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PAPER A — NOVEMBER 27TH, 1990

COMPUTED COLOUR ECHODOPPLER IMAGING TO MONITOR
THE EARLY POSTOPERATIVE PHASE FOLLOWING RENAL
TRANSPLANTATION

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Thirty renal allografts were monitored for the first 15 postoperative days by colour coded doppler imaging. Daily graft status was categorised as: stable, rejection, acute tubular necrosis and cyclosporin toxicity. All forms of graft dysfunction showed significantly raised resistive indices ($p < 0.01$) and pulsatility indices ($p < 0.01$), in the arcuate, interlobar and main renal arteries. It was not possible to use the doppler imaging to differentiate the true cause of graft dysfunction. However, stable grafts with high cyclosporin levels had significantly higher RI ($p < 0.04$) and PI ($p < 0.04$) in the 3 vessel groups than the stable grafts with cyclosporin levels in the recommended range. Colour coded doppler imaging can be effectively combined with standard graft monitoring techniques in the diagnosis of graft dysfunction, but may be more suitable to observe serial changes.

PAPER B

COMPARISON OF COLOUR DOPPLER ULTRASOUND AND ISOTOPE
IMAGING IN THE DETECTION OF RENAL TRANSPLANT
REJECTION

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For over a decade serial radio-isotope scanning with technetium labelled pertechnetate has been used to assess changes in blood flow to aid in the detection of rejection in non-functioning renal allografts. Serial colour Doppler ultrasound investigations also allow the detection of changes in the pattern of blood flow through the transplant kidney.

A prospective study was carried out to compare colour Doppler ultrasound and isotope imaging in 46 renal transplant recipients. All patients had both examinations performed within two days of transplantation and further examinations carried out as dictated by the clinical course. On the basis of an abnormal scan by either technique twelve episodes of acute rejection were confirmed by histological changes on renal biopsy. Ten of the twelve were identified by a pulsatility index of greater than 1.5 (ratio of peak systolic flow minus minimum diastolic flow divided by mean flow) on colour Doppler ultrasound and seven by isotope imaging. Four of the five false negative isotope studies were probably related to the absence of a satisfactory baseline investigation. Colour Doppler ultrasound appeared to be particularly useful in the early post-transplant period when the kidney may be poorly perfused leading to difficulties in interpretation of the isotope studies.

Colour Doppler ultrasound and isotope imaging are valuable and complementary techniques for the detection of early transplant rejection in non-functioning renal grafts.

PAPER C

DUPLEX DOPPLER US AND DTPA ISOTOPE SCANS IN THE EVALUATION OF PAEDIATRIC RENAL TRANSPLANTS

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Four children, (age range 16 months - 6 yrs) were examined with serial duplex doppler US and DTPA isotope scans in the post renal transplant period. The resistive index (RI) was determined from the sonographic studies, and the renal blood flow (RBF) as a% of cardiac output was calculated from the isotope scans. The clinical status of the child and the corresponding plasma creatinine levels were assessed together with these two parameters. RI ranged from 40-145% and RBF from nil to 16.8%. A fall in RBF preceded a rise in plasma creatinine and a clinical and histologically proven rejection episode on 2 occasions and a rise in RBF preceded a fall in plasma creatinine level on 3 occasions. In 1 patient the RBF remained low throughout a period of acute tubular necrosis and increased as primary function was established and the plasma creatinine fell. The RI did not show much variation within each patient and the changes did not appear to reflect changes in plasma creatinine. There was however considerable variation in the values obtained for RI between patients, reinforcing the need for establishing a normal range within the paediatric population. From our preliminary data the RBF predicts changes in graft function more accurately than does the RI.

PAPER D

ROLE OF DUPLEX IMAGING AND FINE NEEDLE ASPIRATION CYTOLOGY OF RENAL ALLOGRAFTS IN MONITORING RESPONSE TO ANTI-REJECTION THERAPY

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The role of duplex imaging and fine needle aspiration cytology (FNAC) in monitoring the response to anti-rejection therapy was investigated in 14 of the 22 rejection episodes which occurred in 30 renal allograft recipients. In 9 of these 14 episodes of rejection, with good resolution, both resistive (RI) and pulsatility (PI) indices decreased by significant proportions ($p < 0.05$). The mean FNAC scores also fell significantly from 5.6 ± 5.0 with anti-rejection therapy to 2.9 ± 2.2 . In a small group of patients both FNAC and Doppler predicted rejection.

