplantation and of the quality of organ being offered.

Replies were received from 47 individuals representing 24 transplant units. Consent was usually obtained by a consultant surgeon or higher surgical trainee. 17 units offered a preassessment meeting/clinic for discussion of risks. The major risks associated with transplantion - rejection, malignancy, infection, graft failure and drug side effects were discussed by most surgeons. CMV status and risk was discussed routinely by only 27 (57%) of consultants.

The quality of individual organs is becoming increasingly relevant with the necessity to use marginal donors to meet rising demand. The quality of individual organs was discussed prior to transplantation by 32 consultants (68%) – particularly HLA matching, sensitisation and cold ischaemia time. Of those who did not currently raise these issues prior to transplantation only 3 of 15 thought their practice would change in the future.

Patients may in the future, particularly if provided with more detailed information on organ quality, make specific requests regarding this prior to accepting an organ – currently 17 (36%) of consultants allowed such requests. Two individuals thought that their unit policy on this issue would change in the next 5 years.

Overall consent issues are covered broadly within GMC guidelines. However with increasing evidence that organ quality is important to longterm outcomes it is clear that patients may expect more details of individual organ quality. It is important not to over-burden patients with information, yet guard against a paternalistic attitude. The evidence of this questionnaire supports a view that greater detail is being given by some and to quote from the case of Bolam this group may become the "proper standard of competent, professional opinion in deciding whether to warn or not."

Intrathoracic

C72

FACTORS INFLUENCING RECIPIENT SELECTION IN ADULT CARDIAC TRANSPLANTATION – AN ANALYSIS OF 466 TRANSPLANTS

AC ANYANWU, C JONES, CA ROGERS, AJ MURDAY

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

Background: When presented with a prospective donor heart, a transplant unit often has to select one from several potential recipients. Some recipients may be at a disadvantage by virtue of their characteristics, and thus less likely to be selected.

Aim: To identify factors associated with recipient selection.

Methods: Multi-centre prospective study. 466 consecutive adult donor hearts transplanted in 8 centres were studied. For each donor, all ABO compatible patients more than 10 years old and registered on the waiting list at the unit where the heart was transplanted at the time the organ was donated were identified. Conditional logistic regression was used to identify factors associated with the selection process.

Results: The median number of potential recipients for a donor heart (ABO compatible patients within a transplant centre) was 15 (interquartile range 7-29). Factors found to be associated with a reduced likelihood of selection were non-identical blood group (Odds Ratio 0.27, 95%CI 0.19-0.37), size mis-match (large donor 0.43 (0.30-0.61); small donor 0.23 (0.16-0.31)), previous surgery (0.71 (0.56-0.89), primary heart disease valvular (0.62 (0.34-1.09)) or congenital (0.33 (0.17-0.62)) and waiting time. Contrary to expectation, longer waiting times were associated with a significantly reduced chance of selection (p=0.0001). Patients on ventilatory support (5.96 (2.25-15.8)) or UNOS status 1 at registration (3.03 (2.08-4.41)) were more likely to be selected. Distance from home to transplant centre, previous transplant, raised PVR (3 units), CMV mis-match and recipient age (>60 years) were not found to be significantly associated with the selection process.

Comment: If recipient selection is largely governed by clinical characteristics, some waiting-list patients will be continually disadvantaged. Such patients are less likely to receive a transplant and are therefore are more likely to die awaiting a transplant. Debate might focus on how best to balance clinical priority, optimisation of outcome, and equity, in deciding which recipient should be allocated a donor heart.

C4

BILATERAL LUNG TRANSPLANT : THE PROCEDURE OF CHOICE FOR END-STAGE SEPTIC LUNG DISEASE

Rao J, DeSoyza A, Forty J, Hasan A, Hilton CJ, Ledingham S, Parry G, Wardle J, Gould FK, Corris PA, Dark JH

Department of Cardiopulmonary Transplantation, Freeman Hospital, Newcastle upon Tyne.

OBJECTIVE: We reviewed our experience with a specific procedure, the bilateral lung transplant (BLTx), and in particular its use in septic lung disease.
METHODS: Retrospective analysis of the first 100 consecutive BLTx performed at our institution since August 1990, with complete follow-up.
RESULTS: Of the 100 patients, 85 had septic conditions - 63 Cystic Fibrosis (CF) and 22 other Bronchiectasis. Age range was 12.5 to 59.2 years(Mean 31.4 years). Age range for CF patients was 12.5 to 49.5 years(Mean 25.8 years) and for Bronchiectatic patients was 25.1 to 56.9 years(Mean 46.3 years). One CF patient received a living related donor lobar transplant. Overall survival is 75.8%, 68.8% and 64.2% at 1, 3 and 5 years. For septic lung disease, two cohorts were examined 1990-1995 (n=41, Group 1) and 1996 to date (n=44, Group 2). The 1 year and 3 year survival was 70.7% and 61% in Group 1 and 83.6% and 79% in Group 2. 30-day mortality was 12% in Group 1 falling to 2.3% in Group 2; there have been no early deaths in the last 23 patients. Of the 170 bronchial anastomoses, there has been 1 dehiscence and 1 required stent insertion (1.8% airway complication rate) The last 126 consecutive bronchial anastomoses have been uncomplicated. Improved pulmonary function was seen in all surviving patients.
CONCLUSIONS: Survival after BLTx for septic lung disease in our hands is comparable to or exceeds the best published figures for combined heart and lung transplantation, without the burden of a denervated, allografted heart.

C61

INDIRECT RECOGNITION OF DONOR ENDOTHELIAL CELLS FOLLOWING CARDIAC TRANSPLANTATION

Linke, A.T., Batten, P., McCormack, A., Banergee, R., Yacoub, M.H. and Rose, M.L.

N.H.L.I., Imperial College School of Medicine, Harefield Hospital, Harefield, Middlesex, UB9 6JH.

Indirect recognition of donor antigens is likely to be a major pathway of allorecognition after solid organ transplantation. Studies have demonstrated long term survivors of solid organ transplantation become hyporesponsive to donor antigens presented via the direct route, but some patients with chronic rejection still have intact responses to donor antigens presented indirectly. It has therefore been postulated that indirect recognition of donor antigens is associated with development of chronic rejection. Most studies have used donor specific MHC class II peptides to demonstrate unresponsiveness. Here we reasoned that the most likely source of MHC molecules in donor grafts would be endothelial cells. We have therefore developed an assay whereby we can test the frequency of T-helper cells recognising donor endothelial derived antigens via the indirect route and compare it to recognition of third party endothelial cells in recipients of cardiac allografts.

T helper precursor frequencies were assessed by limiting dilution analysis (LDA) using PBL from normal volunteers and transplant recipients. Briefly, purified CD4 positive T cells were added at varying concentrations to $5x10^4$ irradiated T cell depleted antigen presenting cells (APC) in the presence of freeze/thawed endothelial cells previously stimulated with 500U/ml IFNγ for 4 days. Endothelial cells were obtained from donor aorta at the time of transplantation. Following three days incubation the presence of IL-2 was determined by conventional CTLL-2 bioassay. In some experiments endothelial cells were freeze/thawed in the absence of IFNγ.

Confirmation that presentation of endothelial cell fragments proceeded via the indirect pathway in this assay was obtained by using 5uM chloroquine a concentration found to inhibit indirect but not direct allorecognition. In addition purified T cells were unable to respond to endothelial cell fragments in the absence of APC.

In normal subjects measurable precursor cell frequencies were found for indirect presentation of untreated endothelial cells (range undetectable to1/168,329) and a significant increase in precursor cell frequency is obtained when endothelial cells are IFNγ activated (range 1/137,000 - 1/17,644 , n=3). Experiments are currently underway to determine whether this is exclusively due to indirect recognition of MHC class II alloantigen.

To date we have examined four cardiac transplant patients without chronic rejection and two lung transplant patients. Current results indicate that in two patients there was a significant hyporesponsiveness to IFNγ treated donor endothelial cells as compared to third party cells with an equivalent HLA mismatch. In four patients the frequencies were <1/500,000 for both donor and third party endothelial cells. We are currently accumulating a larger cohort of transplant recipients for use in this system to estimate Th precursor cell frequencies to donor specific and third party endothelial cells to determine their association with chronic rejection.

IN VIVO MONITORING OF MITOCHONDRIAL NADH DURING HEART TRANSPLANT: AN INDICATOR OF FUNCTION AND ORGAN VIABILITY

HARAMIS H, BALOGUN E*, SHUREY C* & THORNILEY MS
Department of Instrumentation & Analytical Science, UMIST, PO Box 88, Manchester M60 1QD. *Department Surgical Research, Northwick Park Institute for Medical Research, Northwick Park Hospital, Harrow, Middlesex, HA1 3UJ

Introduction Ischaemia and reperfusion injury is a consequence of storage and transplantation of organs and is associated with a fall in pH, ATP and a rise in mitochondrial NADH levels. Surface fluorometric (SF) measurements of NADH have been shown to correlate with histological findings, ATP levels and viability post transplant, in rat liver and rabbit renal transplant models[1,2,3]. The aim of this study was to determine whether changes in the redox state of NADH could be measured in an *in vivo* beating transplanted heart model, and if this could be used as an indicator of mitochondrial function and hence organ viability pre-transplant. To achieve our aim we have used an *in-vivo* heterotopic rat heart transplant model and used several methods to measure NADH: emission spectra, time analysis and ratiometric methods in response to alterations in the vascular supply.

Methods All procedures were carried out according to the Animals (Scientific Procedures) Act, 1986. *In-vivo Heterotopic Heart Transplantation Model* Cardiac isografts were carried out in Male Lewis rats (200-300g) using a modified procedure of Ono and Lindsey (1969)[4]. Surgical anaesthesia was induced with 0.6ml/kg Hypnorm(fentanyl/fluanisone) i.m. and 2.5 mg/kg diazepam i.p.; maintained with 0.1ml injections as necessary. SF measurements of NADH were made using a Perkin Elmer LS 50 with a fibre-optic probe, placed on the surface of the heart. The effect of arterial occlusion of the transplanted heart on the NADH emission at 470 nm (excitation 366nm) was determined in 6 isografted hearts. Measurements were made continuously and between 2 to 5 arterial occlusions were made in the same animal (n=22 occlusions). Results presented in terms of relative fluorescence units (arbitrary values)

Results Repeatable values were obtained for the effect of arterial occlusion on NADH measurement of a beating *in-vivo* heart. The baseline values in 6 in situ transplanted hearts was 7.05±0.186 (mean±SEM) compared to 10.29±0.33 in the occluded heart. (n=22 occlusions in total). The difference pre- and post- release of occlusion were highly significant ($p < 0.0001$). This represented a change of 3.23±0.226 fl units upon occlusion, release of occlusion resulted in a return to baseline values in less than 30 seconds.

Conclusions In the heterotopic heart transplant model, reproducible NADH measurements can be made in response to arterial occlusion. We will also present measurements of the rates of NADH production and loss during clamping. These measurements are a function of blood flow, and any degree of congestion. These measurements should enable us to determine whether this could be used as an indicator of mitochondrial function and hence organ viability pre-, during and post-transplantation and possibly enable early intervention resulting in a better prognosis.

References

1. Thorniley MS, Lane NJ, Green CJ 1994a. *Kidney Int* **45**, 1489-1496.
2. Thorniley M, Simpkin S, Fuller B, Jenabzadeh M, Green C 1995a. *Hepatology* **21**, 1602-9.
3. Okamura R, Tanaka A, Uyama S, Ozawa K 1992. *Transplant Int* **5**, 165-169.
4. Ono K, Lindsey ES 1969. *J Thoracic Cardiovascular Surg* **57**, 225-229.

INHALED NITRIC OXIDE ATTENUATES ENDOTHELIAL INJURY AND THE DAMAGING EFFECTS OF FLUSH SOLUTION IN THE LUNGS.

Sudarshan C D, Bell A, Pritchard G E, Orr H, Flecknell P A, Shenton B K, Dark J H.

Cardiothoracic Centre, Freeman Hospital, Department of Surgery and the Comparative Biology Centre, University of Newcastle upon Tyne, Newcastle upon Tyne NE7 7DN.

Aims: We have established the beneficial effects of pre-treating the donor with inhaled Nitric Oxide (NO) in a porcine single lung transplant model of acute lung injury. We have gone on to study the interactions between NO and cold modified Eurocollins flush solution, the standard preservation technique in clinical lung transplantation.

Methods: Healthy pigs weighing 50-55 kg were anaesthetised. A left-sided human double-lumen tube was inserted through a tracheostomy incision. NO at 20 ppm was delivered to one of the lungs for a period of twenty minutes. Serial blood samples for neutrophil counts were taken from pulmonary artery (PA) and vein (PV) on each side during this period. 5 million 3µm microspheres were injected into the PA immediately prior to flushing the lungs with cold modified Eurocollins solution. Broncho-alveolar lavage (BAL) was performed in basal segments of both lungs. Lymphocyte, neutrophil & microsphere counts were made of the effluent & albumin, as a further marker of endothelial leakage, was measured. Biopsies were obtained from both lungs to identify the expression of Intercellular Adhesion Molecule-1 (ICAM-1).

