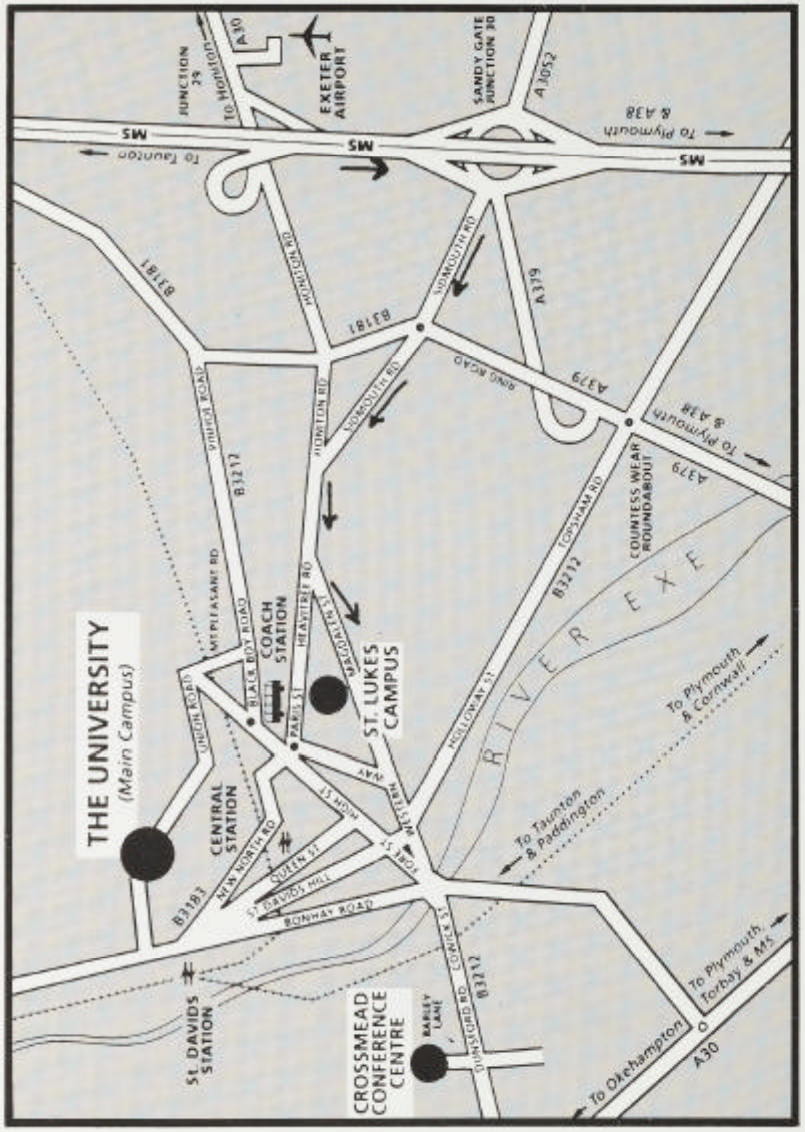
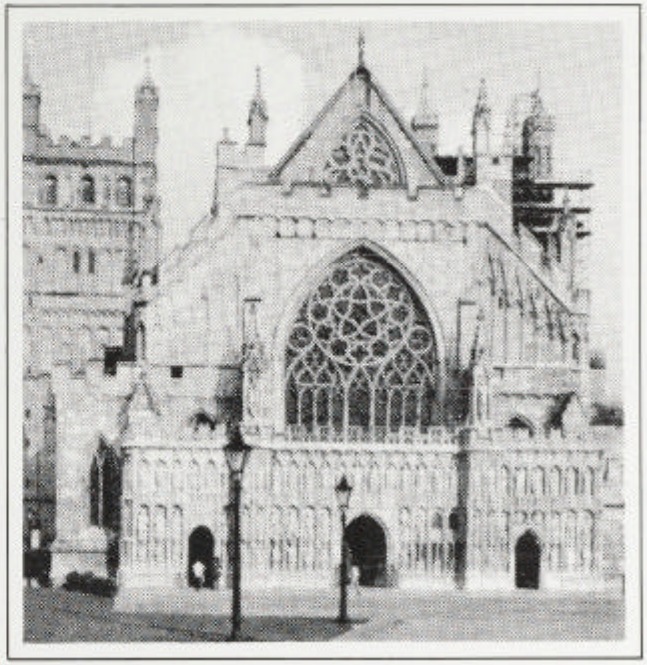
HOW TO GET THERE



THE BRITISH TRANSPLANTATION SOCIETY



NKRF 25th ANNIVERSARY

ST LUKE'S COLLEGE
EXETER

6th & 7th April 1993

25 YEARS OF TRANSPLANTATION IN EXETER

BRITISH TRANSPLANTATION SOCIETY
STATEMENT OF ACCOUNTS FOR THE YEAR ENDED 30 SEPTEMBER 1992

RECEIPTS	1991	1992
	£	£
Subscriptions Received	7,324	7,217
Immunology Letters	–	63
Bank Interest	2,025	2,166
Net Surplus from Meetings		
Autumn	2,600	575
Spring	4,092	2,047
Accounting in Transplantation (Liverpool)	–	111
Summer School	3,999	–
Net Surplus from Bursaries	1,400	700
	21,400	12,879
Balance as at 30 September, 1991		37,216
Add Excess of Receipts over Payments		8,419
		45,635

PAYMENTS	1991	1992
	£	£
Postage and Stationery	2,354	2,375
Bank Charges	33	50
Secretarial Honoraria	1,000	1,000
Audit Fee	30	35
Engraving Medawar Medal	45	–
Transplant Training Meeting – to send delegate	157	–
Summer School 1993 Advance	–	1,000
	3,619	4,460
Excess of Receipts over Payments	17,821	8,419
	21,440	12,879
Represented by:		
Ulster Bank Ltd. Business Reserve		42,500
Current Account		3,135
		45,635

The Statement of Accounts and Balances are in accordance with information and records provided.

Derek Middleton – *Honorary Treasurer.* M. Richards – *Honorary Auditor*

Of monies in Business Reserve Account £7,945 is for use for Bursaries.

PAPER 1

ORGAN SHARING BASED ON HLA MATCHING SAVES DIALYSIS YEARS AND PATIENT LIVES

J Thorogood[1,2], G G Persijn[1], G M Th Schreuder[3] and J J van Rood[1]

Eurotransplant Foundation[1], Department of Medical Statistics[2] and Department of Immunohaematology and Blood Bank[3], Leiden University Hospital, Leiden, The Netherlands

Within our international organ exchange organisation, sharing of kidneys is mandatory for 0 HLA-A+B+DR mismatches and centres are requested to offer kidneys if a minimum match of 2 HLA-B+DR antigens, including at least 1 HLA-DR antigen, cannot be obtained locally. We have examined the effectiveness of our exchange criteria over the last 5 years, with respect to graft and patient survival.

8847 recipients of first, unrelated kidney grafts, transplanted in 50 centres between 1 January 1987 and 31 December 1991 and treated with cyclosporine, were analyzed. Serological typing was performed using a standardized serum set. Overall 1-year survival was 85% and 96% for grafts and patients, respectively. 4396 (49.7%) kidneys were transplanted locally and 4451 (50.3%) were shipped to another transplant centre. The mean cold ischemic period (CIP) was longer in shipped (26 hours) than locally used kidneys (20 hours, p=0.0001). Graft survival was similar for local and shipped kidneys, and patient survival was slightly higher for shipped kidneys (Table 1). Overall, 90% of all transplants were performed according to the requested minimum match criteria, 85% of local and 93% of shipped kidneys. 4161 kidneys shipped and meeting the minimum match criteria fared better than kidneys transplanted locally with a poor match (due to reasons such as clinical urgency, positive crossmatches for potential recipients at distant centres, etc), for both graft (p=0.0280) and patient (p=0.0066) survival. If the 4161 kidneys had been transplanted locally with a poorer match, graft and patient survival would have been up to 4% and 3% lower, respectively. Sharing, thus, saved up to 166 dialysis-years and 125 patient lives 1 year post-transplant.