In 5 episodes of rejection, where the graft continued to deteriorate, there was no significant fall of RI and PI ($p > 0.2$). Biopsy data in this group indicated a vasculitic type of rejection. One graft in this group was lost despite conversion to triple drug therapy.

In conclusion, both duplex imaging and FNAC have a role in selection and optimal modulation of drugs in the treatment of acute renal allograft rejection.

PAPER E

COLOUR FLOW MAPPING AND VELOCITY MEASUREMENTS IN RENAL ALLOGRAFT REJECTION

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Colour flow mapping greatly facilitates Doppler examination of the transplanted kidney. The main advantage, however, is that it enables serial measurements to be made in the same vessels and makes velocity measurements, corrected for the angle of insonation possible.

Detailed studies of velocities and systolic diastolic ratios in native and transplanted kidneys show that values change at each level of branching, the change being related to the branch angle. It is this that explains why a range of value is normally found when sampling different parts of the kidney. This limits the interpretation of Doppler examinations. Vascular and severe cellular rejection are not a problem as they produce very abnormal values. Mild or moderate cellular rejection, however, may produce values which overlap with the normal range. Colour flow enables serial measurements to be made in the same vessels. We believe that this may increase the sensitivity of the test in cases of mild or moderate cellular rejection.

Velocity measurements give a further dimension to the Doppler study. Chronic rejection gives normal systolic diastolic ratios, but a gradual reduction of velocities. Acute tubular necrosis gives reduced velocities in the main branches but more normal values in the peripheral vessels. Acute rejection only gives reduced systolic velocities in prolonged severe episodes.

PAPER F

COLOUR DOPPLER FLOW VELOCITY MEASUREMENTS IN RENAL TRANSPLANT DYSFUNCTION.

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This paper presents a novel method of estimating renal transplant perfusion using measurements of intra-renal peak arterial velocities.

Several centres have reported the use of Doppler ultrasound to assess circulation changes within renal transplants during episodes of poor function. To date, much reliance has been placed on descriptions of flow waveform shape (e.g. pulsatility index and resistive index) because of the inability to measure renal blood flow, or absolute blood velocities within the small vessels of the kidney. While these indices have been shown to be reliable in cases of acute rejection, ambiguous results have been reported in cases of chronic rejection, acute tubular necrosis and Cyclosporin toxicity.

Colour Doppler imaging offers several improvements on conventional duplex Doppler ultrasound. Areas of high or low perfusion are readily appreciated and the colour flow image may be used to speed the pulsed Doppler measurements. Furthermore, by showing the course of the vessels, the pulsed Doppler cursor may be aligned with the vessel, thereby permitting Doppler angle correction to give absolute velocity measurements.

We have employed colour Doppler imaging to investigate 134 new renal transplants and over 145 established grafts. Colour Doppler imaging has been shown to be a reliable method of examining large vessel causes of renal dysfunction; for the screening of renal artery stenosis and to monitor arteriovenous fistulas and venous problems.

In measuring changes to renal circulation during poor function, we have observed that there is a wide range of pulsatility indices (PI = 0.8 to PI = 1.8) between patients with 'normal' renal function (plasma Cr < 150 $\mu\text{mol/l}$), and that these values overlap those of grafts undergoing rejection reported elsewhere. Pulsatility indices are affected by many factors including heart rate, and the condition of the proximal and distal vasculature.

We have utilised intra-renal velocity measurements as a further indication of change to renal perfusion. Provided a sufficient length of vessel (>6mm) is imaged, peak flow velocity can be measured. The site of the vessel is measured as a depth from the renal capsule. By plotting peak velocity vs. depth from the capsule, an index of perfusion is measured. Index values for normal function range from 10-15 cm/s/cm over the outer medulla and cortex. This index has been observed to fall as low as 4cm/s/cm in chronic rejection despite pulsatility indices remaining within the normal range.

Absolute velocity measurements of intra-renal flow have also been helpful in assessing the impact of arterial stenoses and fistulas on the circulation when indices of flow waveform pulsatility become unreliable.

PAPER G

USE OF MAGNETIC RESONANCE IMAGING IN THE DIAGNOSIS OF EARLY TRANSPLANT DYSFUNCTION

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Twenty patients who showed signs of graft dysfunction within 2 months of renal transplantation were assessed by both magnetic resonance imaging (MRI) and ultrasound (US). All were receiving Prednisolone and Cyclosporin and 2 had grafts from living related donors. Sixteen of the 20 patients were investigated because of primary non-function and the remaining 4 during episodes of secondary dysfunction.