Results: Neutrophil sequestration, indicated by differences in PA and PV cell counts, was observed in both lungs. Significant differences in loss of white cells into lungs were noted at 10, 15 & 20 minutes of NO treatment ($p=0.029$, $p=0.017$ & $p=0.002$ respectively). White cells were found in the BAL from both lungs. The number of cells in the NO treated lung were significantly less when compared to the control lung ($p=0.001$). The mean albumin content in the lavage of the control lung was 5.46µg/ml and this was significantly reduced in the treated lung which was 1.91µg/ml ($p=0.021$). There were significantly more microspheres in the control lung when compared to the NO treated lung ($p<0.001$). The expression of ICAM-1 which was very obvious in the control lung was virtually absent in the NO treated lung.

Conclusion: Surgical trauma - sternotomy and cannulation - leads to endothelial activation and this is exacerbated by cold modified Eurocollins solution leading to leakage of cells and protein into the alveoli even in this non-brain-stem-dead model. The striking reduction of sequestration and leakage seen with NO may explain some of its benefits in attenuating acute lung injury after single lung transplantation.

Laboratory

EFFICACY OF DIFFERENT BONE MARROW LINEAGES IN PROLONGATION OF ALLOGRAFT SURVIVAL

J.Stephen Billing, Wilson Wong*, Peter J. Morris and Kathryn J. Wood

Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU
* Department of Nephrology and Transplantation, Guy's Hospital, London SE1 9RT

Objective: Donor-specific bone marrow (BM) has been shown to be highly effective at inducing operational tolerance to allografts in experimental models. Administration of donor bone marrow, either T cell-depleted or whole BM, is being actively investigated as a clinical strategy to prolong allograft survival in conjunction with conventional immunosuppression. It is not known whether the beneficial effect of bone marrow treatment is attributable to one or more specific lineages within the BM; some cell types may even accelerate rejection. In this study we have investigated the ability of different BM lineages to prolong the survival of murine cardiac allografts.

Methods: Recipient CBA ($H2^k$) mice were pretreated with $B220^+$, Mac-1^+ and Gr-1^+ BM cells from transgenic CBK mice, which express the donor MHC class I molecule K^b on a recipient-type $H2^k$ background. Each subpopulation was positively selected using MACS Microbeads and was administered at a range of doses from $1x10^6$ to $5x10^4$ cells 27 days before transplant. The BM infusion was covered by 2 doses of depleting anti-CD4 monoclonal antibody on days -28 and -27. Fully allogeneic C57BL/10 ($H2^b$) hearts were transplanted on day 0.

Results: The $B220^+$ subpopulation was the most effective in prolonging graft survival, with 100% long-term graft survival induced by $1x10^6$ or $5x10^4$ cells. Mac-1^+ cells were also tolerogenic, with long-term graft survival of 100%, 83% and 75% after $1x10^6$, $2.5x10^5$ or $5x10^4$ cells, respectively. Gr-1^+ cells proved less effective, with even $1x10^6$ cells failing to prolong survival significantly in relation to antibody controls.

Conclusions: $B220^+$ and Mac-1^+ cells from the donor-specific transgenic strain CBK were highly effective in prolonging allograft survival whereas Gr-1^+ cells were relatively ineffective. This difference may be due to cell trafficking, antigen persistence or the ability to present donor antigens. These results identify BM cell types which may have particular potential to prolong graft survival clinically.

LABORATORY

IMMUNE CONTRIBUTIONS TO CHRONIC ALLOGRAFT NEPHROPATHY: DIRECT MECHANISMS

Baker, RJ ; Hernandez-Fuentes, MP; Brookes, PA; Chaudhry, AN; Lechler, RI.

Department of Immunology, ICSTM, Hammermith Hospital,
Du Cane Rd, London W12 ONN, UK.

Acknowledgements: MRC, MEC from Spain

The main cause of late renal graft loss is chronic allograft nephropathy (CAN). Despite major improvements in short term graft survival there has been little impact on indolent attrition of grafts due to CAN. There is evidence to support the contribution of immune mechanisms to the development of CAN. In order to assess the immunological contribution to CAN we have studied the T cell responses in 22 recipients of live-related renal allografts (median time since transplantation 13 years). 9 of these patients have developed CAN (8/9 confirmed by biopsy) while 14 have maintained good graft function.

We have enumerated the CD4+ T cell responses to both donor and control alloantigens by the technique of limiting dilution analysis. CD4+ T cell frequencies have been determined according to the secretion of the cytokines, IL-2, IL-5 γ-IFN. We have also examined the frequencies of CTL precursors in PBMCs.

The results are shown in the table below:

	CAN	Not CAN	Total
IL-2	9/9	13/13	22/22
IL-5	7/9	7/13	14/22
IFN-γ	6/9	12/13	18/22
CTLp	5/7	12/12	17/19

Figures shown represent the proportion of patients who had significantly lower frequencies of responding T cells against donor when compared to third party.

In conclusion there was a global donor-specific hyporesponsiveness according to IL-2 secretion. There was also evidence of widespread donor-specific hyporesponsiveness when the cytokines IL-5 and g-IFN were measured. In addition donor-specific cytotoxicity was specifically reduced in most patients. There were no significant differences between patients with or without chronic allograft nephorpathy. This confirms our earlier impression that direct allorecognition is markedly reduced in a donor-specific manner in recipients of longstanding renal allografts. If immune mechanisms are contributing to the development of CAN then we believe that they are more likely to be operating through the indirect pathway.

Category: LABORATORY

THE EFFECT OF DIFFERING IMMUNOSUPPRESSIVE REGIMES ON THE FUNCTIONAL AND MORPHOLOGICAL CHANGES IN A RAT RENAL ALLOGRAFT MODEL OF CHRONIC REJECTION.

McGrath, JS and Shehata, M.

Nottingham Transplant Unit, NG5 1PB.

Background: Chronic rejection (CR) represents the leading cause of late graft loss and there are currently no effective measures for its prevention or treatment. We assessed the effectiveness of cyclosporin (CyA), tacrolimus (FK), mycophenolate mofetil (MMF) and SDZ-RAD on the development of CR in the F344-LEW renal transplant model.
Methods: Orthotopic renal transplant was performed from F344 donors into unilaterally nephrectomised LEW recipients. Isografts (F344 to F344) served as controls (n=8). Groups of animals (n=8) received one of the following drug regimes; monotherapy (CyA or FK) or dual therapy (MMF+CyA or MMF+FK or SDZ-RAD+CyA). Animals were sacrificed at 2 and 4 months and both kidneys were retrieved for histological and morphometric analysis. Serum creatinine and 24-hour urinary protein were measured before transplant and at 3,7 and 10 days and at 1,2,3,months and at sacrifice.
Results: Untreated allografts developed functional and morphological changes typical of chronic rejection by 2 months. Further progressive changes were observed at 4 months. There was no significant deterioration in serum creatinine in all groups except rats treated with CyA SDZ-RAD in combination. Significant proteinuria was only noted in untreated allografts and those treated with CyA.
Tissues from animals treated with CyA displayed arteriolar hyalinosis (AH), proximal tubular vacuolation (TV) and thickened glomerular basement membrane with no evidence of cellular infiltrate at 2 months. At 4 months, AH progressed and was associated with interstitial fibrosis (IF) and glomerulosclerosis (GS). Tissues from FK-treated rats showed minimal TV and AH but a moderate degree of interstitial infiltrate was noted at 2 and 4 months. Sections from animals treated with MMF+CyA showed minimal TV and AH. Rats treated with MMF+FK did not show any histological changes except a mild interstitial infiltrate. The most significant histological changes were seen in animals treated with SDZ-RAD+CyA. In these animals severe AH, extensive TV and atrophy and IF were noted. Native kidneys of rats from all groups showed no histological evidence of nephrotoxicity except those treated with CyA or SDZ-RAD+CyA. The former group showed evidence of AH, TV and interstitial oedema and the latter demonstrated tubular atrophy and IF, but only minimal hyalinosis.
Conclusions: These experiments suggest the following:

1. The F344-LEW combination is a reproducible model of CR.
2. All drugs used are effective in preventing CR during the treatment period.
3. Interstitial infiltrate noted in FK-treated rats suggests insufficient dose of FK.
4. The combination of SDZ-RAD+CyA is effective in preventing CR but appears nephrotoxic.
5. The presence of hyalinosis in both native and allograft kidneys treated with CyA suggests that these changes are due to CyA nephrotoxicity.

EFFICIENT GENE DELIVERY TO VASCULAR SMOOTH MUSCLE CELLS USING A NON-TOXIC, SYNTHETIC PEPTIDE VECTOR SYSTEM TARGETED TO MEMBRANE INTEGRINS

Jian-Mei Li[1], Louise Collins[1], Xiaohong Zhang[1], Kenth Gustafsson[2] and John W. Fabre[1]

[1]Department of Clinical Sciences
The Institute of Liver Studies
Guy's, King's and St Thomas' School of Medicine
King's College Hospital
Bessemer Road
LONDON SE5 9PJ

Background
Chronic rejection is now the major cause of allograft failure, and remains an untreatable condition. A prominent characteristic of the histopathology is extensive intimal proliferation of vascular smooth muscle cells (VSMCs). Targeting VSMCs by gene therapy techniques offers a possible avenue for arresting or reversing chronic rejection. Defining suitable DNA vectors for VSMCs is the objective of this study.
Methods
A 31 amino acid synthetic peptide has been evaluated as a DNA vector for VSMCs of man, rabbit and rat. The vector comprises the 15 amino acid integrin-binding domain of molossin (the venom of the American Pit Viper *Crotalus molossus molossus*) and a chain of 16 lysines for electrostatic binding of DNA.
Results
Initial binding studies on frozen sections showed that the integrin-binding domain of molossin binds strongly to vascular smooth muscle cells, but not to vascular endothelial cells, in all 3 species. Primary cultures of vascular smooth muscle were therefore studied, looking in particular at chloroquine, cationic lipids and a fusogenic peptide to promote endocytic exit of vector/DNA complexes. The polylysine molossin vector complexed to β galactosidase and luciferase reporter genes was by itself ineffective at gene delivery. The use of chloroquine to assist endocytic exit, which works well on immortalised cell lines, was of little value because of toxicity to the primary vascular smooth muscle cells. The addition of cationic lipids to polylysine-molossin/DNA conjugates gave excellent gene expression, but required mildly toxic doses of cationic lipid, and resulted in some loss of integrin specificity of the vector system. The optimal system involved the use of the amino terminal 20 amino acids of the haemagglutinin of the influenza virus. This peptide, when added to polylysine-molossin/ DNA complexes at an optimal w/w ratio of 5:1:2 (polylysine-molossin/DNA/fusogenic peptide) resulted in 25-30 % transfection of vascular smooth muscle cells with good levels of gene expression and no toxicity.
Conclusion
This represents an effective and safe DNA vector, comprised entirely of synthetic peptides, and therefore readily standardised for clinical and experimental application.

LABORATORY

COSTIMULATORY BLOCKADE BY THE INDUCTION OF AN ENDOGENOUS XENOSPECIFIC ANTIBODY RESPONSE.

Rogers, N.J., Mirenda, V., Jackson, I., Dorling, A. and Lechler, R.I.

Department of Immunology, Imperial College School of Medicine, Hammersmith Campus, Du Cane Road, London, W12 0NN.

Long-term survival of xenografts will depend on the induction of T cell tolerance across species barriers. The response of human T cells against porcine tissues is of comparable magnitude to that induced by alloantigens. This reflects productive interactions between key accessory molecules across the species barrier, and the delivery of costimulation by porcine CD86 through human and murine CD28. Here, we describe a novel approach to inhibit the costimulation of murine T cells with direct anti-porcine xenoreactivity by inducing porcine CD86-specific antibodies in the recipient prior to grafting, a strategy which removes the need for repeated injection of antibodies or fusion proteins. C57BL/6 mice were immunised with chimeric peptides incorporating an ovalbumin T cell epitope and 8-12 amino acids corresponding to nine hydrophillic sequences sequences predicted to lie in the N-terminal domain of porcine CD86. T cell immunity in these mice was specific for the ovalbumin epitope, and no proliferation was seen to the CD86 sequences. Sera from mice immunised with two of the chimeric peptides recognised native porcine CD86 on transfected cell lines. Furthermore, the antisera inhibited direct mouse anti-porcine T cell responses but had no effect on the delivery of costimulation by murine CD86, demonstrating the species-specificity of this approach. In vivo experiments involving transplantation of porcine islets into normal or peptide-sensitised mice demonstrate that the induced anti-porcine CD86 antibodies prolong islet graft survival. A similar approach is being employed for the generation of endogenous porcine CD40. This strategy of endogenous costimulatory blockade appears to be effective, graft specific and may have direct clinical application.

LABORATORY

CONTRIBUTION OF T CELL ANERGY TO DONOR SPECIFIC HYPO-RESPONSIVENESS IN RENAL TRANSPLANT PATIENTS

W.F. Ng[1], R. Baker[1], M.P. Hernandez-Fuentes[1], A. Chaudhry[2] and R.I. Lechler[1]
Departments of Immunology[1] and Renal Medicine[2], Hammersmith Hospital, Imperial College School of Medicine, London, United Kingdom

Long-term allograft survival is the ultimate goal of transplantation. It has been demonstrated in our laboratory previously that the direct allospecific precursor frequencies of $CD4^+$ T cells against donor antigens were reduced in the majority of renal transplant patients. The mechanism(s) for this donor-specific hypo-responsiveness, however, were unclear. Following transplantation, donor bone-marrow-derived antigen presenting cells (APCs) are eliminated within a few weeks and the transplanted tissue is repopulated with recipient bone-marrow-derived APCs. From then on, the only cells displaying intact donor molecules are the parenchymal cells on the graft; these cells are unable to provide crucial additional signals that are required for T cell activation. In the absence of co-stimulatory signals, MHC alloantigen recognition by direct pathway T cells not only fails to induce activation, but results in T cell anergy.