TABLE 1: 1-year graft and patient survival according to match criteria

Minimum match	1-Year Graft Survival Local (N)	Shipped (N)	logrank p-value	1-Year Patient Surv. Local	Shipped	p-value
Yes	86% (3758)	86% (4161)	0.9318	96%	97%	0.0327
No	82% (638)	76% (290)	0.0276	94%	94%	0.8104
Total	85% (4396)	85% (4451)	0.7942	95%	96%	0.0254
Poor local match vs shipped and well-matched	82% (638)	86% (4161)	0.0280	94%	97%	0.0066

In conclusion, the exchange criteria in use lead to similar graft survival in locally used and shipped well-matched kidneys and a slightly improved patient survival in those shipped, despite prolonged CIP. The gain in graft survival through exchanging organs based on HLA matching covers the costs incurred, through savings in dialysis years and patient lives.

PAPER 2

ARE "UNSUITABLE" DONORS UNUSABLE ?

D.R.Wheeldon, C.D.O.Potter, M Jonas, J.Wallwork, S.R.Large.

Transplant Unit, Papworth Hospital, Papworth Everard, Cambs CB3 8RE

From a series of 100 donors between October 1990 and July 1992 there were 21 that on initial inspection fell well outside our current transplant guidelines: (Mean Arterial Pressure {MAP} > 60 mmHg, Central Venous Pressure {CVP} < 12 mmHg, Pulmonary Capillary Wedge Pressure {PCWP} < 12 mmHg on inotropes < 5 mcg/kg/min). 13/21 donors had a MAP < 55 mmHg, 6/21 had a CVP > 15 mmHg and 2/21 were on > 20 mcg/kg/min of inotropes with a MAP < 60 mmHg. Following full haemodynamic monitoring and evaluation, another 5/100 donors had an unacceptable Left Ventricular Stroke Work Index {LVSWI} of < 15 g.m. (normal resting LVSWI is approximately 30 g.m.). A further 9/100 donors had a PCWP > 15 mmHg (mean PCWP = 19.6 mmHg with a mean CVP = 10.3 mmHg thus producing a mean atrial differential of 9.3 mmHg which would not have been detected without full monitoring). Following our management regime these 35 initially unsuitable donors yielded 19 hearts and 11 heart-lung blocks for transplantation. Despite our best efforts 5/35 hearts were eventually deemed unsuitable for transplantation due to left ventricular hypertrophy [2], inotrope dependency [2], poor function [1]. 25/30 transplant recipients (83.3%) are alive and well from 2 - 23 months post transplant. Four deaths occurred within 30 days due to arrythmia (heart), acute respiratory distress syndrome (heart), cerebrovascular event (heart and lung) and infection (heart,lung and liver). One death occurred at 90 days due to tamponade (heart). Aggressive donor management and the use of initially "unsuitable" donors has enabled us to maintain our level of transplant activity without adverse effects on outcome (30 day mortality 16.2% in 1989, 11.8% in 1990 and 6.8% in 1991). Optimal management and evaluation of a multi-organ donor must include the use of full haemodynamic monitoring to avoid retrieval of unsatisfactory organs or refusal of potentially usable organs given optimum intervention. Careful donor management can enable initially unsuitable organs to be successfully transplanted.

PAPER 3

DONOR SPECIFIC BONE MARROW INFUSION FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION. A RANDOMISED CONTROLLED STUDY. PRELIMINARY RESULTS.

K. Rolles, A. Burroughs, B. Davidson, H. Prentice, M. Hamon

Liver Transplant Unit and Bone Marrow Transplant Unit, Royal Free Hospital, London.

The quest for donor specific unresponsiveness has never been more intense. Donor specific bone marrow infusion following solid organ grafting has been shown to induce a tolerance state in a variety of animal models and more recently in man. At the Royal Free Hospital, 9 of 25 patients were randomly selected to receive an infusion of cryopreserved red cell depleted donor bone marrow at a dose of 2-3 x 10^8 nucleated cells/kg five days after completion of a ten day course of ATG (Merieux) at 2.5 mg/kg/day. Maintenance immunosuppression was with Cyclosporin A only, maintaining whole blood levels between 50 and 150 nannograms/ml.

8 of the 9 patients (89%) receiving donor bone marrow are currently alive between 95 and 370 days post-transplant compared with 12 out of 16 of the control patients (75%).

Three rejection episodes per patient were seen in the bone marrow group, compared to 3.125 in the control group.

No evidence of chimerism was seen in the blood of any patient between 20 and 100 days post-transplant by means of erythrocyte antigen studies.

Y-probe in situ hybridisation showed no chimerism in one female recipient of male donor bone marrow in peripheral blood between days 30 to 80 or in recipient bone marrow at day 90 post-transplant.

No evidence of graft versus host disease has been seen in any patient. The preliminary results give no encouragement or justification for the eventual withdrawal of immunosuppressive therapy in the hope that tolerance has been achieved.

PAPER 4

Randomised Clinical Trial comparing nephrotoxic potential of FK506 vs. Cyclosporin (CyA) triple therapy.

Umana JP, Ayres RCS, Harrison JD, Davies MA, Mayer D, Buckels JAC and McMaster P.

Liver Unit, Queen Elizabeth Hospital, Edgbaston, BIRMINGHAM, West Midlands. B15 2TH.

Patients undergoing orthotopic liver transplantation (OLT) for end-stage liver disease commonly have impaired renal function and the nephrotoxicity of Cyclosporin A (CyA) is well recognised. The macrolide antibiotic FK506 is a powerful immunosuppressive agent and studies suggest it may be less nephrotoxic than CyA.
Objective-To evaluate the nephrotoxic potential of FK506 compared to CyA.
Subjects-45 patients undergoing OLT.
Design-Randomised study comparing the efficacy and nephrotoxicity of these two drugs following OLT. Group C comprises 16 patients receiving CyA and group F 18 patients received FK506 in addition to steroids and azathioprine. We have compared the Glomerular filtration rate (GFR) and effective renal plasma flow rate (ERPF) pre-OLT and 3 months post-OLT in each of the groups. A nephrotoxic event (NE) was determined to have occurred when there had been a fall of over 16 mmol/l on consecutive days and the mean change in creatinine during these events was compared between groups. Regression analysis was applied to the levels of CyA and FK506 with creatinine change as the dependent variable.
Results-There was a significant fall in GFR at 1 (86*mls/min) and 3 (66*mls/min) in group C compared to pre-OLT values (106*mls/min,$p<0.05$). A similar trend was seen in group F with a significant fall in GFR/ERPF at 3 months post-OLT. In a regression analysis there was no correlation between CyA and FK506 levels and creatinine change. There were more NE in group F (162v92 events).
Conclusion-FK506 at a dose of 0.15 mg/Kg has a similar nephrotoxic potential to CyA. There were probably more NE in group F due to the initial use of intravenous FK506

*Medial values

PAPER 5

CYCLOSPORIN, NIFEDIPINE AND GINGIVAL HYPERPLASIA: A RANDOMISED CONTROLLED STUDY

JDT MORGAN, MJ SWARBRICK, CM EDWARDS, PK DONNELLY

Department of Surgery, Leicester General Hospital, Gwendolen Rd. Leicester.

Gingival hyperplasia is a common and distressing side-effect of Cyclosporin (CyA) therapy, with reported incidences of between 7 and 80%. It has been linked in this context with nifedipine, a calcium channel blocker, frequently used in transplantation for control of hypertension.
We have studied the incidence of gingival hyperplasia within the framework of a randomised trial comparing 3 immunosuppressive regimens, with nifedipine treatment in one arm. At transplantation patients were randomised to either Group A: high dose CyA and prednisolone, Group B: high dose CyA, prednisolone and nifedipine, or Group C: low dose CyA, prednisolone and azathioprine. Assessment of gingival hyperplasia was made 3 months after transplantation and graded as none, mild, moderate or severe. Endentulous patients do not develop gingival hyperplasia and were excluded from this analysis.
Results show that Group B were at significantly higher risk of developing gingival hyperplasia than either Group A or C.

	n	None/Mild	Moderate/Severe	
Group A	15	15	0	
Group B	17	8	9*	*p<0.001
Group C	17	14	3	

This confirms that the combination of high dose CyA and nifedipine predisposes to gingival hyperplasia. Importantly there have been reports of malignancies arising within areas of gingival hyperplasia, in a group already at high risk of *de novo* cancers.