MRI was performed by a radiologist who was not aware of the clinical diagnosis using a 0.15 Tesla resistive magnet. The initial examination was carried out 6 to 47 days after transplantation and repeated a week later in 4 patients. Ultrasound examination was undertaken within 48 hours of each MRI scan and in 19/24 instances combined with Doppler assessment (DA).

For each patient a clinical diagnosis was ascribed on the basis of the clinical and biochemical picture, cyclosporin levels, response to therapy, serial technetium scans and in 12 cases transplant biopsy. Diagnoses included acute tubular necrosis (n = 5), acute rejection (11), cyclosporin nephrotoxicity (1), combined rejection and cyclosporin nephrotoxicity (2) and infection (1). MRI agreed with the clinical diagnosis in 14/24 (58%) episodes of dysfunction and US in 15/24 (62%) episodes. When DA was combined with US the presumed correct diagnosis was made in 14/19 (74%) cases.

The results of this study suggest that MRI is no better than US alone in the assessment of early renal transplant dysfunction and is inferior to combined US and Doppler examination.

PAPER H

DOPPLER ULTRASOUND FOR THE ASSESSMENT OF HEPATIC ARTERY PATENCY IN LIVER TRANSPLANT PATIENTS

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Hepatic artery occlusion threatens graft survival and leads to significant morbidity and mortality in liver transplant recipients. It occurred in 25 of the first 300 liver transplants performed in Birmingham.

This incidence accords with published experience from other centres. When thrombosis of the artery causes fulminant hepatic failure early retransplantation is required (unless, exceptionally, thrombolytic therapy is successful). In less severe cases retransplantation may not be necessary but biliary complications and sepsis are common.

Duplex Doppler offers the potential for non-invasive diagnosis and should reduce the need for contrast angiography in the post transplant patient. This study aims to prospectively assess all liver transplant patients within 24-48 hours of surgery by real-time and duplex Doppler ultrasound. The examinations are repeated at one week, one month and three months. Interval examinations are performed as clinically indicated. In cases with equivocal findings the ultrasound is repeated within 24 hours. Angiography is performed to confirm hepatic artery occlusion if predicted by Doppler or if doubt persists.

Details of the surgical anatomy, operative complications, intra-operative flow measurements and post-operative events such as shock or rejection and other complications within the first three months were recorded. We have now studied 50 patients. There have been 6 hepatic artery occlusions.

The results of the studies will be presented and discussed.

PAPER 1

ULTRASOUND STUDIES OF PANCREATIC ALLOGRAFTS

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Pancreatic transplants are performed concomitantly with renal transplants in diabetic patients. The pancreatic vessels are anastomosed to the recipient's iliac vessels and the pancreatic duct is implanted into the bladder.

Doppler examination of the transplanted pancreas is more difficult than of the kidney, as the pancreas is a less vascular organ, and the main vessels in the body lie at a disadvantageous angle to the Doppler beam. Colour flow mapping greatly facilitates the examination however.

Rejection episodes are characterized by reduction in diastolic flow or, in severe episodes, reversed diastolic flow. The pattern of changes is identical to that in the transplanted kidney, except that the normal values are different. Venous thrombosis gives absent venous flow and, in the arterial signal, a typical narrow systolic peak and reversed diastolic flow. Prolonged rejection also produces pancreatic swelling and fluid around the pancreas.

Ultrasound with duplex Doppler may, therefore, be used to monitor the progress of pancreatic allografts, though colour flow mapping is very desirable to facilitate the examination.

PAPER 1 — NOVEMBER 28TH, 1990

TISSUE TYPING - STATE OF THE ART

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Spleen or lymph node from the renal donor and white blood cells from the corresponding renal recipient were sent from 82 centres to one laboratory. DNA was extracted from these samples and examined for HLA-DR antigens by Restriction Fragment Length Polymorphisms (RFLP) in a number of laboratories.

A total of 2252 individuals were tested and an overall discrepancy rate of 29.5% was found between the serological type and the RFLP type.

The discrepancies varied by centre ranging from 0% (in centres who had submitted data on less than 10 individuals) to 81%. The maximum variation in replicate samples by RFLP was 5.1%.

The discrepancy rate in HLA-DR antigens detected ranged from 6.7% (DR7) to 79% (DRw13-DQw3). However this was markedly reduced when laboratories with an overall high discrepancy rate were removed from the analysis.