Based on these observations, this study was designed to determine whether the maintenance of the observed donor-specific hypo-responsiveness in renal transplant recipients is due to T cell anergy in the direct allospecific pathway. If anergic T cells were responsible for the maintenance of the observed reduction of direct allospecific frequencies against donor cells, one would predict that treatment with interleukin-2 (IL-2) would reverse the anergy and hence result in complete or partial restoration of anti-donor frequencies. To exclude the possibility of any non-specific enhancement of immune response by the treatment with IL-2, the allospecific frequencies against third party antigens were used as control. Thus, in this study, peripheral blood samples were obtained from renal transplant recipients at least 1 year post-transplant in a single transplant centre in London. $CD4^+$ T cells were then purified from the blood by negative selection with magnetic beads. A proportion of the obtained $CD4^+$ T cells were used immediately in limiting dilution analysis against third party or donor cells. The remaining $CD4^+$ T cells were incubated with 30 units of recombinant human-IL-2 in 10% human AB serum supplemented culture medium for 72 hours at 37°C followed by resting for 24 hours without IL-2. Limiting dilution analyses were repeated with the IL-2-treated $CD4^+$ T cells as responder cells and the frequencies of IL-2-secreting $CD4^+$ T cells against donor and third party antigens measured.

8 out of 12 patients studied were found to have significantly lower frequencies against donor cells compared to third party cells. The anti-third party $CD4^+$ T cell frequencies were unaffected by the treatment with IL-2 ($p=0.48$ using Wilcoxon's Rank test). For the 8 patients in whom donor-specific hypo-responsiveness was demonstrated, the frequencies against donor antigens were significantly increased after IL-2 treatment in 5 patients. These findings suggested that in some patients, IL-2 specifically reversed the hypo-responsiveness of recipient $CD4^+$ T cells against donor antigens, consistent with the existence of anergic T cells in these patients.

Category: LABORATORY

LOCAL AND SYSTEMIC CTLA-4Ig PROLONGS RAT CORNEAL ALLOGRAFT SURVIVAL

Comer RM[1,2], King WJ[1], George AJT[1], Larkin DFP[2]
[1]Department of Immunology, Imperial College School of Medicine, Hammersmith Hospital and [2]Moorfields Eye Hospital, London, UK

Graft rejection is a T cell dependent process. CTLA-4Ig is a fusion protein, which binds with high affinity to B7 co-stimulatory molecules, resulting in inhibition of T cell activation, through CD28.
Purpose To determine whether systemic and/or local treatment with CTLA-4Ig, could prolong graft survival in a rat model of corneal transplantation. **Methods** Brown Norway donor corneas (3.5mm) were grafted into Lewis recipients (3.0mm), n=6 in all treatment and control groups. Treatment groups were as follows Group 1. Lewis rat recipients injected intraperitoneally (i.p.) with 500μg of CTLA-4Ig in divided doses, with 100μg given on day 2 post-op and 50μg daily thereafter until day 10. Control animals received an identical regimen with inactive human IgG. Group 2. Brown Norway donor corneas were cultured overnight at 4°C in corneal medium containing varying concentrations of CTLA-4Ig; a) 20μg/ml b) 2μg/ml c) 0.2μg/ml and then grafted into Lewis recipients. Control corneas were cultured overnight in 2μg/ml of human IgG. **Results** Systemic treatment with CTLA-4Ig resulted in significantly prolonged corneal allograft survival (median 13 days) compared to controls (median 9 days). Local treatment of donor corneas showed a dose-dependent effect on graft survival with significantly prolonged survival seen at 2μg/ml (median 13 days) but no significant effect at 20μg/ml (median 10 days), 0.2μg/ml (median 10 days) or in the control group (median 8 days). **Conclusion** As corneal allograft survival can be prolonged by both local and systemic treatment with CTLA-4Ig this indicates that the B7-CD28 pathway of costimulation has a role in corneal allograft rejection and that this protein may have therapeutic potential.

Supported by The Wellcome Trust (Grant 050966)

Posters

Wednesday 22 March

C38

THE EFFECT OF NIFEDIPINE ON LONG TERM SURVIVAL OF RENAL ALLOGRAFTS

M Metcalfe, SA White, GJ Murphy, R Saunders, J Moorehouse, PS Veitch, ML Nicholson

Department of Surgery, University of Leicester & Leicester General Hospital, Leicester

AIM

The beneficial effects of nifedipine for renal transplant recipients as measured in terms of renal function and graft survival up to 4 years has previously been reported. The aim of this study is to assess whether these beneficial effects persist long-term.

METHOD

Over a 3 year period 103 renal transplant recipients were recruited into a randomised controlled clinical trial. Patients were allocated to receive either nifedipine (n=35) or not (n=68), with cyclosporin based imunosuppression. The dose of nifedipine was titrated according to diastolic blood pressure. Controls were administered other classes of anti-hypertensives as necessary. The minimum length of time a patient remained in their allotted group was 6 weeks.

RESULTS

Patients who received nifedipine continued to demonstrate significantly superior graft suvival compared to controls. A log rank analysis using Kaplan-Meier survival showed a signficant advantage ($p<0.05$). Overall the 10 year graft survival rates were 56% in the nifedipine treated group compared with 38% for the controls.

CONCLUSION

These results suggest that the short term benefits of nifedipine in ameliorating the detrimental effects of cyclosporin toxicity are still apparent at 10 years.

C17

SPLENECTOMY AND LIVER TRANSPLANTATION - OUTCOME AND SEPSIS RELATED MORTALITY

Kumar N*, Grant DR, Wall WJ (Jurewicz WA)
University Hospital of Wales, Cardiff *,
London Health Sciences Center, London, Ontario, Canada.

The outcome of patients who have had splenectomy at the time of orthotopic liver transplantation (LT) is not well documented. We analyzed the incidence of rejection and long-term survival in patients who had splenectomy at the time of LT and compared this to a matched group without splenectomy.

METHODS

Of a group of 843 liver recipients sixteen adult patients had splencetomy at the time of LT. Two controls were selected for every case, matching for the UNOS status at the time of transplant, age and year of transplant. Actuarial survival was calculated using the Kaplan Meier method and compared with log rank test. The rates of acute rejections were compared with unpaired t test.

RESULTS

The indications for splenectomy were ABO incompatible graft (4), large spleen (3), splenic artery aneurysm (2), idiopathic thrombocytopenic purpura (2), to reduce antibody formation in a sensitized liver/kidney recipient (1), spleen dependent portal hypertension (1), intraoperative rupture of spleen (1), previous Warren shunt (1) and granuloma of spleen (1). There were two retransplants in the study group and one in the control group. The rate of acute rejection in the patients with splenectomy was 31% compared to 40% in the control group (P=0.54). The ten year actuarial survival in the study and control groups were 74.6% and 51.3% respectively (P=0.21). Two of the three deaths in the study group were due to sepsis and one of them died within 30 days of LT. Four of the 12 deaths in the control group were due to sepsis and 75% of these were within 30 days of LT.

CONCLUSION

We conclude from our study that splenectomy does not affect survival following LT compared to liver recipients who have not had their spleen removed. The rate of rejection is not affected and in this experience splenectomy did not increase the mortality from sepsis following LT.

C24

ALLOCATION OF LIVERS FOR TRANSPLANTATION

M Akyol, M Ho, P C Hayes,

Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Lauriston Place, Edinburgh

At the time of conducting this study there were no nationally agreed guidelines or system in the UK for the allocation of livers for transplantation. The views of all UK consultant liver transplant surgeons and hepatologists working in liver transplant units were assessed with an anonymous postal questionnaire. There were 43 respondents (82% response rate) who, on a visual analogue scale (VAG) indicated their willingness to offer liver transplantation to 7 potential recipients. Case histories for the 7 hypothetical recipients were written to explore views on liver transplantation for Jehovah's witnesses, for HIV positive individuals, for reformed IV drug abusers on Methadone programmes, retransplantation for recurrent hepatitis C, retransplantation for recurrent chronic rejection, living donor transplantation for young children and living donor transplantation for young adults in the setting of fulminant hepatic failure. The median score (expressed from 0 to 10 on the VAS) was greater than 5 for all 7 cases. There was considerable variation in the distribution of scores for each case and between the respondents for most of the cases. The results also point to a discrepancy between opinions of transplant clinicians and actual practice in the UK. Allocation of cadaveric donor livers merits further study to determine whether a national system or guidelines are required to ensure equity of access to this scarce and life saving resource.

C31

IMPROVED HbA1c AFTER PANCREATECTOMY AND ISLET AUTOTRANSPLANTATION WITH A MARGINAL ISLET MASS FOR PATIENTS WITH END STAGE CHRONIC PANCREATITIS

White SA, London NJM, Davies JE, Pollard C, Clayton HA, Swift SM, Dennison AR

Department of Surgerys, University of Leicester & Leicester General Hospital, Leicester

As a last resort, patients with chronic pancreatitis (CP) may need pancreatectomy (TP). In order to prevent diabetes some patients may be suitable for simultaneous islet autotransplantation (IAT). The aim of this study was to assess glycaemic control after IAT.

Over a 5 year period 25 patients (14 F: 11 M) have undergone pancreas resection (23 total {2 completion total}, 2 subtotal) combined with IAT in an effort to provide pain relief and prevent diabetes. After resection islets were immediately isolated using collagenase distension combined with a semi-automated pancreas digestion. Where possible (70%) islets were also purified on a density gradient. These patients were compared with 10 further patients who also underwent TP without IAT. Attempts at islet isolation in 3 of these patients failed because of severe pancreatic fibrosis and calcification.

In 20 patients islets were embolized directly into the liver via the portal vein (PV), 3 received combined splenic and PV autografts and 2 splenic alone (total median volume 10ml). The median number of islets transplanted was 1820 IEQ/kg (range 320-9240). Portal pressure was transiently raised during the transplant procedure . One patient died 4 weeks post-operatively after a CVA.

Post-operative islet autograft function was demonstrated in all patients by elevated serum C-peptide levels. Eight patients have developed transient insulin independence (range 2 days to 3 years) and 3 patients are currently insulin independent (2 > 1year). Despite the need for exogenous insulin IAT recipients have significantly lower HBA1c's (median 7.4 % versus 9.8%)and daily insulin requirements compared to patients having TP alone. The majority are also completely free of pain (80%).

In conclusion TP when combined with and IAT can be a safe procedure with acceptable rates of pain relief . Compared to patients having TP alone, IAT recipients have significantly lower daily insulin requirements and HbA1c's. Whether the effect of good pain relief at the expense of diabetes will improve patients' quality of life, in preference to lower rates of pain relief with insulin independence offered by a lesser, subtotal resectional procedure will need further evaluation.

C39

SPOUSAL KIDNEY DONATION AND TRANSPLANTATION – A SINGLE CENTRE'S EXPERIENCE

Dudley C, Hamilton K, Mathieson P, Harper S, Tomson C, Feest T, Lear P.

Richard Bright Renal Unit, Southmead Hospital, Westbury-on-Trym, Bristol BS10 5NB

Because of the shortage of cadaveric donor kidneys, we have for a number of years pursued an active programme of transplantation using living donors. Since 1995 we have performed 46 living kidney transplants of which 9 (20%) have been from spousal donors. The details of these nine transplants are provided below.

Age	Sex	Matching grade	Immuno-suppression[a]	AR[b]	Current creatinine umol/l	GFR[c] mls/min	Length of follow up (weeks)
61	M	2-2-0	(P) C A	0	151	48	259
46	F	0-2-1	P C (A) (ATG)	0	104	50	220
54	M	1-2-1	P C (ATG)	1	157	52	145
54	M	2-1-1	P T	0	166	61	109
52	M	1-1-1	P C	2	172	46	93
55	F	1-2-2	P T	0	112	53	92
42	F	2-2-2	P T (M) (ATG)	1	122	63	70
37	F	0-1-1	P (C) T	0	91	61	18
41	F	1-1-1	P T	1	150	46	2

[a] Immunosuppression: P=prednisolone, C=cyclosporin, A=azathioprine, T=tacrolimus, M=mycophenolate, ATG=anti-thymocyte globulin. ( )=drug now discontinued
[b] Number of acute rejection episodes
[c] Creatinine clearance using Cockroft and Gault formula

Over this same period a total of 43 potential spousal kidney donors who were blood group compatible with the recipient were assessed according to a protocol. Thirty-one (70%) spouses have not proceeded to donor nephrectomy for a variety of reasons:

REASON FOR EXCLUSION FROM DONOR NEPHRECTOMY	
Positive cross-match	10
Recipient received cadaveric transplant during donor work up	5
Another better matched living donor	4
Medical contraindication	5
Other	7

One donor has been successfully assessed and has a date for nephrectomy. Two donors are currently undergoing assessment.