PAPER 6

PREVALENCE OF ANAL INTRAEPITHELIAL NEOPLASIA IN RENAL ALLOGRAFT RECIPIENTS

O.A.Ogunbiyi, J.H.Schofield, J.H.F.Smith, A.Raftery, F.Sharp, K.Rogers.

Departments of Surgery, Gynaecology, Pathology, and The Sheffield Kidney Institute, Northern General Hospital, Sheffield.

An increase in the prevalence and incidence of human papillomavirus (HPV) associated genital intraepithelial and invasive neoplasia occurs in immunosuppressed organ transplant recipients. Although a similar trend has been suggested for the aetiologically-related anal squamous neoplasia, there have been no studies of the prevalence of anal squamous neoplasia in organ transplant recipients. The present study tests the hypothesis that renal transplant recipients are at higher risk of intraepithelial neoplasia of the anus than are control patients of similar age.

Ninety-eight renal transplant recipients (59 males;mean age 45.6 years,and 39 females;mean age 47.8 years) were recruited and underwent anal colposcopy. A histological diagnosis of anal intraepithelial neoplasia (AIN) including one anal cancer was made in 24.5% of the study group. The distribution of the lesions is shown in the table below.

	male	female	Total
AIN 1	13	6	19
AIN 2	-	2	2
AIN 3	1	1	2
Anal Cancer	-	1	1

HPV 16 DNA was identified in anal biopsies in 30 (41.6%) of 72 patients in the study group. No evidence of AIN was found in the control group (50 women and 20 men). HPV 16 DNA was identified in 7 (10%) anal biopsies in the control group.

These results suggest that AIN is more prevalent in renal transplant recipients. The need for anal examination in screening for AIN in these patients requires further investigation.

PAPER 7

MALIGNANCY IN 14 YEARS OF HEART AND LUNG TRANSPLANTATION

J.Parameshwar, N.Cary, T.Wreghitt, C.Dennis, N.Briffa, L.Hoff, S.Stewart, P.Schofield, S.Large, F.Wells, J.Wallwork.

Transplant Unit, Papworth Hospital, Papworth Everard, Cambridge. CB3 8RE.

Malignancy is known to occur more commonly in immunosuppressed patients. We have analysed the incidence of malignancy in 548 patients who survived for more than 3 months after a heart, heart-lung or lung transplant performed between January 1979 and September 1992. Cyclosporine (CsA) has been part of the immunosuppressive regime in all but the first 29 patients. Antithymocyte globulin was used in the induction phase in all patients. During this period there have been 49 malignancies in 48 patients (9%). These consisted of lymphoproliferative disease (LPD) in 17 patients (35%), skin malignancy in 13 (27%), gastrointestinal tumours in 7 (14%), genitourinary tumours in 5 (10%), lung tumours in 4 (8%), carcinoma of the larynx in 1 (2%), malignant melanoma in 1 (2%) and acute monocytic leukaemia in 1 (2%). In 8 of the 17 patients with LPD, disease was confined to the heart and lungs. Evidence of Epstein-Barr virus (EBV) infection on serology was present in 11 patients (65%) contrasting with a 23% infection rate in our total transplant population. Treatment consisted of a reduction in CsA dosage and oral acyclovir in all patients; in addition 4 patients received chemotherapy and 2 received radiotherapy. In 6 patients reduction in CsA and acyclovir lead to clinical and radiological resolution. The early response to chemotherapy (upto 18 months) has been encouraging in 3 patients. 9 of the 17 patients with LPD have died; in 3 of these the diagnosis was only made at autopsy. LPD occured more commonly and earlier in recipients of a lung than a heart transplant (incidence of 5% vs 2.5% and mean time to diagnosis 8 months vs 41 months). This may reflect the higher doses of cyclosporine commonly used in the former group. Of the patients with non-lymphoid malignancy, the lesion was the cause of death in 15 of the 19 patients who had lesions other than dermatological. In 3 of these the diagnosis was not made antemortem. Malignancy is an important problem in the follow up of patients after heart and lung transplantation and the incidence of LPD is higher than in other solid organ transplants. Early diagnosis and treatment of LPD with reduction of immunosuppression, acyclovir, and chemotherapy where necessary is effective in a significant proportion of patients; thus a high index of suspicion and aggressive investigation including early biopsy is warranted.

PAPER 8

THE USE OF CREATINE KINASE MB ISOFORMS IN PREDICTING ACUTE ALLOGRAFT REJECTION FOLLOWING CARDIAC TRANSPLANTATION

M Hossein-Nia, J Mascaro, SH Jennison, PA Brown, AS Adams, AJ Murday, DW Holt

Dept. of Cardiological Sciences, St. George's Hospital Medical School, London SW17 0RE

Frequent routine right ventricular endomyocardial biopsy is the standard procedure to detect significant acute allograft rejection in cardiac transplant patients in their first post-operative year. There are, however, still no non-invasive methods available to detect or predict significant acute rejection. Detection of the creatine kinase (CK) isoforms (MB2 & MB1) in plasma, as a sensitive marker of myocardial cell injury, is now possible. The aim of this study was to investigate the usefulness of CK-MB isoform measurement as an early non-invasive marker of acute rejection following cardiac transplantation. Plasma samples (n=236) were collected from 55 orthotopic heart transplant recipients prior to biopsy, during hospitalisation, and at out-patient visits. CK-MB isoforms were determined by high resolution agarose gel electrophoresis and quantified by densitometric scanning. 20/55 patients suffered 22 episodes of significant acute rejection with myocytolysis. At the time of biopsy there was no significant difference in MB2/MB1 ratio between patients with grades 2 and 3 rejection compared to those with grades 0 and 1 rejection (medians 1.65 vs 1.33, respectively). However, MB2/MB1 ratio was significantly increased prior to histological changes seen on biopsy in 13/16 patients with significant acute rejection (mean 14 days), in whom consecutive samples were collected ($r=0.23$, $p<0.001$). This rise in MB2/MB1 ratio indicates that either myocardial cell injury was present, but had been missed by biopsy, or that it was developing before histological changes were apparent. We conclude that analysis of CK-MB isoforms may provide a sensitive and more global estimate of myocardial cell injury than relying solely on the histological appearance of the biopsy. Its usefulness as a predictive non-invasive marker of significant acute rejection warrants further evaluation.

PAPER 9

CYTOKINE GENE EXPRESSION IN ALLOGENEIC AND SYNGENEIC PANCREATIC ISLET GRAFTS IN THE MOUSE.

M.R. Newton, S. Shimizu, M.J. Dallman, D.W.R. Gray and P.J. Morris.

University of Oxford, Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford.

Certain cytokines, in particular IL1ß, have been implicated in the destruction of allografted islets. However, there is little data on the expression of cytokines within allografted islet tissue. C57BL mouse islets were prepared by intraductal collagenase digestion of the pancreas, separated using a BSA density gradient and transplanted under the kidney capsule of either syngeneic or allogeneic C3H recipients. At 1,3,5 or 7 days post transplantation the recipient was killed, the kidney removed and the islet graft carefully dissected away from the kidney. RNA was prepared from the graft using the guanidine isothiocyanate method followed by density gradient centrifugation over CsCl. A cDNA copy of this RNA was made and analysed for cytokine gene expression using the polymerase chain reaction and cytokine specific primers. To date 3 cytokines have been examined in detail; Il-1 , IL-2 and IL-10. In contrast to previous findings in vascularised organ isografts, IL-2 was found in syngeneic islet grafts. However this expression was variable, never as much as in the allografts and differed between individuals. In the allografts the results were more consistent with increasing expression from D1 to D7. IL-1 was expressed at all time points in both isografts and allografts, with maximal expression at D1 in both, decreasing more quickly in the isograft than the allograft. IL-10 was expressed at approximately equal levels in both isografts and allografts at all time points studied. The findings are relevant to phenomena that affect transplanted islets such as primary non-function and susceptibility to rejection.

PAPER 10

ALLOGRAFT REJECTION OF RETRANSPLANTED KIDNEYS IN RATS PRIMED TO INDIRECT RECOGNITION.