These results have serious implications for the sharing of kidneys. For the majority of antigens the discrepancy rate is due to the false detection of another antigen rather than to the antigen being typed as a blank. Thus even in 6 - antigen matched grafts there are problems in the accuracy of the typing.

PAPER 2

DONOR CROSS-MATCH MATERIAL PREPARATION AND STORAGE: A SURVEY OF PRACTICES IN THE UNITED KINGDOM (UK) AND IRELAND

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In our recent experience a kidney was received as a "beneficial match" having been positively cross-matched at another centre. Whilst the organ was potentially viable the cross-match was impossible as all available lymphocytes were dead. Is cross-match tissue viability a problem elsewhere? A survey of 26 transplant units in the UK and Ireland covered the following; experience of non-viable cross-match material, donor pre-treatment, quantity, storage medium, and timing of lymph node and splenic biopsies.

Replies were obtained from 73% (19) of transplant units. Thirty seven percent of units had received kidneys with non-viable cross match lymphocytes as a result of which kidneys were not transplanted in all but one case. Fifty-three percent of units routinely give renal donors drug treatment prior to organ retrieval. Three transplant units routinely took the whole spleen for cross matching whilst the majority (53%) allocated 2 cm³ with each kidney. The mean number of lymph nodes taken was 4.9 (range 3-10). The storage medium for both spleen and lymph node specimens varied widely; 63% used saline (0.9%), 21% organ preservation fluid and 16% tissue culture medium. All splenic specimens and 95% of lymph node biopsies were taken after in-situ perfusion at the end of the organ retrieval. Conclusion: Donor kidneys are being wasted because of inadequate cross-match material. Further efforts to improve graft survival by optimising HLA matching and organ preservation will be thwarted by inadequate collection and storage of cross-match lymphocytes.

PAPER 3

HISTOCOMPATIBILITY AND LIVER TRANSPLANT OUTCOME

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Although HLA matching has a beneficial effect in other solid organ transplants, in liver transplantation the effect of HLA matching on allograft survival is unclear. We have performed prospective HLA typing on 369 primary liver transplants recipients between June 1985 and June 1990. Donor/recipient data were available for 324 patients. Of 369 pairs typed for HLA A and B, 160 pairs were matched for 1 and 16 pairs for 2 A antigens, and 101 pairs were matched for 1 and 6 pairs for 2 B antigens. Of 324 pairs typed for DR, 136 pairs were matched for 1 antigen and 9 pairs for 2 antigens. We compared graft survival using Breslow (early effect) and Mantel-Cox (late effect) tests, in donor/recipient pairs receiving one or two matches at either HLA A, B or DR with pairs receiving zero matches at either HLA A, B or DR. Whilst there was no effect of HLA A matching ($p = 0.8107$ Breslow, and $p = 0.958$ Mantel-Cox), there was a highly significant benefit of B matching on survival ($p = 0.0082$, Breslow and $p = 0.0251$ Mantel-Cox). DR matching had no effect on transplant survival ($p = 0.9506$ Breslow, and $p = 0.8876$ Mantel-Cox).

These data indicate that HLA matching at the B locus may prolong graft survival in primary liver transplants. The absence of a significant effect of HLA DR matching in this series, together with the earlier observation that HLA DR matching increases the risk of chronic graft rejection (1), support the Pittsburg suggestion that there may be a dual effect of HLA matching in liver transplantation (2).

(1) O'Grady J, et al. Lancet 1988; *ii*: 302-305.

(2) Markus B, et al. Transplantation 1988; *46*, 3: 372-377.

PAPER 4

IS THERE A RELATIONSHIP BETWEEN HLA HISTO-COMPATIBILITY AND ACUTE LIVER ALLOGRAFT REJECTION?

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Although the importance of HLA in renal transplantation is well recognised, the importance of HLA in liver transplantation remains uncertain. Previous studies have shown no beneficial effect of HLA compatibility on 1 year survival or the development of chronic rejection (1). In this study we looked at HLA histocompatibility and the incidence of biopsy proven acute rejection in the first 12 weeks post-transplant. Complete typing data for HLA-A, B and DR antigens were available for 32 donor recipient pairs; 18 patients (56.3%) developed acute rejection (grade II or above) of which 5 had steroid resistant rejection requiring OKT3 and 6 required re-transplantation for severe rejection.