Assessment of the spousal donor is time-consuming and demanding. However, the outcome both in terms of donor, recipient and graft survival is excellent as is the quality of graft function. In addition, spousal donors may obtain greater psychological and social benefit from kidney donation than living-related donors.

C45

NEUROLOGICAL COMPLICATIONS FOLLOWING LUNG TRANSPLANTATION

Sudarshan CD, Crackett R, Corris PA, Dark JH.

Department of Cardiopulmonary Transplantation, Freeman Hospital, Newcastle upon Tyne NE7 7DN.

273 Lung and Heart-Lung Transplantations have been carried out in 269 patients at a regional Cardiopulmonary Transplantation Centre in the United Kingdom between July 1987 and September 1999. 51 neurological complications developed in 44 patients following transplantation (16.35%). 133 patients received Cyclosporin A (Cy A) and 87 received Neoral (Neo) as one of the three immunosuppressants for at least a month following transplantation. 15 patients in the Cy A group (11.3%) and 29 in the Neo group (33.3%) developed neurological complications (p=0.002). Of the 16 who developed seizures 8 had Cy A (6.0%), 8 had Neo (9.2%, p=0.31). The details are as follows:

Complication	No.	Predisposing Factor/s
Epileptiform seizures	16	Large rises in Cyclosporin levels - 10
Intractable Headaches/Migraine	12	Cyclosporin - 3, Hypertension - 1
Dystonic reactions	2	Cyclosporin - 1, Metoclopramide - 1
Intracerebral bleed	3	Hypertension -1, Thrombocytopenia - 1
Prolonged confusion	3	Multi-organ failure (MOF) - 3
Peripheral sensorimotor neuropathy	6	MOF - 3
Transient motor weakness	3	
Encephalopathy	1	Cyclosporin
Common peroneal neuropathy	1	Prolonged bed rest due to MOF
Acute confusional state	2	Cyclosporin - 1
Transient blurring of vision	1	
Intra cerebral Abscess	1	

3 in the Neo group who had seizures underwent cranial MRI scanning. These demonstrated multiple areas of non-enhancing increased T2 signal, predominantly affecting the white matter and in a patient who serial scans there was resolution of these lesions achieved by reduction in the dose of Neo thereby aiming for lower trough levels. This was compatible with posterior leukoencephalopathy syndrome, a condition that is associated with hypertension and immunosuppressive therapy. Another patient had to be ventilated following the seizures and eventually died of multi-organ failure. 4 patients (2 with intra cerebral bleeds, 2 due to multi-organ failure not related to their neurological complication) died, 1 has persistent left sided weakness, 2 are on long term anti-convulsants and 2 have mild persisting neuropathy.

Epileptic seizures following transplantation seem to occur in the younger age group (mean +/- SEM 25.3 +/- 4.0 years vs. 38.6 +/- 0.95 years; p=0.03), within 2 months of transplantation, are related to a combination of high Cyclosporin trough levels (either CyA or Neo), low Magnesium levels, hypertension and recent augmentation with intravenous steroids for acute rejection. It is commoner in patients treated with Neo although the difference is not significant. Neurological complications following Lung transplantation are not uncommon and seem to be commoner in the Neo treated group. Long term sequelae however are few.

C51

THE SELECTION OF AN ACCIDENT & EMERGENCY DEPARTMENT FOR A NON HEART BEATING KIDNEY DONATION PROGRAMME

Buckley P E

Freeman Hospital, Newcastle upon Tyne

The selection of an appropriate Accident & Emergency (A&E) department is paramount if a non heart beating donation (NHBD) programme is to succeed. Whilst factors such as support of the staff, location in respect of distance from the transplant team base, availability of space within the unit to perform the NHBD procedures are clearly a requirement, the numbers of potential donors in the department must be assessed to ensure that the effort will be worthwhile and to review the subsequent workload for the transplant team. It has been speculated that an A & E department should be selected by its numbers of new patients per annum as this will indicate the numbers of deaths and potential donors. To assess the potential from the A & E unit chosen for our NHBD programme an audit of new patients per annum, deaths in the department by age group, day and time of death were performed for the first 6 months of 1999. Comparison was made with data from 1995. Suitability as a potential donor was assessed as - aged 18-60, no medical contra-indications to kidney donation and total WIT <30 mins.
Method - Data was obtained from the A & E treatment cards in respect of day, date and time of death and age of all deceased patients.
Results - Table 1. There was a 16% increase in admissions to the department between 1995 and 1999. However, the number of deaths have increased by 75%. Forty four potential donors were identified in 1999 as opposed to 16 in 1995.

Table 1. Deaths in the Department and Potential Donors

	1995	1999*
Number of new patients in department	52,105	60,600
Number of deaths in department	161	282
Number of deceased aged 18-60	26	68
Number of potential donors	16	44

* Data from 1999 calculated from 6 months (01/01/99 - 30/06/99)

The pattern of deaths in 1999 in respect of day and time is described in Table 2.

Table 2. Day and Time of Death

n (%)	Mon-Fri 0000-0759	Mon-Fri 0800-1759	Mon-Fri 1800-2359	Sat/Sun 0000-0759	Sat/Sun 0800-1759	Sat/Sun 1800-2400	N/K
All deaths = 141 (100)	21 (15)	43 (31)	25 (18)	6 (4)	26 (18)	11 (8)	9 (6)
18-60 years 34 (100)	7 (21)	11 (32)	8 (24)	1 (3)	3 (9)	1 (3)	3 (9)

67% of deaths in the 18-60 age group occurred outside the Monday-Friday, 0800-1759 time period. This would equate to 30 donors per annum in on-call duty periods.
Comments - The department selected has adequate numbers of potential donors. The increase in deaths in the department was due to the centralisation of all medical admissions on to this hospital site. We would recommend that those wishing to establish and A & E bases NHBD programme should perform a comprehensive audit of deaths in the department to ascertain the numbers of potential donors.

C65

USE OF LIVER GRAFTS FROM DONOS WITH BACTERIAL MENINGITIS

Satoi S, Solomon M, Bramhall S, Gunson B, Buckels JAC, Candinas D, Mayer AD, McMaster P, Mirza DF

Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, United Kingdom

Introduction: Bacterial meningitis is an infrequent cause of brainstem death. Use of cadaveric donors with bacterial meningitis may be associated with an increased risk of sepsis. We audited the results of liver transplantation (LT) from 27 such donors between 1982-1999.

Methods. During this period, 27 of 1790 livers were retrieved from donors with bacterial meningitis: meningococcal (n=13), haemophilus influenzae (n=1), pneumococcal (n=3) and unknown (n= 10)} and 28 OLT (9 males, 15 adults) were performed including 1 split LT. The indications were PBC (n=8), biliary atresia (n=6), fulminant hepatic failure (n=5), retransplantation (n=4), malignancy (n=3), familial cholestasis (n=1), α1 antitrypsin deficiency (n=1). LT was elective in 21 patients and emergency LT in seven. All donors received adequate antimicrobial therapy before organ procurement and all recipients received prolonged postoperative broad spectrum antibiotics. The mean post-transplant follow-up was 36 ± 7 months (range: 0-106). The data was expressed as mean ± SEM.

Results. No infectious complications caused by the meningeal pathogens were observed. Peak AST and bilirubin levels on post-operative days 1-5 were 2170 ± 614 IU/L (range: 210-9592, 95% CI: 884-3455) and 160 ± 22 μmol/L (range: 21-364, 95% CI: 114-206) respectively. The mean ITU stay was 4 days (range: 1-15). There were 9 deaths (5/21 elective patients and 4/7 emergency, 4/15 adults and 5/13 children). The causes of death were multiple organ failure (n=3, 2/3 emergency LT), haemorrhage (n=2), metastatic cancer (n=2), chronic rejection (n=1; emergency LT) and cerebral (n=1; emergency LT). Two patients were re-transplanted and are still alive. Overall actual patient survival rate was 68 % (19/28). The survival rate in elective cases (76 %, 16/21) was significantly better than emergency cases (43 %, 3/7, $p<0.05$, log-rank test).

Conclusion. Liver transplantation from donors with bacterial meningitis is a safe procedure provided both donors and recipients receive adequate antimicrobial therapy.

C69

SELENIUM DEFICIENCY: AN IMPORTANT RISK FACTOR FOR THE DEVELOPMENT OF CHRONIC GRAFT NEPHROPATHY ?

G.J. Morris-Stiff, S. Smith[1], I. Quiroga, H. Stockdill, G.Jones, A. Janezic, R Lord, C. Darby, D. Oleesky[1], W.A. Jurewicz.

Welsh Transplantation Research Group, Department of Surgery, and Department of Biochemistry[1], University of Wales College of Medicine, Cardiff, CF4 4XN.

AIMS: : Lipid peroxidation by free radicals is a key step in the development of atherosclerosis. Chronic graft nephropathy (CGN) is a common cause of allograft failure and shares many histological features with atherosclerosis. While hyperlipidaemia is a common finding in renal transplant recipients, not all patients develop CGN. We hypothesise that the degree of damage sustained is related to recipient antioxidant status and only those who are antioxidant deficient succumb to free radical attack and develop CGN. The aim of this study was to determine the antioxidant profiles of patients with biopsy-proven CGN and to compare their profiles to transplant patients with good renal function.

METHODS: Plasma selenium and Vitamin A and E concentrations were measured in 10 patients with biopsy-proven CGN and 10 contemporaneous, sex-matched patients with normal renal graft function, who received identical cyclosporin-based immunosuppressive therapy. Plasma selenium was measured by electrothermal graphite furnace atomic absorption and concentrations of vitamins A and E were determined by high performance liquid chromatography. Data regarding immunological and non-immunological risk factors was gathered from a prospectively maintained database.

RESULTS:

	Controls (n=10)	CGN (n=10)	Range
Selenium (μmol/l)	0.94 ± 0.23 (0.47-1.22)*	0.67 ± 0.17 (0.33-0.92)	0.8-1.4
Vitamin A (μmol/l)	2.52 ± 0.86(1.61-4.39)	3.70 ± 1.09 (1.28-4.94)	1.1-2.6
Vitamin E (μmol/l)	22.44 ± 4.72 (15.9-29.47)	28.39 ± 3.88 (16.6-38.8)	11-47

* $p<0.05$ Student's paired *t* test

There was no difference in the prevalence of any of the immunological or non-immunological risk factors.

CONCLUSIONS: Patients with CGN have evidence of selenium deficiency, suggesting that impaired antioxidant status may contribute to the development of CGN. Further studies are in progress to determine whether selenium deficiencies at the time of transplantation predict those individuals who will subsequently develop CGN.

C73

EFFECT OF RACE ON TRANSPLANT OUTCOME AND LIKELIHOOD OF KIDNEY ALLOCATION.

PJ Sinnott[1], A Gupta[1], MJ Raftery[2] and M Yaqoob[2]

[1]Tissue Typing Laboratory and [2]Department of Renal Medicine and Transplantation, Barts and the London NHS Trust, London E1 1BB

The demographics and ethnic diversity of the population served by this unit are unique in the United Kingdom. We have attempted to determine differences in transplant outcome in Caucasoid (C) and non Caucasoid (NC) patients.

One hundred and eighty-nine patients who had received their first transplant during the last five years were analysed. We included waiting time (from entry onto waiting list to date of transplant), the *match prognosis index* (MPI) and ABO blood group as pre transplant indicators. Post transplantation, the mismatches for HLA-A, -B (-Cw) and -DR (-DQ) as per the UKTSSA organ sharing scheme and biopsy proven rejection episodes in the first year were recorded. The patients were divided into Caucasoid (n=114) and non Caucasoid (n=67) groups which included north European and Mediterranean Caucasoids in the former and Africans, Caribbean blacks, Indo-Asians (Indian, Pakistani and Bangladeshi) in the latter. The two ethnic cohorts were compared using chi squared analyses.

Both groups were comparable with regard to age, gender distribution and waiting time on the transplant list (mean waiting C vs. NC, 35 vs. 32 months $p=ns$). The number of mismatches at the HLA-B, -C and -DR loci were similar in the two groups but the there were significantly more mismatches at the HLA-A and -DQ loci in the NC group compared to C $p<0.01$. This is reflected in a mean MPI score of 3.9 in NC and 5.5 in C group. Despite apparent poorer matching at these loci, total number of rejections were significantly decreased in the NC compared to C group in the first twelve months post transplantation (31/55 vs. 67/99 $p=0.02$). This significant difference could be explained due to fewer patients having 2 rejections in the NC compared to C group (2/55 vs. 16/99 $p=0.03$). Interestingly our current waiting list of 332 patients includes only 138 Caucasoids suggesting an increased likelihood of transplantation in this group.

We therefore conclude that despite apparent poorer HLA matching, NC patients had fewer rejection episodes than C patients. It also suggests that the current UK matching system, results in reduced transplantation rates in NC group.

ANALYSIS OF CYTOKINE PRODUCING T CELLS FOLLOWING RENAL TRANSPLANTATION MAY PREDICT CHRONIC REJECTION

Aurelia Zamauskaite [*1], Shara Cohen[*2], Alejandro Madrigal [*2], Paul Sweny [*1], Zac Varghese[*1], Adam McLean[*1] Stephen H Powis[*1]. [*1]Centre for Nephrology, Royal Free & University College Medical School, London UK; [*2] The Anthony Nolan Research Institute, The Royal Free Hospital, Pond Street, Hampstead, London, NW3 2QG. [*2]The Department of Haematology, The Royal Free Hospital, Pond Street, London, NW3 2QG.