Adam Benham, Greta Sawyer and John Fabre

Transplantation Biology Unit, Institute of Child Health, 30, Guilford Street, London, WC1N 1EH.

We have used a retransplant protocol to provide additional evidence for the role of indirect recognition in allograft rejection of kidney grafts. Retransplantation allows mobile dendritic cells in the donor kidney to emigrate whilst in the first recipient. A kidney allograft free of dendritic cells is slowly rejected in appropriate strain combinations, with direct recognition likely to play a less dominant role. It is therefore a better (and clinically more relevant) model than the skin allografts used in previous work.

LEW ($RT1^1$) rats were primed with uncongugated peptides derived from the hypervariable regions of the DA $RT1^a$ class I α helixes. A second set of LEW rats received a (DAxLEW) F1 left orthotopic kidney transplant under Cyclosporin A cover. After >100 days the F1 kidneys were retransplanted into the LEW rats primed with peptides for indirect recognition. Control rats received Freunds adjuvant before retransplantation.

Biopsies were taken at d7 post-retransplant and blood urea levels monitored to ascertain kidney graft function. The peptide primed rats showed marked acceleration of kidney graft rejection, high blood urea levels and poor kidney histology when compared to control rats. Moreover, sera taken from the peptide primed rats showed accelerated kinetics of antibody formation to intact DA class I molecules when compared to the controls.

These results demonstrate that indirect recognition can play an important role in the effector mechanisms of kidney graft rejection.

PAPER 11

UNIVERSAL HETERODUPLEX GENERATORS FOR RAPID HLA-DQA1, DQB1 AND DPB1 MATCHING.

T.M.Clay, D.Culpan, B.A.Bradley, J.L.Bidwell

Molecular Research Division, Dept of Transplantation Sciences, University of Bristol

DNA heteroduplex analysis is a rapid and technically simple method for matching HLA-DR-Dw allotypes (1-3). Using the polymerase chain reaction (PCR), second exon sequences of all HLA-DRB genes within a given haplotype are co-amplified using generic DRB PCR primers. At the end of each PCR cycle, heteroduplexes are formed which are mismatched at allele-specific positions. These mismatches affect the helical structure and charge of these molecules and permit their separation in nondenaturing PAGE. HLA-DR-Dw allotype match or mismatch between individuals may therefore be examined by visual comparison of heteroduplex banding patterns. For the DQA1, DQB1, and DPB1 loci, DNA heteroduplex analysis cannot readily be used.

For these loci, we have developed "universal heteroduplex generators" (UHGs), which are synthetic, PCR-amplifiable DNA constructs containing deliberate nucleotide substitutions and deletions contiguous with hypervariable nucleotides in the genomic DNA. When amplified and subsequently "crossmatched" with PCR products from the DQA1, DQB1 and DPB1 loci, characteristic heteroduplexes are formed which permit the rapid matching of allotypes. These tests are particularly useful in the rapid selection (or elimination) of HLA matched, unrelated marrow donors.

1 Bidwell JL and Hui KM (1990) *Technique* 2:93-100

2 Clay TM, Bidwell JL, Howard MR and Bradley BA (1991) *Lancet* 337:1049-1052

3 Wood NAP, Clay TM and Bidwell JL (1991) *Eur J Immunogenet* 18:147-153

PAPER 12

THE IMMUNOPHARMACOKINETICS OF CYCLOSPORINE IN VIVO

J Hyam, M Rowland*,IV Hutchinson

Immunology Research Group, Depts of Cell & Structural Biology & Pharmacy
Stopford Building, University of Manchester, Manchester M13 9PT, UK.

We routinely add cyclosporine (CsA) to tissue culture experiments to modulate the functions of lymphocytes during their activation in vitro. We wanted to know if the same functional modulation occurred in vivo. However, the two systems are quite different - the available concentrations of CsA are constant in vitro over many days while, in vivo, the level of CsA varies markedly according to the bolus dosage regimen. To allow direct comparison we: a) studied the pharmacokinetics of CsA in rats, b) derived a 'bolus plus infusion' protocol to provide long-term a range of constant CsA-levels in vivo, c) transplanted CsA treated rats with renal allografts to compare T cell activation and the generation of cytotoxic (CTL) and suppressor (Ts) cells in the spleen and lymph nodes, according to CsA levels in vivo and against corresponding responses at the same CsA levels in vitro and d) compared to the outcome of treating transplanted rats with the same total dose over 7 days but given as a constant infusion, or as boluses given once a daily, every other day or as one big dose at the outset. Constant levels of available CsA greater than 0.08µg/ml abrogated specific CTL activation both *in vitro* and *in vivo*, although histological evidence of rejection was not affected until CsA levels of 0.72µg/ml were achieved. *In vivo* non-specific, non-T cell cytotoxicity in transplanted rats was not affected by CsA levels up to 1.0µg/ml. Activation of Ts was increased in CsA treated rats, as it was *in vitro*, at all CsA levels tested (0.35 - 1.00µg/ml). Except for high CsA doses, bolus administration was more effective at preventing rejection than constant infusion. The critical parameter was that the unbound CsA level should not fall below 800ng/ml by constant infusion or 300ng/ml by bolus administration. These conditions also abrogated specific CTL activation, but spared Ts cell activation. Our results show that the effects of CsA on T cell activation and on CTL and Ts generation differ dramatically in vivo depending on the level of available CsA and on the dosage regimen, and that some of the immunological changes are very sensitive to CsA concentration and time.

PAPER 13

PLATELET-DERIVED GROWTH FACTOR (PDGF) AND CYCLOSPORIN-A (CyA) NEPHROTOXCITY.

M Shehata, A M EL Nahas, G H Cope, A T Raftery

Sheffield Kidney Institute, Sheffield.

PDGF is a pluri-potential growth factor capable of inducing vasoconstriction, stimulation of smooth muscle cells proliferation and the synthesis of extracellular matrix. As these are features of CyA nephrotoxcity, we carried out this study to investigate the role of PDGF in the pathogenesis of these changes in rats injected with CyA.Adult male Sprague-Dawley rats were injected daily with either CyA (25 mg/Kg body weight) or equivalent volume of its olive oil vehicle. Groups of rats (n=6) were sacrificed at different time intervals (1, 2, 3 and 4 weeks). At the beginning and end of the experiments, serum urea and creatinine as well as whole blood CyA levels (RIA) were estimated. At sacrifice, kidney tissue was processed for the immunohistochemical detection of PDGF by the avidin-biotin peroxidase method. Three different polyclonal antibodies were used against PDGF homodimers AA and BB and the heterodimer AB. Mean CyA level was 2093 ng/ml (± 292 SEM) at 1 week and increased to 3500 ng/ml (± 325 SEM) at 4 weeks. No difference was observed in serum creatinine between CyA and vehicle-injected animals at any time interval, whilst serum urea was raised in CyA animals (18 ± 3.27 mmol/l) compared to controls (6.1 ± 0.23 mmol/l. $P < 0.01$)
Immunoreactive PDGF-B chain was detected within the media of both glomerular and interlobular arterioles. Staining was detected within one week, reached peak intensity by week 2 and was still present at 4 weeks. This study demonstrates an increased expression of immunoreactive PDGF in kidneys of rats injected with nephrotoxic doses of CyA and suggests that this peptide may play a role in the early haemodynamic changes and the late structural damage to the kidney characterised by interstitial fibrosis.

PAPER 14

TOLEROGENICITY OF ALLO-MHC CLASS I MOLECULES CORRELATES WITH INDIRECT PRESENTATION OF ALLO-PEPTIDES BY RECIPIENT DENDRITIC CELLS

DA Shoskes, PJ Morris, KJ Wood.

Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford OX3 OHN.

Cardiac allograft prolongation can be achieved in the mouse by the preoperative injection of recipient type L cells transfected with donor class I MHC genes. We have shown that a hierarchy exists with regard to the ability of individual class I molecules to injuce graft prolongation that is dependent on the strain combination used (ie $K^b > D^b$ but $D^d > K^d > L^d$). In addition, the amount of allo-antigen delivered during pretreatment is also critical for optimal induction of unresponsiveness. We have also shown that following IV injection with transfected L cells, recipient splenic dendritic cells (DC) can stimulate a primary mixed lymphocyte culture (MLC) against naive recipient T cells, demonstrating that indirect presentation of MHC class I allo-peptides is occuring in vivo. Here we correlate the degree of MLC stimulation by indirect presentation of allo-peptides with cardiac allograft survival.