These patients had a mean of 1.9 DR mismatches, 1.8 mismatches at the A locus and 1.9 mismatches at the B locus. In contrast 14 patients (43.7%) who had no rejection in the first 12 weeks had a mean of 0.7 mismatches on the DR locus, ($p = <0.05$ compared to rejectors); 1.3 mismatches on the A locus and 1.4 mismatches on the B locus (N.S.). These preliminary results indicate that in contrast to other authors HLA matching and in particular DR matching may have a beneficial effect on the incidence of acute rejection in the liver allograft recipient. With the possibility of improved organ storage, distribution of livers according to match may be desirable for semi-elective cases.

1. O'Grady et al. Lancet 1988; ii: 302-305.

PAPER 5

THE INTRA-OPERATIVE USE OF TRASYLOL (APROTININ) IN LIVER TRANSPLANTATION

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Orthotopic liver transplantation is frequently complicated by severe coagulopathy and blood loss. Accelerated fibrinolysis has been identified as an important component of the haemostatic disorders that contribute to perioperative bleeding. Aprotinin has been reported to reduce blood loss in difficult cases requiring cardiopulmonary bypass surgery and more recently in orthotopic liver transplantation.

In a pilot study we have compared the effects of Aprotinin on transfusion requirements and coagulation profiles in 12 patients under going OLT for end stage liver disease with an equal number of consecutive transplants in patients with similar pathology who did not receive Aprotinin. Aprotinin was given as a loading dose of 2,000,000 Kallikrein inhibitory units (KIU) followed by an infusion of 500,000 KIU/h until return to the ITU. In addition 70,000 KIU were given with each unit of blood transfused.

Intraoperative transfusion of blood products was reduced to less than one third in the Aprotinin treated group. Operative times were also significantly reduced and as would be expected, the improved perioperative course resulted in a significant decrease in ITU stay. Results of our haematological data confirm that Aprotinin causes profound inhibition of fibrinolysis and this is likely to be the major mechanism by which blood loss is produced. Thrombelastography (TEG) revealed severe fibrinolytic changes in the anhepatic stage in 4 out of 6 control patients which accelerated in 3 following reperfusion of the new graft. The degree of fibrinolysis was related to the degree of blood loss. By contrast in the Aprotinin treated group only 1 out of 12 patients showed fibrinolytic activity on thrombelastography in the anhepatic period and there was no evidence of fibrinolysis in any patient following reperfusion.

PAPER 6

RESUSCITATION OF LIVER METABOLISM BY BRIEF HYPOTHERMIC
PERFUSION - NMR SPECTROSCOPIC STUDIES IN RAT LIVER UP TO 48 H.

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Phosphorus NMR spectroscopy provides a unique, non-invasive method for studying organ metabolism during cold preservation. We have previously shown in rat liver (1) that short (20 min) periods of cold reperfusion are capable of restoring liver adenine nucleotides (ATP + ADP) and correcting acidosis following flush preservation. The present study was undertaken to compare the recovery potential of flush-stored livers using a lactobionate / raffinose (UW or Wisconsin-type) solution and an extracellular (Ex) solution after prolonged periods of ice-storage (24 & 48 h).

Donor rat livers were harvested as described previously (1), and placed on the cold perfusion circuit in the bore of an 8.5T magnet attached to a Bruker AM360 spectrometer for baseline ³¹P NMR study. The vascular bed of the organs was flushed with UW or Ex. Thereafter the organs were stored in ice for 24 or 48 h (n=5 in each group). Subsequently, the livers were returned to the magnet for metabolic evaluation.

Immediately after harvesting the livers exhibited signals for ATP + ADP and pH similar to in vivo values. By 24 h cold storage, all signals for ATP + ADP had disappeared, inorganic phosphate (Pi) had accumulated and pH fell (<6.8 in both). A similar picture was seen after 48 h. During hypothermic reperfusion after 24 h storage ATP + ADP recovered to 85% of initial values, Pi fell by 70% and pH recovered to 7.2. After 48 h in UW ATP + ADP returned to 80% , Pi fell by 60% and pH to 7.1. However in Ex, ATP recovery was only 30%, Pi only fell by 20% and pH returned only to 6.93. Thus UW protected metabolic recovery in long-term (> 24 h) storage. Brief hypothermic reperfusion may be useful as a method for resuscitating liver metabolism following storage and may also be developed in future as a viability assessment.

Work supported by the Leverhulme Trust and the MRC.