Chronic rejection remains the major cause of graft failure following renal transplantation. The aim of this study was to investigate whether changes in the pattern of cytokine production might allow prediction of patients at risk of developing chronic rejection . We studied the intracellular cytokine profile of peripheral blood T (CD3+) cells from 31 patients with biopsy proven chronic rejection and 26 patients with well functioning grafts. We also analysed 10 healthy individuals as normal controls. Cytokine profiles were determined before and after 4 hours stimulation with mitogen phorbol-12-myristate-13-acetate and ionomycin, in the presence of monensin. We observed that IL-2, IFNg, and TNFa were increased following mitogen stimulation in all groups analysed. When we compared healthy controls and recipients with chronic graft rejection (Tx-chr) we found a difference in expression of the Th2 cytokines IL-4 after stimulation with mitogen (controls 3.08±1.21; Tx-chr 15.48±14.49; p=0.011; all values mean±SD), and in Th1 cytokines TNFa after stimulation (10.03±4.90; 18.02±12.06; p=0.049), IFNg after stimulation (13.53±3.93; 22.45±11.57; p=0.022) When we compared healthy controls and patients with well functioning grafts (Tx-N) we found a significance difference in IL-4 production before (controls 1.72±1.31; Tx-N 8.13±8.97±7.71; p=0.014) and after stimulation (controls 3.08±1.21; Tx-N 14.92±13.81; p=0.011); and in TNF-a after stimulation (controls 10.03±4.90; Tx-N 21.32±15.65; p=0.033). When we compared the two transplant groups we found a difference in IL-10 expression before stimulation (Tx-N 0.30±0.23; Tx-chr 2.75±3.96; p=0.003). These preliminary results suggest that renal transplant patients exhibit changes in cytokine profiles which may be used as non-invasive monitoring of the immune response and might help to predict chronic rejection.

LABORATORY

IS QUANTITATIVE MEASUREMENT OF INTRA-GRAFT PERFORIN AND GRANZYME B mRNA TRANSCRIPTS OF VALUE IN DIAGNOSIS OF ACUTE RENAL ALLOGRAFT REJECTION?

Khanna R, Bukilica M, Janezic A, Jones G, Morris-Stiff G, Stockdill H, Baboolal K, Jurewicz WA.

Welsh Transplant Research Group, Department of Surgery, University of Wales College of Medicine, Cardiff CF14 4XN

INTRODUCTION

One of the mechanisms proposed for T cell mediated acute renal allograft rejection is release of perforin granules in the inter-cellular cleft between T cell and target cell. Perforin polymerises and inserts into target cell membrane forming pores, which permit ion flux and entry of granzyme B that activates the apoptotic machinery via the interleukin 1B converting enzymes. Reports in the literature on the value of intra- graft perforin and granzyme B mRNA detection for the diagnosis of acute renal allograft rejection are conflicting.

AIM

The newly developed technology of SYBR green based real time quantitative PCR was used to investigate the intra-graft mRNA expression of perforin and granzyme B molecules and correlate it with the histological Banff scores.

METHODOLOGY

Needle biopsies were obtained from 37 renal allografts whenever indicated due to graft dysfunction. The mRNA was extracted, reverse transcribed to cDNA which was subjected to real time quantitative PCR for perforin and granzyme B. The biopsies were allocated a Banff score as per histological findings.

RESULTS

A wide range of cDNA copy numbers for perforin and granzyme B were obtained by quantitative PCR.(0-70,000 copies of cDNA / 25 ng. of mRNA). The positivity rates of the genes as per Banff score are tabled below.

	Banff Score		
	1 (Normal)	4(Acute rejection)	3(Borderline)
Granzyme B & perforin negative	75%	25%	38%
Either or both genes positive	25%	75%	62%

The combined sensitivity and specificity of presence of perforin and / or granzyme B to diagnose a histologically proven borderline or acute rejection episode was 67% and 75% respectively. The predictive value of a positive and negative test for both genes in combination was 78% and 63% respectively.

CONCLUSIONS

We found that granzyme B and perforin appeared to be playing a role in 75% of histologically diagnosed acutely rejecting kidneys and 62% of borderline cases. The absolute copy numbers of the genes showed little correlation with the histological severity of the rejection process. The involvement of other T cell mediated mechanisms including Fas L activation are currently under investigation.

Laboratory

THE ROLE OF NITRIC OXIDE IN SURVIVAL OF MOUSE CARDIAC ALLOGRAFTS

Smith XG, Bolton EM, *Wei X-Q, *Liew FY, Frick TW, Bradley JA.

University of Cambridge Department of Surgery, Box 202, Addenbrooke's Hospital, Cambridge CB2 2QQ; *University of Glasgow Department of Immunology, Western Infirmary, Glasgow G11 6NT.

Nitric oxide (NO) has a range of biological effects, including potentially opposing roles as an immune effector or immunoregulatory molecule. During allograft rejection, cytokine-dependent upregulation of inducible nitric oxide synthase (iNOS or NOS2) leads to increased NO production, but because of its biological diversity the influence of iNOS on graft survival is difficult to predict. We have previously found that iNOS-deficient mice showed an enhanced in vitro proliferative response to alloantigen and increased Th1 cytokine production, but no net influence on the kinetics of skin allograft rejection.

In this study we have used recipient 129 ($H\text{-}2^b$) mice homozygous for the disrupted iNOS gene to determine the role of iNOS in cardiac allograft survival. iNOS-deficient 129 ($H\text{-}2^b$) mice received vascularised heterotopic cardiac allografts from minor HC-mismatched, C57BL/10 ($H\text{-}2^b$) mice or from MHC-mismatched, BALB/c ($H\text{-}2^d$) mice.

Allograft survival was prolonged in minor HC-mismatched, iNOS-/- recipients (MST 15 days vs 7d in iNOS+/- controls) but was the same in MHC-mismatched iNOS+/- and -/- mice (MST 7d). Treatment with anti-CD4 plus anti-CD8 depleting mAbs resulted in prolonged survival (MST 40d) which was not NO-dependent, while combined anti-CD8 plus anti-TNF-α mAbs failed to extend graft survival, suggesting that NO does not influence the contribution of DTH to acute allograft rejection. Administration of 3×10^6 donor lymphocytes at the time of transplant, together with a single dose of non-depleting anti-CD4 moderately prolonged survival of MHC-mismatched heart grafts in iNOS+/- (MST 20d) but not iNOS-/- mice (MST 8d).

These results suggest that NO is involved in rejection of mHC-mismatched cardiac allografts but its role in MHC-mismatched, acute graft rejection is eclipsed by other effector mechanisms. They also suggest that immunomodulation by treatment with donor MHC antigen appears to involve NO.

LABORATORY

POST-TRANSPLANT CYTOMEGALOVIRUS DISEASE AND THE LEVELS OF TUMOUR NECROSIS FACTOR ALPHA AND INTERLEUKIN 6

A. Bakran[1], C. Y. W. Tong[2], H. Williams[2], L. Cuevas[3], M. Peiris[4], C. A. Hart[2]
Renal Transplant Unit[1] and Department of Medical Microbiology[2], Royal Liverpool University Hospital, Statistics and Epidemiology Unit[3], Liverpool School of Tropical Medicine, Liverpool, U.K. and Department of Microbiology[4], University of Hong Kong, Hong Kong.

Background. Cytokine production has been implicated as being important in the reactivation and pathogenesis of post-transplant cytomegalovirus (CMV) disease. CMV infection increases the production of tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). At the same time, TNF-α is known to stimulate the replication of CMV. High level IL-6 expression has been demonstrated in CMV-infected lungs. IL-6 level has recently been found to be independently associated with CMV disease after bone marrow transplantation.
Aims. To study the relationship of TNF-α and IL-6 levels with CMV disease after renal transplantation.
Methods. The plasma levels of TNF-α and IL-6 were studied using weekly samples collected from 34 prospectively recruited renal transplant recipients during the first 12 weeks after transplantation. The patients were matched and divided into three groups: group A - patients with CMV disease (n = 12); group B - patients with CMV DNA detection but no CMV disease (n = 11); group C - patients with no detectable CMV activity (n = 11).
Results. The peak TNF-α level in group A patients were significantly higher than that in group B ($p < 0.01$) or group C ($p < 0.02$). A significantly higher proportion of group A patients had a TNF-α level of above 100 pg/ml ($p< 0.01$) compared to those in group B and group C. The peak IL-6 levels in group A and group B patients were significantly higher than those in group C (group A vs group C: $p < 0.04$; group B vs group C: $p < 0.03$). None of the patients in group C had IL-6 levels above 15 pg/ml whereas 25% and 18.2% of patients in group A and B respectively had this level ($p = 0.04$). A TNF-α level of above 100 pg/ml was significantly associated with CMV disease and high plasma CMV loads, but not with other confounding factors including HHV-6 and -7 DNA detection or rejection episodes. An IL-6 level of above 15 pg/ml was significantly associated only with CMV DNA detection but not with CMV disease, plasma CMV loads, HHV-6 and -7 activities or rejection episodes.
Conclusions. A high TNF-α level is significantly associated with CMV disease and high plasma CMV loads. TNF-α levels may be useful as a non-specific predictor of CMV disease. A high IL-6 level is significantly associated with the presence of CMV DNA. However, unlike TNF-α, it is not associated with CMV disease or CMV loads.

THE ASSOCIATION OF HUMAN HERPESVIRUSES 6 AND 7 WITH CYTOMEGALOVIRUS DISEASE AFTER RENAL TRANSPLANTATION

C. Y. W. Tong[1], A. Bakran[2], H. Williams[1], C. Y. Cheung[3], M. Peiris[3]. Department of Virology[1] and Renal Transplant Unit[2], Royal Liverpool University Hospital, Liverpool, U.K. and Department of Microbiology[3], University of Hong Kong, Hong Kong.

Background. Human herpesvirus 6 (HHV-6) and 7 (HHV-7) together with cytomegalovirus (CMV) are members of the Betaherpesvirus subfamily. Activities of all three viruses are frequently detected after solid organ transplantation. CMV disease is established as a major cause of infective complication after transplantation. Both HHV-6 and HHV-7 have also been linked with the occurrence of post-transplant complications. Two recent studies have shown that serological response to HHV-6 is associated with CMV disease after liver and kidney transplantation.
Aims. To study the association of HHV-6 and 7 activities with CMV disease after renal transplantation.
Methods. 37 renal transplant recipients were monitored weekly for CMV, HHV-6 and 7 activities using serology and the polymerase chain reaction (PCR) for 12 weeks after renal transplantation. The occurrence of CMV disease was related to viral activities. Univariate and multivariate analyses were carried out to identify factors significantly associated with CMV disease.
Results. CMV disease was identified in 12 patients (32.4%). One patient had a viral syndrome associated exclusively with HHV-6 variant A. Univariate analyses showed that pre-transplant CMV serostatus of the recipients, HHV-6 and 7 serological responses and HHV-7 DNA detection were all associated with CMV disease. However, only the detection of HHV-7 DNA and CMV serostatus of the recipients were found to be independently associated with CMV disease in a logistic regression analysis. Patients with detectable HHV-7 DNA also had significantly higher peak plasma CMV loads ($p = 0.01$).
Conclusions. HHV-6 could be responsible for a viral syndrome that resembles CMV disease. HHV-7 DNA detection is significantly associated with CMV disease. The action of HHV-7 may be effected via an increase in CMV load.

AN IMPROVED SOLUTION FOR LIVER PRESERVATION IN ISOLATED PERFUSED LIVER MODEL.

Ahmed I, Song S B, Attia M S, Corps C L, Potts D J, Lodge J P A.

Department of Organ Transplantation, St James's University Hospital Leeds; School of Biomedical Sciences, University of Leeds.

There remains a need for an improved flush preservation solution for multi-organ use. University of Wisconsin solution (UW) is the present gold standard but is not ideal in all situations. Phosphate buffered sucrose (PBS 140) is a clinically proven preservation solution for kidney. We now report that a modification of this solution (PBSI) is very effective for liver preservation as judged by an isolated perfused liver model.

Methods: Rats were anaesthetized, a laparotomy carried out and the bile duct cannulated with a fine (0.28 mm internal diameter) cannula. The liver was flushed with either cold UW or PBSI via the aorta and portal vein.. After flush the liver was removed and stored in 60 ml of the same preservation solution at a temperature below 4°C for 24 hours. The liver was then re-perfused at 37° C at a rate of 15 ml/min. Observations were made of gross appearance of the liver, weight change after storage and re-perfusion, bile flow, oxygen consumption, liver enzymes (ALT, ADH, LDH) and pressure in the portal vein throughout the experiment.

Results: Following preservation with PBSI gross appearance of the liver was better, liver weight gain was lower and bile flow was superior. Liver enzyme release was lower suggesting less cell damage during preservation with PBSI. Oxygen consumption was slightly higher and pressure dropped slightly in both the groups but this difference was not significant.