C3H mice ($H\text{-}2^k$) were injected IV with L cells transfected with single MHC class I molecules (K^b, D^b, K^d, D^d, L^d) at dosages optimal for heart allograft prolongation as well as over a dose range. Two weeks later, spleens were removed and low density adherent cells isolated according to the DC enrichment protocol of Steinman. These were used as stimulators in a primary MLC against naive C3H splenic T cells. In the $H\text{-}2^b$ group, $L\text{-}K^b$ treatment gave higher MLC stimulation than D^b or treatment with an irrelevant transfectant. The proliferative response in the MLC also correlated with antigen load delivered. The dose response showed higher counts with the optimal L cell dose ($5x10^6$) than with a higher ($1x10^7$) or lower ($1x10^6$) dose. These results correlate directly with C57BL10 -> C3H heart graft survival (K^b vs D^b, 47 vs 12 days; 10^6 vs $5x10^6$ vs 10^7 of K^b, 9 vs 47 vs 17 days respectively). Similarly, in the $H\text{-}2^d$ group, the haplotype hierarchy for MLR stimulation ($D^d > K^d > L^d$) and D^d dose response directly mirrored the in vivo BALB/c -> C3H heart graft survival. To test the functional relevance of DC presenting MHC class I peptides in vivo, C3H DC were isolated 14 days after injection with transfected L cells, injected SQ into naive C3H mice and transplanted with a BALB/c heart 7 days later. $L\text{-}D^d$ treated DC sensitised C3H recipients resulting in accelerated graft rejection, which was specific for BALB/c hearts but was not seen after pretreatment with an irrelevant transfectant. These results suggest that the tolerogenicity and immunogenicity of MHC molecules may be related to the recipient T cell response and to the peptide repertoire that can be processed and presented by recipient antigen presenting cells.

PAPER 15

Pretreatment of kidney allografts with monoclonal antibodies to CD45: results of a multi-centre study.

Anti-CD45 Study Group presented by Dr L.Goldberg.

Transplantation Unit, St Mary's Hospital, London.

Monoclonal antibodies to CD45 have been used in the past to pretreat kidneys prior to transplantation in order to inactivate passenger leucocytes and reduce the incidence of acute rejection. The present study used a pair of rat monoclonal antibodies that synergistically induce lysis of leucocytes in the presence of human complement. The antibodies were made by cells derived from the cell lines YTH 24.5 and YTH 54.12, and manufactured in accordance with Good Manufacturing Practice. In an open label, safety study of 40 patients from four centres, 2mg of each antibody in 50 ml of chilled hypertonic citrate were injected into the renal artery with the renal vein clamped and the kidneys maintained at 4°C for at least 40 minutes. Prior to wound closure a biopsy was taken to measure antibody uptake using a double labelling immunohistological technique. The patients were followed for three months. There were no patient deaths, though four kidneys were lost: one from unremitting rejection; one from failure of reperfusion at the time of the transplant, probably due to pre-existing renal disease in the donor; and two from a thrombosis of the renal artery, at least one of which was mechanical in aetiology. Only two patients had an antibody response to rat immunoglobulin (HARA) and one of these had HARA activity prior to treatment. There was a significant inverse correlation [$p<0.05$] between antibody uptake and the incidence of acute rejection episodes. This study confirms the previous results of Brewer et al (Transplant.Proc. *21*, 1772, 1989) and suggests that appropriate pretreatment of kidneys with anti-CD45 monoclonal antibodies will reduce the incidence of acute rejection.

PAPER 16

ELUCIDATION OF KEY PEPTIDE DETERMINANTS INVOLVED IN AN INDIRECT RECOGNITION PATHWAY OF RAT KIDNEY ALLOGRAFT REJECTION.

Adam Benham and John Fabre

Transplantation Biology Unit, Institute of Child Health, 30, Guilford Street, London, WC1N 1EH.

In our previous studies we have used 22-24 amino acid peptides from the variable α helices of DA ($RT1^a$) MHC class I molecules to prime for indirect recognition in LEW rats receiving allografts. These peptides must be processed by host APCs and presented in association with host MHC class II molecules. The optimal size of peptide found in the class II groove is between 10-16 amino acids, suggesting that in vivo our peptides undergo further processing. To elucidate the biologically relevant part of one of our peptides (P1, 24 amino acids), we synthesised a nested set of 15mers that span the original peptide from the N to the C terminus.

T cells from LEW ($RT1^l$), WAG ($RT1^u$) and PVG ($RT1^c$) rats, immunised in vivo with the whole P1, were tested in vitro for their responsiveness to individual 15mers. LEW rats responded to all 15mers, but the greatest response was against the N and C terminal peptides. WAG T cells gave a different response, in that C-terminal 15mers stimulated relatively poorly. PVG T cells did not respond to P1 or the 15mers.

These data suggested 2 possibilities: (1) the presence of multiple T cell epitopes within P1, especially as viewed by LEW T cells. (2) an epitope involving the central hexamer of P1, common to all 15mers. To study this further, T cells from LEW rats immunised in vivo with single 15mers were tested in vitro against all the 15mers. They responded predominantly to the immunising 15mer, with some proliferation to adjacent 15mers in the series. This result is consistent with the existence of multiple, discrete T cell epitopes within P1. However, stimulation by adjacent peptides suggests flexibility in the way peptides bind to class II molecules.

PAPER 17

CHROMOSOMAL MAPPING OF MINOR HISTOOMPATIBILITY ANTIGEN GENES USING MICROSATELLITES AND T CELL CLONES

GA FOWLIS, S FAIRCHILD, K TOMONARI AND E SIMPSON

Transplantation Biology section, MRC Clinical Research Centre
Watford road, Harrow, Middlesex HA1 3UJ

Minor histocompatibility (mH) antigens are the targets of host versus graft (HVG) reactions and graft versus host disease (GVHD) in Major histocompatibility Complex (MHC) matched individuals. mH antigens are recognised exclusively by T cells and this has made their molecular identification difficult. These T cells recognise mH antigens in an MHC restricted manner: MHC molecules present components of mH antigens to specific T cells in a manner analogous to the presentation of viral peptides to antiviral T cells.

Genes encoding mH antigens have been shown to be scattered through the genome in mice from mapping studies with mH congenic strains, but their accurate chromosomal localisation has proved difficult. Two C3H-derived T cell clones (1D7 & 2E2) specific for CBA mH antigens have been isolated and characterised in our laboratory. We have also identified which microsatellites (simple DNA sequence repeats) show polymorphism between 10 inbred mouse strains, and in particular between pairs of inbred strains of special interest to us (such as C3H/CBA). These polymorphisms are all demonstrable by agarose gel electrophoresis of the DNA products obtained by the polymerase chain reaction (PCR), allowing for simplicity and rapid analysis.

Using microsatellites polymorphic between C3H and CBA and the 1D7 & 2E2 T cell clones, it has been possible to accurately map the 1D7 & 2E2 mH genes by a classic genetic backcross approach, using (C3HxCBA)F1 x C3H mice. Chromosomal mapping of mH genes is one route towards identification of mH antigens important in HVG reactions and GVHD in clinical transplantation.

PAPER 18

THE EFFECT OF HLA MATCHING ON INCIDENCE OF ACUTE REJECTION IN RENAL TRANSPLANTATION

IJ Beckingham[1], MJS Dennis[2], SJ Smith[3], ML Nicholson[2]

Depts of Surgery Nottingham City Hospital[1] and University Hospital[2], and Dept of Epidemiology University Hospital[3].

Multi-centre studies have shown that the degree of HLA matching achieved between donors and recipients in renal transplantation exerts a powerful effect on allograft survival. Smaller centre studies often show no correlation between HLA matching and survival because there are insufficient patient numbers and graft losses to reach statistical significance. We have examined the influence of HLA matching on the incidence of acute rejection and subsequent graft function and survival in 181 consecutive cadaveric transplants from our unit, where we have a policy of DR matching patients as closely as possible.