(1) Fuller B, Busza A & Proctor E, 1990. Transplantation : In Press

PAPER 7

CHANGES IN SMALL INTESTINAL MICROFLORA FOLLOWING SMALL BOWEL
TRANSPLANTATION IN THE RAT

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Quantitative and qualitative changes in rat small intestinal microflora were studied following small bowel isotransplantation. Graft vessels were perfused with Marshall's solution and the lumen flushed with 0.05% chlorhexidine before transplantation. Both ends of the graft were exteriorised. All animals received gentamicin 1 mg s.c. per-operatively. Groups of 6 animals were sacrificed at 2, 7 and 28 days. The host and graft bowel were flushed with 10 ml sterile saline. Effluents were mixed, serially diluted and 1 ml of 5 dilutions cultured aerobically and anaerobically at 37C. Colonies were counted and identified.

	Bacterial counts(per gram dry weight bowel)		
	2 days	7 days	28 days
Host	6.3x10 ⁶	9.0x10 ⁶	6.6x10 ⁶
Graft	170x10 ⁶ *	350x10 ⁶ *	9.6x10 ⁶

* p<0.05 compared to host

Aerobic faecal organisms predominated at 2 and 7 days in both graft and host bowel but an overgrowth of Flavobacterium meningosepticum occurred at 28 days in both the transplant and host bowel. The grafted bowel is inevitably denervated and the change in bacterial flora could be caused by either this factor or ischaemic damage. Microsurgical division of all nervous tissue around the superior mesenteric artery was undertaken in 6 animals and bacteriological changes studied at 7 days. The mean colony counts were lower but not statistically different from controls at 1.7x10⁶. The observed changes in bacterial flora following small bowel transplantation are therefore due to the late effects of ischaemia and reperfusion injury.

PAPER 8

HLA-C ANTIGEN MISMATCHES - ARE THEY RELEVANT IN TRANSPLANTATION?

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There is good evidence that HLA matching improves graft survival. The role of matching for the antigenic products of the different MHC genes has been studied. HLA-DR exerts the strongest influence on graft survival followed by the B locus. The role of the A locus products is unclear.

Matching for HLA-C locus products has received little attention mainly because of the difficulties with which these antigens are detected by serology. Rejection of renal grafts due to C locus mismatches has been documented as has the production of specific anti-Cw antibodies post rejection.

We have investigated two unsuccessful cases of bone marrow transplants by novel techniques involving one dimensional isoelectric focusing (1D-IEF) for variants of Class I antigens, including HLA-C locus products. Patient NS died from acute GvH disease and patient MH rejected the graft. The 1D-IEF revealed C locus mismatches in both patients. The mismatch in patient NS was in the GvH direction and in patient MH, the mismatch was in the host versus graft direction. No other Class I mismatches were detected.

Whether C locus mismatches contributed to the clinical outcome in the two patients is unclear. The possible role of Cw antigens in marrow transplantation and in solid organ transplants requires further study.

PAPER 9

GRAFT T LYMPHOCYTE DEPLETION IN THE PREVENTION OF GRAFT VERSUS HOST DISEASE (GVHD) IN SMALL BOWEL TRANSPLANTATION

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In this study graft pre-treatment with a specific T cell immunotoxin was used in an attempt to prevent GVHD in a rat model of small bowel transplantation. The immunotoxin consisted of a conjugate of A-chain ricin with the mouse anti-rat CD5 monoclonal antibody OX19. Small bowel grafts were perfused with the immunotoxin *ex vivo* before transplantation.

Five groups of parental strain to F₁ hybrid strain animals were transplanted with vascularised Thiry-Vella loops of small bowel.

	Control	OX19	Immunotox	CsA	Immunotox + CsA
Median Survival	20	21	29*	32*	49#

* p<0.01 cf control, # p<0.01 cf other groups

Immunotoxin infusion was compared with mesenteric lymphadenectomy (MLE) in fully allogeneic transplants between DA and PVG rat strains. Graft cell migration to host tissues was studied by immunoperoxidase staining using the strain specific monoclonal antibodies MN4 (DA ClassI) and OX27 (PVG ClassI).

	Percentage migration of host cells to graft tissues		
	spleen	MLNode	Peyers patches
No therapy	0	3	25
CsA	47	47	41
CsA + immunotoxin	40*	41*	32* * p<0.05 compared
CsA + MLE	32*	27*	26* with CsA group

Although the use of immunotoxin was unable to prevent the development of GVHD it significantly delayed its onset and enhanced survival when combined with low dose cyclosporin. In the fully allogeneic combination immunotoxin significantly reduced graft cell migration to host tissues but not to the extent seen with mesenteric lymphadenectomy.