Parameter	PBSI	UW
Bile flow (µL/min/g)	0.72 ± 0.03	0.38 ± 0.05*
Oxygen consumption (µmoles/min/g)	0.71 ± 0.04	0.67 ± 0.03
LDH release (U/L)	411 ± 41.1	612 ± 58.2*
ALT release (U/L)	42.9 ± 2.15	62.11 ± 6.92*
ADH release (U/L)	39.8 ± 3.67	68.2 ± 5.67*
Pressure in portal vein (kPa)	3.2 ± 0.05	3.1 ± 0.05
Weight change after storage (%)	-2.97 ± 0.12	-3.56 ± 0.89
Weight change after re-perfusion (%)	0.09 ± 1.43	4.49 ± 0.94*

*$P<0.05$: *Values are expressed as means ± SEM.*

Conclusion: PBSI provided better preservation than UW solution in this experimental model.

I Ahmed is supported by the Royal College of Surgeons of England

Laboratory

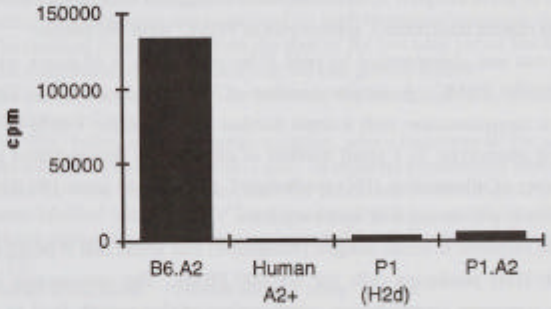
RAPID REJECTION OF HLA-A2 TRANSGENIC SKIN GRAFT DUE TO INDIRECT ALLORECOGNITION

Jurcevic S., *Chandler P., Sacks S H. and *Simpson E.

Department of Nephrology & Transplantation, Guy's Hospital, Guy's, King's and St Thomas' Medical School, King's College, London
*MRC Clinical Sciences Centre, ICSM Hammersmith Hospital, London

Skin transplanted from HLA-A2 transgenic mice (B6.A2) to B6 littermates was rejected rapidly, in 12-14 days. While naive B6 mice did not respond to B6.A2 splenocytes in vitro, transplanted mice showed strong proliferative responses. A B6vsB6.A2 T cell line from transplanted mice made proliferative responses to B6.A2 splenocytes, but did not recognise HLA-A2 on human cells or transfected allogeneic mouse cells P1.A2 (Figure 1).

Figure 1



The indirect, self-MHC restricted recognition of HLA-A2 implied by this was confirmed by finding that lysates of HLA-A2 positive, but not HLA-A2 negative human B cells were stimulatory, and by inhibition of B6vsB6.A2 proliferation with anti-mouse MHC class I and anti-MHC class II antibodies. In conclusion, indirect recognition of xenogeneic MHC antigen results in rapid graft rejection.

LABORATORY

CHARACTERISATION OF THE ALLORESPONSE FOLLOWING HUMAN RENAL TRANSPLANTATION USING THE ELISPOT ASSAY.

Farmer CKT, Monk NJ, Sacks SH and Jurcevic S.

Department of Nephrology and Transplantation, Guy's Hospital, Guy's, King's and St. Thomas' School of Medicine, King's College, London

Many methods have been employed in an attempt to assess the risk of rejection in renal transplant recipients, including, limiting dilution assay (LDA), proliferation assays and staining for intracellular cytokines. None of these methods have proved applicable in clinical transplantation.

We have developed an ELISPOT assay to detect alloreactive IFNγ and IL-2 producing T-cells. ELISPOT is more sensitive than ELISA and using T cell clones we have shown that it can detect virtually every single cytokine producing T cell. PBMCs from normal volunteers and renal transplant recipients, both prior to transplantation and during episodes of acute allograft dysfunction, were stimulated with donor PBMC (in the case of living related transplants), splenocytes or PBMC from 3rd parties.

The naive alloresponse was characterised by peak IFNγ production at 48 hours with 5-15 spots per 100,000 PBMC. A similar number of cells produced IL-2. This suggests that prior to transplantation, only a small number of alloreactive T cells have a cytokine producing phenotype. In a small number of patients, we demonstrated an increase in the number of alloreactive IFNγ producing T-cells (30-40 spots/100,000) in the presence of biopsy proven acute allograft rejection.

We also analysed the response to recall antigen (tuberculin) and found that it peaks at 24 hours with 40-60 IFNγ producing cells per 100,000 PBMC. The introduction of immunosuppressive treatment (prednisolone, azathioprine, cyclosporine), lead to a decrease in the number of T cells responding to tuberculin (<20 spots per 100,000) and in the small number of patients on the high dose of tacrolimus or prednisolone, this number was further reduced (0-5 spots per 100,000).

In conclusion, we have shown that prior to transplantation only a small number of alloreactive T cells (5-15 per 100,000 PBMC) produce cytokines which is in striking contrast to the estimated number of alloreactive T cells (1-10%) able to proliferate in MLR.

LABORATORY

RAPID CLOTTING AND ISLET DAMAGE OBSERVED WHEN MOUSE ISLETS ARE INCUBATED IN HUMAN BLOOD

Titus, T., Badet, L., Handa, A., Horton, P., Chang, L-W., Gray D.W.R.

Nuffield Department of Surgery, John Radcliffe, University of Oxford

The mouse has many attractions as a donor for experimental islet xenotransplantation, because of the availability of transgenic and knockout lines. Mouse pancreatic islets transplanted beneath the kidney capsule of cynomolgus macaques are usually rapidly destroyed with a neutrophil infiltration seen on histology. However, the mechanism of this early destruction remains unclear. Previous attempts to reproduce the destruction in vitro by use of human serum, complement and neutrophils failed to produce consistent changes in islet viability. A recent report has described damage to porcine islet xenografts associated with accelerated clotting when freshly isolated islets are exposed to fresh human blood. We sought to ascertain whether the phenomenon was measurable using thromboelastography (TEG), a measure of clotting in common clinical practice which evaluates the viscoelastic properties of whole blood clot formation.

METHODS

Freshly isolated mouse islets (C57 Bl6, 150 IEQ) were incubated in human citrated/recalcified blood and TEG performed. The result was compared to TEG on incubation of blood alone as negative controls.

Four standard measurements were analysed on each thromboelastograph tracing:

- The reaction time R, time from the start of the test until initial fibrin formation
- The coagulation time K identifying the clot growth kinetic
- The maximum amplitude MA measuring the strength of the clot (interaction of platelet and fibrinogen)
- The TEG index, a mathematical equation, which combines all the parameters.

The results were analysed by a paired-T test.. In separate experiments islets were incubated with human blood for 4 hrs then formalin fixed and processed for histological examination. Further experiments labelled islets with Cr^{51} and incubated with human blood and mouse blood as control. Human blood mediated cytotoxicity was evaluated by Cr^{51} release assays of the supernatant.

RESULTS:

N=15	Human Blood alone	Human Blood + islets	p value
R	11.73	7.08	<0.001
K	5.73	4.38	<0.001
MA	56.5	58.7	<0.001
Teg	1.61	2.32	<0.001

The measurement of human blood cytotoxicity to islets was partly obscured by retention in the clot. Nevertheless specific lysis of the xenogeneic islets was demonstrable by increased release of Cr into the supernatant (Mean = 48.2 % $p<0.05$) after 4 hours incubation in human blood. Histological examination confirmed striking necrosis with neutrophil infiltration.

CONCLUSION

: Mouse islets incubated in human blood induce rapid activation of the clotting cascade which is followed within 4 hours by approximately 50% lysis of islets. This in vitro model could be used to study the mechanism of early destruction of xenogenic islets in primate recipients and ways of circumventing it.

THE ALLOGENEIC B CELL AND T CELL RESPONSE IS STRONGLY DEPENDENT ON COMPLEMENT

Marsh JE, Farmer CTK, Jurcevic S and Sacks SH,

Department of Nephrology and Transplantation, Guy's Hospital, King's College, London, UK

Complement augments humoral responses to threshold doses of T-dependent antigens, but debate surrounds its potential physiological significance as it has been suggested that increasing the antigen dose can overcome any effect of complement deficiency. We have investigated the B and T cell responses to tissue antigen in the form of a skin graft.

Following skin grafting, the production of alloantibodies in complement-deficient mice was compared (n=12-15 in each group) by two colour flow cytometry. In addition, the activation of recipient T cells was assessed by mixed lymphocyte culture and ELISPOT assays for IL-2 and interferon-γ producing T cells exposed to irradiated donor-strain splenocytes.

Mice deficient in either complement components C3 or C4 demonstrated a marked impairment of allospecific IgG production ($p<0.0001$) and defective ability in producing a range of IgG isotypes other than the non-complement fixing IgG1. In contrast, the IgM response was not impaired. There was a striking defect in memory B cell responses when the mice were exposed to a second skin graft ($p<0.0001$), or primed with donor splenocytes. In contrst, mice deficient in C5 had a normal alloantibody response, and brisk skin graft rejection. Of interest, C3- but not C4-deficient mice failed to demonstrate accelerated rejection to a second skin graft ($p<0.0001$). In addition, T cells from C3-deficient mice demonstrated a profound defect in mixed lymphocyte responses and cytokine production, whereas those from C4 deficient mice were less affected.

In conclusion, even in the presence of a large antigen dose, complement is an important effector of a normal alloantibody response. The major defect is in class switching, and development of memory, and is mediated through the classical pathway, but the terminal pathway is unimportant. There is evidence that complement also plays a role in T-B cell co-operation in alloresponses.

LABORATORY

Free Communications

Thursday 23 March

11.30-13.00

Clinical

C34

CONTRIBUTION OF CYTOMEGALOVIRUS SEROLOGY FOR LONG TERM GRAFT SURVIVAL IN CADAVERIC KIDNEY TRANSPLANTATION

Gerstenkorn C, Di Franco F, Robertson H, Ali S, Kirby JA, Talbot D

Department of Surgery and Transplant Immunobiology Group,
The Medical School, University of Newcastle upon Tyne,
Newcastle upon Tyne NE2 4HH; United Kingdom

Background. Cytomegalovirus (CMV) serology in donors (D) and recipients (R) is widely held to have an impact on graft survival in kidney transplantation. It is controversial however when prophylactic antiviral treatment should be given and whether the CMV serology should be taken into account whilst allocating donated cadaveric kidneys.
Patients and methods. Cadaveric kidney graft survival rates of 3399 patients, who were transplanted in the UK & the Republic of Ireland between January 1987 and 31 December 1993, were analysed regarding their CMV serology status. Transplants were divided into 4 subgroups with respect to the combination of donor and recipient CMV antibody status (D-/R-; D-/R+; D+/R-; D+/R+). The graft survival was estimated according to Kaplan Meier for 1, 3 and 5 years.
Results. The study can confirm a negative impact of CMV positive donor organs for initial graft survival regardless of CMV recipient status within the first 3 years of transplantation. In respect to the longer follow up of 5 years after transplantation the D+/R+ subgroup showed significantly the poorest outcome (60.7%). This trend continued while analysing 7 year follow up results within a single regional centre, whereas the D+/R- subgroup seems to improve compared to the other groups and had a lower graft loss rate once the first 3 years after transplantation had been overcome and its 5 year graft survival of 67.7% was better than that of the D-/R- subgroup with 66.6%.
Conclusions. In contrast with previous published data from the United States, where CMV positive donors in general were identified as higher risk transplants, this study suggests that this general view needs to be reconsidered. Since diagnostic tools and prophylactic as well as therapeutic treatment of active CMV disease have improved, early graft loss due to infection or reactivation of the virus has decreased. This study shows that there is a lack of correlation between a poor outcome and the D+/R- group of renal transplants so that the concept of avoiding transplantation in this combination particularly with the use of prophylactic antiviral treatment should be ignored. Furthermore, it is necessary to improve the long term outcome of the D+/R+ subgroup. These patients are susceptible for CMV reactivation in the posttransplant period and carry the highest viral load, which could account for subsequent graft loss in the long term. The results of this study indicate that the D+/R+ subgroup may also benefit from prophylactic antiviral treatment. If this long term trend continues nationwide efforts possibly could be made to reduce the size of this subgroup having the poorest long term outcome by organ allocation similar to the scheme in place for HLA matching.

C52

FACTORS AFFECTING THE WAITING TIME TO KIDNEY TRANSPLANT IN THE UK

Belger MA, Johnson RJ, Pugh RJ, Briggs JD
on behalf of the UKTSSA Users' Kidney Advisory Group

Characteristics of patients registered on the active UK kidney transplant waiting list between 1 January 1990 and 31 December 1997 were analysed to examine the factors affecting the chance of receiving a kidney transplant. The first registration of all adults and children in this period were included.

The waiting times to transplant were analysed using Cox's proportional hazards regression. The outcome event was transplant, and registration periods ending in removal or death were censored. Waiting times were also censored for those patients still on the list at the time of analysis. Days spent on the suspended waiting list were excluded from the waiting time.

Results of the analysis of 17,000 registrations showed a significantly longer waiting time ($p<0.0001$) for later registrations, adults, blood groups O and B, registrations for regrafts, HLA-DR homozygotes, females and diabetics. These results are illustrated by univariate median waiting times.