Grafts with better HLA DR and B matching showed significantly lower rejection rates than less well-matched allografts with both univariate (25%, 62% and 82% for 0, 1 and 2 DR mis-matches; p<.001) and multivariate analysis.

	No rejection	1 rejection	2 rejections	3 rejections
0 DR mismatch	70	20	3	0
1 DR mismatch	21	26	6	2
2 DR mismatch	4	11	6	1

Superior matching was also associated with improved graft function one year after transplantation (mean serum creatinine 137, 180 and 225 umol/l for 0, 1 and 2 DR mismatches; p<.05 and p <0.001).

No association was demonstrated between the degree of matching and overall graft survival.

Mean additional drug cost per patient	0 DR mismatch	1 DR mismatch	2 DR mismatch
	£564	£640	£824

Our findings show that good HLA matching can reduce the number of acute rejection episodes, producing significant savings in treatment costs (table). Furthermore long-term graft function is improved and minimising early graft damage is an important factor in reducing later development of chronic rejection.

PAPER 19

T CELL ANERGY: A CONSEQUENCE OF INTERACTION BETWEEN RAT T CELLS AND ALLOGENEIC RENAL EPITHELIAL CELLS

MR Rajasekar, JA Kirby, G Proud & RMR Taylor

Department of Surgery, The Medical School, University of Newcastle upon Tyne, NE2 4HH, UK.

The ability of rat renal tubular epithelial cells (TEC) to present alloantigens to immunocompetent T cells was examined in vitro.

Rat renal TEC were cultured and characterised by staining intracellular cytokeratin. The cells were treated with γ-IFN and induction of the expression of Class II MHC antigens was monitored by flow microfluorimetry; after 4 days maximal induction was observed. At this time allogeneic lymphocytes were added to TEC in 96 well micro-titre plates and proliferation of these lymphocytes was measured after 5 days by quantification of ^{3}H-thymidine incorporation. It was found that the lymphocytes failed to proliferate in the absence of exogenous IL-2.

Lymphocytes recovered after four days in bulk mixed culture with TEC were purified and restimulated with irradiated spleen cells for 5 days prior to measurement of proliferation. These lymphocytes failed to proliferate if the TEC used during the primary stimulation were syngeneic with the splenic stimulator cells. However, if the TEC used in the primary culture were from a third-party donor, the lymphocytes showed a normal proliferative response to allogeneic spleen cells.

It has been demonstrated that γ-IFN stimulated rat renal TEC fail to initiate proliferation of resting allogeneic lymphocytes. Furthermore, these TEC can induce a state of allospecific T cell anergy.

PAPER 20

OCCUPATIONAL REHABILITATION FOLLOWING RENAL TRANSPLANTATION

KM Rigg[1], PE Buckley[2], R Reaich[2], K Russell[2], G Proud[2], RMR Taylor[2]

[1]City Hospital, Hucknall Road, Nottingham, NG5 1PB and [2]Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP

Renal transplantation is cost effective for the health service, but is it cost effective for the community? Many dialysis patients are unable to work but can transplantation improve work prospects?
A self completion questionnaire was designed to record a modified Karnofsky score [KPS] (measure of physical condition on a score 1-7, 1=normal, 7=disabled), ability to work for pay, current work activity/employment status and an assessment of physical and emotional well being. The questionnaire was completed by 145 potential recipients while awaiting their cytotoxic crossmatch (91 were transplanted and 54 continued dialysis) and again at 3 and 12 months. Age, percentage retired patients, duration and mode of dialysis and initial scores were similar in both groups. The McNemar test was used for statistical analysis measuring improvement or worsening of variables. The results are as follows:

Transplant group	0	3/12	12/12	p 0v3/12	p 0v12/12
KPS [mean]	4.0	2.8	2.6	<0.001	<0.001
Ability to work for pay[%]	27	38	51	<0.01	=0.01
In or seeking employment[%]	25	35	40	}	
Unable to work (health) [%]	40	26	18	} <0.01	=0.001

Emotional and physical well-being were also significantly improved at 3 and 12 months post-transplant [p<0.001].

Dialysis group	0	3/12	12/12	p 0v3/12	p 0v12/12
KPS [mean]	3.8	4.0	4.4	ns	ns
Ability to work for pay[%]	24	20	6	ns	ns
In or seeking employment[%]	25	17	6	}	
Unable to work (health) [%]	32	41	55	} ns	ns

Transplantation improves the physical state and the ability to work, but many post-transplant patients remain unable to work for health reasons. Co-existent morbidity and duration of dialysis may account for this. Optimal occupational rehabilitation in end stage renal failure requires early transplantation.

PAPER 21

HISTOLOGICAL FEATURES OF TRANSBRONCHIAL BIOPSIES IN THE FIRST YEAR FOLLOWING LUNG TRANSPLANTATION AND THE SUBSEQUENT DEVELOPMENT OF OBLITERATIVE BRONCHIOLITIS.

Milne DS,[1] Gascoigne AD,[2] Ashcroft T,[3] Dark JH,[2] Svilland L,[1], Malcolm AJ,[1], Corris PA[2]

University Department of Pathology, Royal Victoria Infirmary,[1], Department of Pathology[3] and Sir William Leech Centre for Lung Research,[2] Freeman Hospital, Newcastle upon Tyne.

Twelve patients receiving lung transplants between 1988 and 1992 who developed clinical and histological features of obliterative bronchiolitis (OB) were compared to a group of 13 patients with good stable lung function (FEV_1 more than 80% predicted). Patients were monitored by lung function tests and transbronchial biopsy at regular intervals according to a protocol. Histological features of 180 biopsies were studied from the first post-operative year in order to assess whether any were associated with the development of OB. There was no difference in numbers of biopsies or frequency of clinically and histologically defined rejection in the 2 groups, and interstitial fibrosis and bronchial lymphocytic infiltrates were commonly identified. There was a significantly increased incidence of organising pneumonia in the OB group ($p=0.025$). A significant association was found between non-viral (predominantly bacterial) pulmonary infection, as identified by lavage, and OB ($p=0.028$) unassociated with organising pneumonia.

These findings indicate that organising pneumonia and, independently, non-viral pulmonary infections are more common in the OB group of patients. The hypothesis that OB is solely a manifestation of chronic pulmonary rejection should be questioned.

PAPER 22

DNA HETERODUPLEX ANALYSIS OF HLA-DRB ALLELES IN SEROLOGICALLY MATCHED CADAVERIC KIDNEY TRANSPLANTS.

J K Connolly[2], A J Ivinson[1], P J Sinnott[1], S Martin[1], N R Parrott[2], R W G Johnson[2], and P A Dyer[1].

N W Regional Tissue Typing Laboratory, St Mary's Hospital, Manchester[1] and Renal Transplant Unit, Manchester Royal Infirmary[2].

Current UKTSSA rules for allocation of cadaveric kidneys for transplantation include definition of HLA-DR specificities by serological techniques. Many more HLA-DRB1 alleles can be defined using DNA molecular biological methods. We have shown that even when optimal serological analyses are used prospectively they fail to define or misassign 10% of specificities as revealed by retrospective DRB1 RFLP testing. It is of concern that this lack of definition may conceal alleles of immunological importance.

Using a DNA heteroduplex technique, we have performed reterospective analysis of HLA-DRB alleles using PCR amplification of DNA from donor/ recipient cadaveric kidney transplant pairs which were allocated prospectively on the basis of two HLA-DR antigen matches using serological techniques.

From 400 transplants performed between May 1989 and May 1992 there were 187 first transplants with two HLA-DR matches and to date 67 pairs have been analysed. In 32 (48%) donor/ recipient pairs heteroduplex analysis detected differences in alleles. The alleles which most frequently showed discordance by heteroduplex analysis belonged to the HLA-DR6 and DR7 specificities; there are 16 recognised alleles of DR6 but only 2 of DR7. Interestingly, allelic discordance in HLA-DR3 (3 known alleles) was detected in only 8 of 22 donor/recipient pairs indicating homogeneity of DR3 in this donor population. There was no statistically significant difference between heteroduplex concordant and discordant donor/recipient transplant pairs when they were compared for graft survival, number and severity of rejection episodes and incidence of acute tubular necrosis.