PAPER 10

THE HUMAN RECONSTITUTED (SCID-hu) SEVERE COMBINED IMMUNODEFICIENT (SCID) MOUSE. A MODEL FOR ISOGENEIC AND ALLOGENEIC HUMAN ISLET TRANSPLANTATION

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The purpose of this study was to develop an in vivo model for assessing human islet allograft survival. SCID mice were reconstituted with a human immune system by the intraperitoneal injection of 5×10^7 splenocytes (prepared from organ donor spleens). Ten days after reconstitution the mice had high levels of human immunoglobulin in their sera and responded to a tetanus toxoid injection with the production of human anti-tetanus toxoid IgG. Analysis of the reconstituted SCID spleens showed numerous T cell areas (CD3 +ve) and follicles containing B cells (CD19 +ve). The model of isogenic transplantation was provided by reconstituting with splenocytes from an organ donor and transplanting islets from the same donor to the renal subcapsular space. The model of allogeneic human islet transplantation was provided by splenic reconstitution followed by transplantation with islets from an unrelated donor.

We found that isogenic transplants were accepted with no evidence of rejection. Allogeneic transplants however were rejected and immunohistology showed that the infiltrating cells were of human lymphoid origin. The majority of these cells were T cells (CD3 +ve) of which 80% were cytotoxic effector cells (CD8 +ve) and the remainder CD4 +ve helper T cells. In conclusion the SCID-hu provides an excellent model system in which to study human islet immunogenicity.

PAPER 11

PANCREATIC DIGESTION AND ISLET RELEASE: A DIRECT COMPARISON OF STANDARD AND AUTOMATED TECHNIQUES

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The isolation of human islets of Langerhans for transplantation depends on the production of a pancreatic digest containing large numbers of intact, viable islets, free of surrounding exocrine tissue. Using the porcine pancreas, as a model for the human pancreas, the effectiveness of the two best available digestion techniques was assessed. Seven consecutive pig pancreata were obtained from the local abattoir, distended with warm (34°C) collagenase and sectioned longitudinally, along the line of the duct. The two halves were digested by either a standard or automated technique. The standard technique involved manual teasing apart of the gland when a representative biopsy had shown the start of digestion. The automated technique involved progressive digestion and liberation of islets in a mechanical isolator, which was continually agitated and connected to a circuit of continually flowing medium. The digest in each case was examined microscopically, using dithizone staining. The digests were also placed onto continuous density gradients using bovine serum albumin at 400 mOsm/kg, for visual purity estimations. The results are tabulated below. All values are median (range).

	Mean Islet Volume	Islet Tissue/ gm pancreas	150 nm Islet Equivs/gm	Purity %
Standard (n=7)	$3.1 \times 10^{-4} \text{ mm}^3$ (2.1-4.06)	1.16mm ³ (0.41-1.92)	655 (231-1084)	61 (53-70)
Automated (n=7)	$8.1 \times 10^{-4} \text{ mm}^3$ (4.4-10.5)	3.34mm ³ (1.38-5.3)	1887 (779-2994)	71 (65-78)

The automated digestion technique significantly improved ($p < 0.05$) the number of islets and total volume of islet tissue produced.

PAPER 12

CYCLOSPORIN A IN RAT PANCREATIC ALLOGRAFT REJECTION: HISTOLOGY, FUNCTION AND PROSTANOID SYNTHESIS.

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Cyclosporin A (CYA) is toxic to liver, kidney and pancreatic B cells. Reduced visceral perfusion, consequent upon reduced prostacyclin synthesis may contribute to this toxicity. In a rat model of pancreas transplantation, morphological features of acute allograft rejection were abrogated by enteral administration of CYA 15mg/kg/day from the day of transplantation. Serum glucose values in the CYA-treated allograft group corresponded with those of isograft controls, but untreated allografts were all hyperglycaemic by the 9th post-transplant day with significantly greater values than the other two groups (both $P < 0.01$).

Transplant prostacyclin and thromboxane synthesis was assessed by RIA of their stable hydrolysis products in *in vitro* incubation media. Thromboxane synthesis by allografts significantly exceeded that by isografts from the 5th post-transplant day ($P < 0.01$), but there was no significant difference in prostacyclin synthesis by these groups. Synthesis of both prostanoids in CYA-treated allografts corresponded with isograft, rather than allograft values.

Thus, the immunosuppressive effect of CYA in rat pancreas transplantation was not associated with prostanoid changes which may impair graft perfusion and the transplant function was unaffected.