Patient factor		Median wait (days)	95% CI	Patient factor		Median wait (days)	95% CI
Registered	1990-1992	401	384-422	Previous grafts	None	407	392-419
	1993-1995	471	446-493		One	1026	922-1157
	1996-1997	554	515-592		Two +	1759	1586-2271
Age	0-17 years	190	174-213	HLA-DR Homozygous		949	872-1046
	18+ years	488	470-505	HLA-DR Heterozygous		407	392-419
Blood Group	O	623	593-656	Gender	Female	507	485-538
	A	301	282-318		Male	429	413-448
	B	857	776-981				
	AB	182	158-216	Diabetic		550	481-621
				Non-diabetic		452	439-468

Median waiting times have increased by five months over the analysis period from 13 months in 1990-1992 to 18 months in 1996-1997. Adult patients waited approximately 16 months, while children waited 6 months on average for a cadaveric kidney transplant. The fact that the number of patients registered each year remained relatively unchanged, while the number of cadaveric solid organ donors dropped (from 924 in 1990 to 823 in 1997) would seem to have caused the significantly longer waiting times in recent years.

C59

PRELIMINARY RESULTS FROM THE SOUTHEASTERN ORGAN PROCUREMENT FOUNDATION (SEOPF) MULTICENTER RANDOMIZED CLINICAL TRIAL COMPARING SANGCYA ORAL SOLUTION AND NEORAL CAPSULES IN DE NOVO ADULT RENAL ALLOGRAFT RECIPIENTS.

McCune T, Light J, Adams P, Peters T, Mendez-Picon G, Zibari G, Pruett T, Yium J, Ham J, Thacker L, Thompson J [Introduced by Pascal Diesel]
Thomas R, McCune, MD, Nephrology Associates of Tidewater, Ltd, 907 Medical Tower, 400 Gresham Drive, Norfolk, VA 23507 USA

This study was designed to compare the safety and efficacy of two modified cyclosporine (CyA) formulations, SangCya (Sang-35) oral solution and Neoral capsules, in a prospective clinical trial of de novo renal allograft recipients to establish therapeutic interchangeability.

Methods: At the time of transplant, patients (pts) receiving first or second renal allografts were randomized to receive SangCya or Neoral. The study was powered on 6-month graft survival to demonstrate that SangCya was therapeutically equivalent to Neoral. Immunosuppressive therapy and clinical care were determined by each center.

Results: 70 pts from 9 centers were enrolled between September 1998 and March 1999 with data from 68 pts available for this analysis (2 enrolled pts were not transplanted). 32 pts were randomized to SangCya and 36 to Neoral. Three pts withdrew from the SangCya and 2 from the Neoral groups (unrelated to study drugs) but all 68 pts were included in this analysis. Patient demographics and clinical outcomes are shown in the table below.

Variable	SangCya	Neoral
N	32	36
Males	18/32 (56%)	15/36 (42%)
Caucasian	22/32 (69%)	16/36 (44%)
Cadaver Donor	21/32 (66%)	23/36 (64%)
Primary Transplant	27/32 (84%)	33/36 (92%)
Age (years)	48±15	49±11
Follow-up (months)	3 ± 1	3 ± 1
Follow-up Range (months)	0-6	0-6
3 month Pt Survival	94%	97%
3 month Graft Survival	91%	97%
Acute Rejection	1/32 (3%)	5/36 (14%)
SAE's	9/32 (28%)	12/36 (33%)

Two pts died: 1 from DIC (Neoral) and 1 from a MI (SangCya). There were 6 biopsy-proven acute rejections, 5 (2 mild, 2 mod, 1 severe) resolved and 1 (mod) led to graft loss (SangCya).

Conclusions: From this analysis of de novo renal allograft recipients, no differences were observed in patient and graft survival rates, incidence of acute rejection episodes, or serious adverse events between SangCya oral solution and Neoral capsules. This suggests that these two CyA formulations are therapeutically equivalent when used for the initiation of CyA therapy at the time of transplant.

C47

THE NEW BANFF CLASSIFICATION OF RENAL TRANSPLANT BIOPSIES: A MAJOR IMPACT ON THE ADEQUACY OF THE CORES TAKEN

Quiroga I, Morris-Stiff G, Baboo R, Griffiths D, Baboolal K, Moore R, Darby C, Lord R, Jurewicz A.

Renal Transplant Unit, University Hospital of Wales. Cardiff, CF4 4XW.

In 1999 an update of the classification of renal transplant biopsies was introduced. (Banff 1993 to Banff 1997). Clinical practice and drug studies use this data as major determinant in treatment and reporting of efficacy. We have reviewed the pathology reports of all the renal transplant biopsies performed in our unit over a period of 33 months and compared the reporting on the basis of the two classifications.
There were a total of 616 biopsies. We recorded the number of passes, glomeruli, arteries and levels. All the biopsies were performed under ultrasound control with an 18-gauge needle by transplant and nephrology registrars and SHOs. Radiographers experienced in renal biopsies did the ultrasound scans. A pathology technician was always present to count by inspection under magnification, the numbers of glomeruli in the specimens obtained.

	Banff 1993	Banff 1997
Unsatisfactory	8.1%	12.7%
Marginal	5.4%	22.2%
Adequate	84.9%	63.5%
Unclassifiable	1.6%	1.6%

The new classification has significantly increased the percentage of unsatisfactory and marginal biopsies. As the results are used for clinical decision-making and study reporting, this is a major problem. We believed our process for obtaining biopsies was near optimal. Our pathology technician previously counted six glomeruli on visual inspection as likely to be adequate, this has been increased to ten glomeruli following this study. However, it seems likely that an increased number of passes of the needle will be required from the current 2.13 per patient, to obtain sufficient renal tissue. Importantly, this will be associated with an increased risk of biopsy related complications.
Hence the change in Banff classification of renal biopsies has had an unforeseen and significant impact on clinical practice.

C55

NATIONAL ALLOCATION OF CADAVERIC KIDNEYS TO CHILDREN IN THE UK

Johnson RJ, Belger MA, Armstrong S, Fuggle SV, Postlethwaite RJ, Morris PJ
on behalf of the UKTSSA Users' Kidney Advisory Group

Children have traditionally been given a degree of priority over adults in access to cadaveric kidneys for transplantation in the UK. This has been strengthened in the new National Kidney Allocation Scheme introduced in July 1998 and further options for benefiting children are under consideration by the UK transplant community.

Between January 1989 and July 1998, children were ensured some access to adult donor kidneys through the national allocation Scheme, as they had priority over adults for 'beneficially' matched grafts (0 mismatches at each of the HLA-A, B and DR loci (000), or a total of one mismatch at either HLA-A (100) or HLA-B (010)). Although kidneys from donors under 18 years were allocated preferentially to children, the unsuitability of the donor (size or HLA match) often resulted in an adult receiving the organ. As a result, in this period, over 4 times as many paediatric organs were used in adults than vice versa.

Since 1 July 1998, children have had increased access to adult organs. At each of the 000 and favourable matchgrades (100, 010 and 110 combined) children receive the first offer. Additionally, if two or more 000 mismatched children are identified for a donor, both kidneys are allocated nationally. This has resulted in a significant improvement in the degree of HLA matching achieved in transplanted children in the 12 months since 1 July 1998 ($p=0.04$). For the year before the Scheme changed, 22% of transplants in children used adult donor kidneys compared with 45% for the year since 1 July 1998 ($p<0.001$).

A further change was made in April 1999 to permit children of blood group B to receive blood group O organs, and children of blood group AB to receive donor kidneys of blood groups A and B. This applies only when the child is well matched with the donor (000 or favourably matched) and should increase the chance of a child receiving a well matched graft.

Further options for increased priority to children are being evaluated through computer simulation. Preliminary results show that making the second kidney from an adult donor available to a favourably matched child could increase the proportion of such grafts in children to 43% with very little effect on adult transplants (0.4% fewer transplants). In the first year of the Scheme 38% of transplanted children received a favourably matched graft.

In conclusion, changes in the prioritisation of children in the National Kidney Allocation Scheme implemented on 1 July 1998 have resulted in significant improvements in the proportion of 000 and favourably matched grafts. However, simulation results suggest that further access to adult organs could still improve the levels of HLA matching with negligible adverse effect on adult patients.

C16

SKIN CANCERS IN RENAL TRANSPLANT RECIPIENTS: A SINGLE-CENTRE, 21-YEAR RETROSPECTIVE ANALYSIS

Bordea C[1], Wojnarowska F[2], Doll H[3], Rodriguez L[4],Welsh K[5], Morris P J[1]

[1]Nuffield Department of Surgery, John Radcliffe Hospital, Oxford
[2]Dermatology Department, Churchill Hospital, Oxford
[3]Institute of Health Sciences, Oxford
[4]London School of Hygiene and Tropical Medicine, London
[5]Tissue Typing, Transplant Centre, Churchill Hospital, Oxford

Immunosuppressed renal transplant recipients are at increased risk of developing skin cancers, especially squamous cell carcinoma. Experience in a specialised Transplant Dermatology clinic, suggested that the incidence of skin cancers after transplantation would be higher than previously reported from centres in the United Kingdom. We have carried out a comprehensive analysis of the epidemiological characteristics of skin cancers occurring in a population transplanted in a single centre over a 21-year period.

From January 1975 until January 1996, 1115 patients received 1360 kidney grafts, of whom 979 patients were suitable for the analysis. The lesions recorded for the analysis were histologically confirmed basal cell carcinoma (BCC), Bowen's disease, squamous cell carcinoma (SCC), keratoacanthoma, malignant melanoma, one case of sebaceous carcinoma and one case of Merkel cell tumour. Differences in the number of tumours, age at transplantation and time to presentation between subgroups were calculated by Pearson chi-square test or Student's t-test. Univariate and multivariate risk factor analysis was carried out using Cox's proportional hazards model. The cumulative risk was calculated by life table analysis.

One hundred and eighty-seven patients (19.1%) developed at least one skin cancer. The total number of skin cancers was 1065 giving an average of 6 tumours/ patient. The rate of any skin cancer after transplantation was 141/1000 person years at risk and the rate of SCC was 71.4/1000 person years at risk. Rates for the other types of skin cancer were also calculated. SCC was the most common skin cancer (51%) and the ratio of SCC/BCC was 3.2/1. Sixty-four percent of patients developed multiple skin cancers and the maximum number in a single patient was 50. In patients with multiple skin cancers of different histological types, the most common association was between SCC and Bowen's disease. The mean time to presentation with skin cancer after transplantation was 8 years and SCC was the most commonly presenting skin cancer. Risk factors identified were increased age at transplantation (OR=1.07, 95%CI=1.06-1.08, $p<0.0001$), recipient sex (OR=0.51 for females versus males, 95%CI=0.37-0.69, $p<0.0001$), time of exposure to immunosuppression ($p<0.0001$), creatinine >150μmol/L at 1 year (OR= 1.74, 95%CI=1.25-2.43, $p=0.001$) and graft relation (OR=0.34 for living-related versus cadaver, 95%CI=0.14-0.82, $p=0.02$). The cumulative risk of skin cancer reached 61% at 22 years after transplantation. Five patients developed lymph node metastases and two patients died secondary to their skin cancer. Skin cancers after transplantation represent a significant cause of morbidity and with improved graft survival the number of patients affected is likely to increase.

Laboratory

C62

The impact of recipient cytokine genotype on acute rejection after renal transplantation

Sara E Marshall, Andrew J McLaren, Neil A Haldar, Peter J Morris, Ken I Welsh.

Oxford Transplant Centre and Nuffield Department of Surgery, Oxford Radcliffe Hospitals, Oxford, OX3 7LJ, UK

Background

Acute allograft rejection remains an important cause of morbidity after kidney transplantation, and has been shown to be a crucial determinant of long-term graft function. As cytokines are major regulators of the immune system, genetic variation in cytokine production or activity may influence susceptibility to acute rejection. This study sought to determine the impact of recipient cytokine and cytokine receptor polymorphisms on acute rejection after renal transplantation.

Methods

209 cadaveric renal transplant recipients were selected for analysis according to the presence or absence of graft rejection in the first 30 days after transplantation. DNA was genotyped for 22 polymorphisms in 11 cytokine and cytokine receptor genes using the polymerase chain reaction with sequence specific primers. Polymorphisms typed included the following: IL-1 alpha (-889t/c), IL-1 beta (-511t/c and +3962 t/c), IL-1 receptor (Pst1 970c/t), IL-1 receptor antagonist (MspI 11100t/c), IL-4 (-590t/c), IL-4 receptor (+1902g/a), IL-6 (-174g/c and +3247g/a), IL-10 (-1082a/g, -819c/t, -592c/a TNF (-308a/g, -238a/g, +488a/g), lymphotoxin (+249a/g, +365c/g, +720c/a) and TGF-beta (-880g/a, -509c/t, aa10L/P and aa25R/P).

Results were stratified by incidence and severity of rejection, and by HLA-DR mismatching.

Results

No association between any polymorphism and the incidence or severity of acute rejection was detected. In particular, no association was seen with TNF or IL10 genotype, either alone or in combination.

Conclusions

This study failed to demonstrate any association between any recipient cytokine polymorphism and acute rejection after cadaveric renal transplantation. Thus we have found no evidence to support the use of recipient cytokine genotyping to predict transplant outcome, or to guide immunosuppressive therapy after transplantation.

PORCINE CTLA4-IG PREFERENTIALLY SUPPRESSES HUMAN CD4+ T CELL RESPONSES CO-STIMULATED BY PORCINE BUT NOT HUMAN B7.

Dorling, A. Vaughan, A.N. Malde, P.K. Rogers, N.J. Jackson, I.M. and Lechler R.I.