Against the background of our many transplants performed over a 3 year period when 60% of kidneys were allocated on the basis of no HLA-DR mismatch, we conclude that a larger study using heteroduplex analysis of HLA class II alleles is needed to clarify the nature of these mismatches and their relevance to transplant survival.

PAPER 23

PROSPECTIVE HLA-DR TYPING BY PCR-SSP

D Middleton, D Savage, A Taylor

N. Ireland Tissue Typing Laboratory
City Hospital, Belfast BT9 7AD

DNA-RFLP and PCR-SSO typing have been successfully used for prospective HLA-DR typing of clinically non-urgent samples. However, due to the length of time required to perform these techniques they cannot be applied prospectively to clinically urgent samples, and in particular to renal cadaver donors.
Prospective HLA-DR typing of clinically urgent samples is now possible (in four hours) using PCR-sequence-specific priming (PCR-SSP) (1). This involves detection of allelic polymorphism within HLA-DRB second exons using oligonucleotide primers designed to amplify specific alleles or groups of alleles. The specificity is part of the PCR reaction, which means that post-amplification processing is reduced to a minimum. Assignment of alleles is based on presence or absence of amplified product following agarose gel electrophoresis.
The PCR-SSP system has been applied in this laboratory to rapidly prepared DNAs extracted from the following; (i) liquid nitrogen stored lymphocytes from spleen of renal cadaver donors (n=28), (ii) whole blood (0.5ml) from potential renal transplant recipients (n=26), potential renal cadaver donors (n=9), related bone marrow donors/recipients (n=15) and (iii) fresh spleen cells from renal cadaver donors (n=26). The results show that on 11 occasions (11%) the allele was not amplified, and on 38 occasions (39%) the positive control was not amplified. Eight of the eleven allele failures was due to too high an amount of DNA being used. Modification to resolve these problems have been introduced.

1. Olerup O & Zetterquist H (1992) Tissue Antigens 39, 225.

PAPER 24

MULTI-ORGAN DONORS - OUR EXPERIENCE

D.R.Wheeldon, C.D.O.Potter, M Jonas, J.Wallwork, F.C.Wells, S.R.Large.

Transplant Unit, Papworth Hospital, Papworth Everard, Cambs CB3 8RE

Full invasive monitoring has been carried out in multi-organ donors since October 1990. 100 complete data sets have been collected revealing data following sternotomy and again following the splanchnic dissection. 87/100 donors yielded transplantable organs including 52 hearts and 35 heart-lung blocks. 13/100 donor hearts were not transplantable of which 4/13 were found to have severe coronary artery disease and the remaining 9/13 had persistent poor function, were inotrope dependent and/or suffered from left ventricular hypertrophy. Final mean haemodynamics in the 87 donors with transplantable organs were: Arterial Pressure (MAP) = 76.1 mmHg, Central Venous Pressure = 6.6 mmHg, Pulmonary Artery Pressure (PAP) = 15.4 mmHg, Pulmonary Capillary Wedge Pressure (PCWP) = 9.2 mmHg, Heart Rate = 104 beats/min, Cardiac Index = 3.3 l/min/m^2, Systemic Vascular Resistance (SVR) = 1060 dynes.s.cm^{-5}, Pulmonary Vascular Resistance = 91 dynes.s.cm^{-5}, Left Ventricular Stroke Work Index (LVSWI) = 30.0 g.m., Right Ventricular Stroke Work Index (RVSWI) = 2.9 g.m. Significant mean haemodynamic changes between initial and final measurements in donors yielding transplantable organs (n = 87) were:- PAP: 18.4 - 15.4 mmHg (t value = 4.19, $p < 0.001$), SVR: 870 - 1060 dynes.s.cm^{-5} (t value = 3.68, $p < 0.001$), RVSWI: 4.2 - 2.9 g.m. (t value = 3.65, $p < 0.001$) - using a paired t-test. The variance in all measured and derived indices was initially large but there was a significant fall in standard deviation, although little change in the mean value, by the end of the operation in:- MAP: 21.6 - 12.6 ($z = 3.64$, $p = 0.0003$), PAP: 6.0 - 4.4 ($z = 2.07$, $p = 0.0385$), PCWP: 5.1 - 3.2 ($z = 3.33$, $p = 0.0009$), LVSWI: 16.4 - 12.3 ($z = 2.18$, $p = 0.0293$) and RVSWI: 2.8 - 2.3 ($z = 3.39$, $p = 0.0007$) - using a Wilcoxon test. Through optimum management, an initial haemodynamically very diverse group of donors, can be brought to a more standard haemodynamic state before removal of the organs. Only full haemodynamic monitoring allows complete assessment and evaluation of potentially transplantable organs.

PAPER 25

NON HEART BEATING DONORS: A POTENTIAL POOL OF KIDNEYS FOR TRANSPLANTATION

Rajasekar MR, Buckley P, Reaich R, Russell K, Proud G, Forsythe JLR and Taylor RMR

Transplant Unit, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP.

Graft function following transplantation of kidneys retrieved from non heart beating donors (NHBD) was examined.

Sixteen NHBD kidneys were retrieved by our centre from August 1990 to January1993. The perfusion of organs at retrieval was achieved by rapid perfusion through the aorta or common iliac artery using an aortic cannula or a double balloon catheter. 6 kidneys were transplanted within the region and 7 kidneys were exported to other centres in the UK. Three kidneys were unusable and of the 13 kidneys transplanted one was removed soon after clamp release. The initial warm ischaemic time (WIT), ranged from 4 to 19 minutes with a mean of 11 minutes and 45 seconds.

Examination of function following transplantation showed that there was a 50% incidence of primary non function and the mean creatinine (n = 12), at three months following transplantation was 213 micro moles/L (range: 96 - 483 micro moles/L). The figures shown in this study illustrates the possibility of retrieving kidneys from NHB donors with acceptable warm ischaemic times and that function of such kidneys compare well with those kidneys with no initial WIT. In spite of the urgency of the procedure, logistical difficulties and the organisational stress to those involved, our small series has encouraged us to pursue retrieval of kidneys from NHB donors.

PAPER 26

CORNEAL TRANSPLANT FOLLOW-UP STUDY (CTFS): AN INTERIM ANALYSIS OF FACTORS AFFECTING GRAFT SURVIVAL, REJECTION AND VISUAL ACUITY

B A Bradley, A Vail, S M Gore, C A Rogers, D L Easty on behalf of the collaborators in the CTFS

Department of Transplantation Sciences, University of Bristol

4,560 corneal grafts performed by 428 consultants in the UK and Ireland between 1987-1991 were registered under CTFS. 2385 transplants were followed-up for 450 days during which time 214 failures were observed. Overall graft survival was 95% and 89% at 3 and 12 months. Multifactorial analysis identified risk factors that influenced outcome measured either as graft failure, rejection, or visual acuity at 12 months.

Factors that increased risk of failure were: centre activity, age <10, regraft, non-visual reasons for grafting, diagnosis and pre-operative deep vascularisation. In 542 HLA typed transplants, preliminary analysis indicated that HLA-A,B, mismatched grafts exhibited the conventional and expected relationship of increased risk of rejection with increasing mismatches. HLA-DR matching gave results contrary to our expectations.

Visual acuity (VA). At 12 months VA was compared with preoperative values and shown to be worse, unchanged or improved in 7%, 10% and 83% respectively. Worse VA was associated with surgeons who reported ≤ 9 transplants, patients aged ≤10 or >50 years, regrafting, cosmetic reasons for grafting, diagnosis and preoperative superficial vascularisation.

Significant changes in graft outcome occurred at and beyond 12 months post-transplant. A more detailed analysis of outcome including astigmatism will be conducted on the definitive database after March 1993.

PAPER 27

MIZORIBINE AS AN ALTERNATIVE TO AZATHIOPRINE IN TRIPLE THERAPY IN CADAVERIC RENAL TRANSPLANTATION

LEE HA, SLAPAK M, VENKAT RAMAN G, MASON JC, DIGARD N, WISE M.

WESSEX REGIONAL RENAL UNIT (UNIVERSITY OF SOUTHAMPTON)
ST MARY'S HOSPITAL, PORTSMOUTH, HAMPSHIRE PO3 6AD

Mizoribine 3 mgs/kg of body weight, compared to Azathioprine 2 mgs/kg of body weight, has been compared in cadaveric renal transplantation in patients receiving triple therapy (other two drugs CyA and steroids). Cyclosporin A and steroid dosages have been the same in both arms of the study. Forty patients have been entered, twenty into both arms. This study is part of a multicentre trial. Both Azathioprine and Mizoribine have been well tolerated. Rejection episodes in the Azathioprine group have been one per 12.3 patient months, or an incidence of 55%. This compares with Mizoribine with a rejection rate of one per 38.8 patient months or an incidence of 20%. Mean follow up in both groups has been 7.75 months. In the Azathioprine group, there have been eight episodes of leukopaenia, six within three months, one at seven months and one at between three and twelve months. This is an incidence of 40% or a rate of 1 per 18.4 patients months. In the Mizoribine group there have been two episodes of leukopaenia (both after a course of ATG), representing an incidence of 10% or a rate of 1 per 77.5 patient months. There have been no examples of hepatotoxicity with Mizoribine.

Although there is a slight trend to hyperuricaemia in the Mizoribine treated group, this is easy to treat as Allopurinol can be used in conjunction with Mizoribine although not, of course, with Azathioprine. Our preliminary results suggest that Mizoribine is a significant advance on Azathioprine as an immunosuppressive agent working in the similar metabolic pathway of a purine synthesis inhibitor.

PAPER 28

DOES MILD REJECTION OF THE TRANSPLANTED LIVER REQUIRE TREATMENT?

L J Buist, B K Gunson, S G Hubscher*, P McMaster.

Liver Unit, Queen Elizabeth Hospital, Birmingham and Dept of Pathology, University of Birmingham.

In the early period after liver transplantation since biochemical liver function is frequently deranged many units undertake protocol biopsies to histologically assess rejection. In 338 patients, biopsies on the 7th post-operative day were histologically graded by an objective scoring system without knowledge of the clinical situation. In 71 cases features indicated severe acute rejection, 104 moderate, 109 mild and 54 no rejection. Of the mild group 12 were considered too unwell to tolerate increased immunosuppression, 50 with biochemical dysfunction received 200mg prednisolone x3 and in 47 with stable biochemistry no action was taken. The outcome of untreated, treated and those with no rejection was compared with minimum follow-up of 3 months.

Results	MILD UNTREATED (47)	MILD TREATED (50)	NO REJECTION (54)
Grafts with later acute rej	9	9	8
No of rejection episodes	14	10	9
Rejection episodes in first 3m	5	9	9
Vanishing bile ducts (3 months)	2	2	2
Later chronic rejection	3	3	1
Graft/patient loss at 3 months	2/0	2/10	3/8

These observations in clinical liver transplantation support the results of animal studies suggesting that histological features of mild acute rejection may resolve spontaneously and be of little significance, having no detrimental effect on the ultimate acceptance of the graft.

PAPER 29

SUCCESSFUL TREATMENT OF DIABETIC DOGS USING DIFFUSION-BASED HYBRID ARTIFICIAL PANCREAS DEVICES

J.P.A. Lodge, T. Maki, R. Lanza, T.E. Muller, B.A. Solomon, W.L. Chick, A.P. Monaco

Division of Organ Transplantation, New England Deaconess Hospital and Harvard Medical School, Boston; Biohybrid Technologies, Inc., Shrewsbury; and W.R. Grace & Co.-Conn., Lexington, Mass., U.S.A.

This study investigates the use of multiple tubular membrane diffusion chambers ("straws") seeded with islet allografts to normalise severe diabetes mellitus in six dogs that had undergone total pancreatectomy (exogenous insulin requirement 28-41 units/day). Canine pancreatic islets were isolated by collagenase digestion and a ficoll density gradient, suspended in a nutrient matrix and seeded into cylindrical chambers (2-4 cm X 5 mm) fabricated from permselective acrylic membranes with a nominal molecular weight cut-off of 50-80 kDa. The straws were then widely distributed throughout the peritoneal cavity.

Two animals rapidly became diabetic after a transient euglycaemic period (<30 days), probably because of islet isolation and infection problems. A third animal maintained normoglycaemia with small amounts of exogenous insulin (6-14 units/day) for one month but then deteriorated and a laparotomy on day 42 revealed many broken membranes. The remaining three dogs did not require any exogenous insulin for >50 days, with <10 units/day for >100 days. Serial intravenous and oral glucose tolerance tests showed excellent glucose clearances during the early part of the study.

These preliminary results suggest that intraperitoneal implantation of multiple diffusion chambers containing islet allografts provides an excellent potential method of islet transplantation without immunosuppression.

PAPER 30

XENOTRANSPLANTATION: EXAMINATION OF ADHESIVE INTERACTIONS BETWEEN PORCINE RENAL CELLS AND HUMAN LYMPHOCYTES.

HCC Pleass, JA Kirby, JLR Forsythe, G Proud, RMR Taylor.

Department of Surgery, The Medical School, University of Newcastle upon Tyne, NE2 4HH, UK.

It is known that adhesion between T lymphocytes and graft cells plays a vital role in the initiation and development of allograft rejection. This adhesion is mediated by specific receptor systems such as:

Lymphocyte		Graft Cell
CD2	→	LFA-3 (CD58)
LFA-1 (CD11a + CD18)	→	ICAM-1 (CD54)
VLA-4 (CD29 + CD49d)	→	VCAM-1

It is not known whether similar interactions can form between human lymphocytes and porcine cells during the rejection of putative xenografts.

Porcine renal epithelial cells were cultured and characterised by immunocytochemical staining. Cells between passes 3 and 5 were seeded into the wells of flat-profile 96-well plates and were cultured to confluency. Either resting or mitogen-activated human lymphocytes were labelled with ^{51}Cr and were added to the cultured porcine cells for 60 min. After this time non-adherent cells were removed, the remaining cells were lysed and the ^{51}Cr containing supernatant was examined by γ-spectrometry. It was found that lymphoblasts were significantly more adherent than resting lymphocytes. The adhesion of lymphoblasts was significantly inhibited in the presence of monoclonal antibodies specific for the adhesion molecules CD2, CD11a, CD18, and CD49d.

These results indicate that specific adhesion interactions can form between porcine renal cells and human lymphocytes. It is possible that therapeutic blockade of these interactions may ameliorate cellular rejection of xenografted porcine organs.

PAPER 31

RENAL ALLOGRAFT REJECTION: UPREGULATED EXPRESSION OF CLASS I MHC ANTIGENS PROTECTS TUBULAR EPITHELIAL CELLS FROM CYTOLYSIS BY NK CELLS.

JA Kirby, Y Lin, G Proud, RMR Taylor.

Department of Surgery, The Medical School, University of Newcastle upon Tyne, NE2 4HH, UK.

Activated natural killer (NK) cells lyse resting renal epithelial cells in culture and, therefore, have the potential to damage allografted renal tissue. This potential for graft cell damage was further investigated in vitro in the presence of IFN-γ and TNF-α. These cytokines are thought to be produced in vivo during rejection.

Stimulation of renal epithelial cells by culture with IFN-γ significantly enhanced the expression of both Class I and Class II MHC antigens; TNF-α had no effect on MHC antigen expression. Treatment with IFN-γ also significantly reduced the susceptibility of renal cells to lysis by IL-2 activated NK cells; TNF-α had no effect on the sensitivity of renal cells to such lysis.

Treatment of IFN-γ treated renal epithelial cells with citric acid significantly increased the subsequent sensitivity of these cells to lysis by activated NK cells. Citric acid depleted renal cells of surface Class I MHC antigens but had no effect on the expression of either Class II MHC antigens or the adhesion molecules ICAM-1, LFA-3 or VCAM-1. Cold-target inhibition experiments showed that citric acid treatment did not alter the binding between NK and renal cells.

High levels of Class I MHC antigen expression appear to block NK cell triggering after engagement with renal epithelial cell targets. The IFN-γ produced during renal rejection may protect graft cells from lysis by infiltrating NK cells.