Department of Immunology, Imperial College Medical School, Hammersmith Hospital, London, United Kingdom.

The long term survival of porcine xenografts in humans will depend on successful suppression of the T cell anti-graft response. However, unlike allografts, pig organs appear to be especially immunogenic in man because they provoke particularly vigorous 'direct' and 'indirect' xenoresponses. Additionally, pig endothelial cells (EC) stimulate strong 'direct' human T cell xenoresponses due to their constitutive expression of porcine B7 molecules (CD80 and CD86).

It may prove difficult to overcome these intense T cell responses using conventional systemic immunosuppressive agents without unacceptable side effects. In addition, the continued presence, long after the emigration of resident passenger leukocytes, of directly immunogenic (B7+) vascular endothelial cells means there may be no natural mechanism by which the graft can tolerise human T cells with direct specificity. This will have an important bearing on the duration of immunosuppression required to prevent xenograft rejection. Safe and effective strategies for the graft-specific suppression of T cell response are therefore needed.

In this study, we describe the cloning of porcine CTLA4 (pCTLA4) and the characterisation of a derived soluble fusion protein, pCTLA4-Ig, which appears to selectively inhibit human CD4+ T cells responses only when costimulation is provided by porcine B7. pCTLA4 was generated by reverse transcriptase PCR of RNA isolated from activated porcine PBMC. After sequencing, both the cDNA and predicted protein sequences were found to have significant homology to human and cattle CTLA4. However, the MYPPPY motif between residues 97 - 102 of human CTLA4, which has been shown to be important for binding B7, was not fully conserved in the pig sequence. The methionine at position 97 was replaced with a leucine residue, (a substitution which has previously been documented in cattle and sheep CD28). A fusion protein constructed from the extracellular regions of pCTLA4 and the constant regions of human IgG1 (pCTLA4-Ig) was found to bind pCD86 with equivalent affinity to that of human CTLA4-Ig. However, pCTLA4-Ig bound poorly to human B7 molecules expressed on fibroblast transfectants and EBV-transformed human B cell lines. In functional assays, with MHC class II-expressing porcine EC and human B cells, pCTLA4-Ig blocked human CD4+ T cell responses to pig but not human cells whereas control human CTLA4-Ig inhibited responses to both. We are currently examining the hypothesis that the selective binding of pCTLA4-Ig to pB7 molecules is due to the L for M substitution in the LYPPPY motif. Our results indicate that pCTLA4-Ig, by failing to inhibit the delivery of costimulatory signals provided by human B7, may prove to be a relatively specific reagent for inhibiting the direct human T cell response to immunogenic pig tissue. In this way, we have exploited one difference between donor and host species to devise a reagent that should have truly graft-specific potential.

MOLECULAR CHANGES IN EXTRACELLULAR MATRIX TURNOVER AFTER RENAL ISCHAEMIA REPERFUSION INJURY

S Jain, GR Bicknell and ML Nicholson

Department of Transplant Surgery, Leicester General hospital, Leicester, UK

Renal ischaemia reperfusion injury is an inevitable consequence of transplantation and contributes to later graft fibrosis associated with chronic allograft nephropathy. This study aimed to elucidate possible mechanisms behind this by studying the expression of genes associated with extracellular matrix turnover.

Ischaemia reperfusion injury was induced in male Wistar rats (n=6 per timepoint) by clamping of the right renal pedicle for 45 minutes, left nephrectomy was also performed. Control non-operated animals (n=6) were studied for baseline levels. Urinary protein was measured every 4 weeks and used as a marker of renal damage. Changes in gene expression were assessed using quantitative RT-PCR. Messenger RNA levels of genes of interest were expressed as a ratio to those of GAPDH a housekeeping gene and expressed in arbitrary units

Animals in the experimental group developed statistically significant proteinuria from 16 weeks onwards, this did not occur in the control group. Changes in gene expression are displayed in the table, shown as mean (standard error of the mean).

	BASELINE	8 WEEKS	16 WEEKS	24 WEEKS
TGFβ	0.86 (0.06)	0.78 (0.08)	0.97 (0.11)	1.37 (0.15)*
Collagen III	1.62 (0.13)	1.11 (0.13)*	1.38 (0.35)	1.79 (0.55)
MMP2	0.14 (0.02)	0.25 (0.03)*	0.12 (0.04)	0.09 (0.01)
MMP9	0.30 (0.07)	0.49 (0.18)	0.33 (0.07)	0.17 (0.09)
TIMP1	0.37 (0.06)	0.29 (0.07)	0.48 (0.03)	0.64 (0.04)*
TIMP2	1.15 (0.23)	0.84 (0.04)	0.93 (0.22)	1.54 (0.27)

*$p<0.05$ vs baseline (students' t-test)

At 8 weeks matrix degradation was favoured with increased mRNA levels of MMP2 and decreased levels of collagen III. At 24 weeks the balance changed to decreased degradation, TIMP1 being highly expressed. TGFβ expression also rose significantly at 24 weeks.

These changes suggest there are two phases of extracellular matrix turnover after renal ischaemia reperfusion injury, an initial increase followed by changes that favour fibrosis.

THE EFFECT OF CYCOLOSPORINE (CsA) AND TACROLIMUS (TAC) ON THE MOLECULAR AND STRUCTURAL DETERMINANTS OF RENAL ALLOGRAFT FUNCTION.

Baboolal K, Jones GV, Janezic AL, Bukilica M, Khanna R, Stockdill H, Jurewicz WA.
Welsh Transplantation Research Group, UWCM, Cardiff, CF14 4XN, UK

Recent studies have demonstrated increased expression of the pro-inflammatory cytokine TGFβ-1 in established chronic graft nephropathy (CGN). Previously we have shown that interstitial fibrosis (IF), mediated by TGFβ-1, increases in sequential renal biopsies over 12 months. In addition, both our group and others have shown that IF at 6 months predicts renal function at 2 years post-transplantation. A number of potential risk factors have been implicated in the development of IF and CGN including drug toxicity, acute rejection episodes, donor age and sex. The aim of this study was to prospectively assess how these factors influence the molecular and structural determinants of renal allograft injury.
A total of 47 cadaveric renal transplant recipients underwent protocol renal biopsies at 0, 3, 6 and 12 months following engraftment. Biopsies were analysed for mRNA expression of TGF-β1 using quantitative RT-PCR and were processed for morphometric assessment of renal structure. Renal function was assessed using serum creatinine. Results are expressed as mean ±SEM.

	CsA(n=19)			TAC (n=28)		
Time Months	TGFβ1 mRNA Log copy No	IF %	Creat µmol/l	TGFβ1 mRNA Log copy No	IF %	Creat µmol/l
0	1.7±.2	13±1		1.7±.3	13±1	
3	2.2±.4	21±1*	146±6*	1.7±.3	16±1	121±4
6	3.1±.3*◊	36±3*◊	146±6*	1.9±.3	23±2◊	126±4
12	2.6±.3	44±3*	156±7*	2.2±.2	26±2	131±6
18			161±7*			138±7

* $p < 0.05$ for comparisons between CsA and TAC post transplant. ◊ $p<0.05$ for comparison between 0 and 6 months

This study demonstrated that patients treated with CsA had a progressive increase in TGFβ-1 within the first 6 months compared to patients on TAC. The elevated levels of TGFβ-1 in those patients on CsA was associated with accelerated renal fibrosis. This structural damage was reflected by significantly higher creatinine levels at all time points. Multiple regression analysis demonstrated that the difference between CsA and TAC was independent of the number of acute rejection episodes, donor age and sex. It therefore appears that treatment with Tacrolimus is potentially less fibrotic, leading to improved function over the initial post-transplant period.

ALTERNATIVELY SPLICED VARIANTS OF IL-2mRNA IN SEQUENTIAL TRANSPLANT KIDNEY CORE NEEDLE BIOPSIES.

Janezic AL, Bukilica M, Jones GV, Khanna R, Morris-Stiff G, Jurewicz WA.

Welsh Transplantation Research Group, Department of Surgery, UWCM, Cardiff, CF14 4XN, UK

Although Interleukin-2 (IL-2) appears to be crucial for the activation and proliferation of cytotoxic T-cells, the post-transplantation immune monitoring of biopsy samples has failed to show a correlation between the levels of IL-2mRNA and the detection of acute rejection episodes. Recently, alternatively spliced variants of IL-2 mRNA missing exon 2 (IL2δ2) and exon 3 (IL-2δ3) mRNA have been described. The recombinant truncated IL-2δ2 and IL-2δ3 proteins have been shown to inhibit IL-2 induced T cell proliferation. The concept was proposed that alternatively spliced forms of IL-2mRNA may code proteins that act as natural IL-2 antagonists. This potentially important auto-regulatory process has not been explored in a clinical setting.
The aim of this study was to establish the relationship between the levels of alternatively spliced variants of IL-2mRNA and the histologically verified acute rejection. Scheduled renal biopsies were retrieved pre and post-perfusion and at 3, 6 and 12 months post-transplant. Unscheduled biopsies were taken at any time of deterioration of renal function. IL-2L, IL-2δ2 and IL2δ3 specific, SYBR green I based, real time quantitative RT-PCR assays were developed and all 3 genes quantified in all 300 biopsies. Initially, the amplicons obtained from the biopsy samples were cloned and sequenced to test the specificity of the assay and to prove the presence of alternatively spliced variants of gene in kidney biopsies.
The native, non-spliced form of mRNA coding active protein was detected in only 6 biopsies. The alternatively spliced IL-2δ2 form in 74 and IL-2δ3 in 56 out of 300 biopsies analysed. The IL2δ2 variant was detected in 9 pre and 11 post-perfusion biopsies and IL2δ3 in 7 pre and 7 post perfusion biopsies. The level of expression varied considerably between samples (20-46000 copies per 25ng of reverse transcribed RNA). Biopsy samples from the IL-2δ2 and/or IL2δ3 positive patients taken 3; 6 and 12 months later also expressed both variants of gene. The unscheduled biopsy sample analysis did not show a correlation between histologically verified rejection and the expression or upregulation of alternatively spliced variants of IL-2mRNA. If the concept that alternatively spliced variants of IL-2 genes encode a non-functional protein is correct then the lack of correlation between the expression of the spliced variants and rejection is to be expected. The alternatively spliced variants of IL-2 seem to be the dominant form of IL-2mRNA expression in transplanted kidneys. The low incidence of the non-spliced variant of IL-2mRNA needs to be explored. It could suggest that up-regulation of IL-2 gene expression is successfully controlled by immunosuppression (Cyclosporine A or Tacrolimus) and/or that the alternative variants of mRNA have different kinetics of expression. The presence of spliced variants in pre and post perfusion biopsy samples before alloantigen stimulation emphasises the importance of sequential sampling of biopsies so that the right conclusion about the origin of the expression can be made.

EXPRESSION OF NOVEL ANTICOAGULANT FUSION PROTEINS INHIBITS FACTOR XA- AND THROMBIN-INDUCED ACTIVATION OF PORCINE VASCULAR ENDOTHELIAL CELLS.

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

[1]Dept. of Immunology, and [2]MRC Haemostasis Research Group, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN

Intravascular coagulation resulting in organ infarction occurs during hyperacute rejection of allografts transplanted into sensitised patients and remains a major problem in hyperacute and delayed xenograft rejection (DXR). We have previously described a strategy to prevent intravsacular thrombosis using two novel anticoagulant fusion proteins based on human tissue factor pathway inhibitor (TFPI) and hirudin. When expressed on the surface of pig endothelial cells (EC) in vitro, these proteins efficiently inhibit the clotting of recalcified human plasma.

Aside from their critical role in thrombosis, coagulation factors also have inflammatory properties. For example, factors Xa (FXa) and thrombin are each known to activate endothelial cells (EC) during in vitro culture. These effects of Xa and thrombin may be important during delayed xenograft rejection, which is characterised by generalised type II EC activation, an influx of inflammatory cells into the graft and widespread intravascular fibrin deposition. This study assessed whether porcine EC expressing the TFPI and hirudin fusion proteins would be resistant to activation induced by FXa and thrombin.

The two constructs encoding the anticoagulant fusion proteins were transfected singly or together into primary porcine EC using a highly efficient targeted liposome strategy. Transfectants and non-transfected controls were incubated with FXa, thrombin or a FXa/thrombin mix. Whereas control EC upregulated the expression of VCAM, E-selectin and tissue factor (TF) in a time- and concentration-dependent manner, EC expressing the TFPI fusion protein were selectively resistant to these proinflammatory effects of FXa but not thrombin. Likewise, the transfectants expressing the hirudin fusion protein were selectively resistant to the proinflammatory effects of thrombin but not those of Xa.

When combined, the Xa and thrombin had an additive effect on the expression of VCAM, E-selectin and TF after incubation with control EC. In contrast, dual transfectants expressing both hirudin and TFPI fusion proteins failed to upregulate any of these three inflammatory markers after incubation with the Xa/thrombin mix.

These results indicate that expression of novel anticoagulant fusion proteins on the surface of porcine EC can protect against EC activation induced by coagulation factors Xa and thrombin. In vivo, we anticipate that expression of these fusion proteins on the endothelium of transplanted xenografts, besides preventing intravascular thrombosis, will also protect against EC activation induced by trace amounts of thrombin and Xa, thereby further protecting the grafts from DXR.

LABORATORY