PAPER 13

A COMPARISON OF THREE TECHNIQUES FOR RENAL SUBCAPSULAR ISLET TRANSPLANTATION IN THE RAT

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The purpose of this study was to compare the results of three methods of syngeneic renal subcapsular transplantation in the rat. Islets were transplanted under the kidney capsule in one of three ways - (1) islets alone; (2) in an isologous blood clot; (3) in an isologous plasma clot. The recipient rats had been rendered diabetic by streptozotocin. We defined reversal of diabetes as the return of blood glucose to < 10 mmol/l by day 14. The table below details the number of successful transplants in each group:

Number of islets transplanted	Islets alone	Islets in plasma clot	Islets in blood clot
1000	6/6	3/6	3/6
750	6/6	1/6	0/6
500	3/6	1/6	0/6

It can be seen that the most efficient technique was the transplantation of islets alone with 6/6 transplants successful using 750 islets compared to 1/6 and 0/6 for the other groups ($p < 0.008$). We conclude that the optimum transplantation method is islets alone and that any possible benefit from trophic factors in plasma or blood is outweighed by the fact that islets in a blood or plasma clot are distanced from the blood supply on the kidney surface. This finding has important implications for human islet transplantation.

PAPER 14

IN VITRO STAINING OF ISLETS OF LANGERHANS FOR FLUORESCENCE-ACTIVATED SORTING

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Previous techniques for purification of pancreatic islets by fluorescence activated sorting used the dye neutral red (NR) to label the islets. A major drawback with this technique was need to inject the dye intravascularly prior to excision of the pancreas. Preliminary investigations showed that topical NR would only produce selective staining of islets at low concentrations insufficient for sorting.

The chelating agent dithizone (DTZ) produces bright red staining of islets by topical application in vitro. Dithizone stained islets exhibit moderately strong fluorescence that fades too quickly for reliable sorting. By combining both NR and DTZ staining in vitro selective fluorescence of islets was obtained that was sufficient to allow efficient sorting. Using the combined DTZ/NR stain the yield of islets obtained from a single rat pancreas was 569 ± 72 ($n=16$), corresponding to 83% of the islets originally present in the digest. The mean purity of the preparation, confirmed by histological examination, was 80%. The viability of the islets was shown to be good by supravital staining and successful cure of streptozotocin diabetes in syngeneic rats following transplantation of sorted islets.

The development of in vitro staining for fluorescence activated sorting of islets represents a significant advance in the practical application of the technique.

PAPER 15

THE ORIGIN OF HLA-DR/Dw PCR FINGERPRINTS AND APPLICATIONS TO CLINICAL TRANSPLANTATION

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We recently described a new technique for HLA-DR/Dw matching termed PCR fingerprinting (Bidwell and Hui, *Technique* 2:93-100 (1990)), which rapidly identifies DR/Dw compatibility by the polymerase chain reaction (PCR) and non-denaturing polyacrylamide gel electrophoresis (N-DPAG). Unique patterns of PCR DNA products (satellites) are observed for all DR haplotypes so far examined. The 8-hour technique requires no probes, restriction enzymes or isotopes. The present study reveals the exact origin of satellite DNAs. These are formed by the association of heterologous single stranded DNAs derived from different DRB loci, during the final PCR annealing stage. Such "heteroduplexes" vary in number, molecular conformation and gel mobility in a haplotype-specific manner. PCR fingerprints may be reproduced by the denaturation and reannealing of the gel-purified homoduplex progenitor molecules. Conversely, heteroduplexes are reduced to iso-mobile component molecules under denaturing conditions. The degree of nucleotide mismatching between heterologous DNA strands alters the extent of retardation in mobility on N-DPAG gels. We describe how DRw8 and DR4 pseudogenes may act as "universal heteroduplex generators" in cases where DR/Dw non-identity reveals similar PCR fingerprints, and how the technique may be used in the rapid selection of DR/Dw compatible transplant donors.

PAPER 16

POSITIVE FLOW CYTOMETRY CROSSMATCHES ARE IRRELEVANT FOR RENAL TRANSPLANTATION

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The role of the conventional complement dependent crossmatch in renal transplantation is still controversial with a major criticism being its limit of sensitivity. Crossmatching by flow cytometry allows very low levels of immunoglobulin (IgG) binding to be detected but its clinical significance is still to be determined.

From the last 51 consecutive renal transplants a total of 74 conventional crossmatches were performed. Flow cytometry crossmatch (FAC) were performed prospectively in 54 cases but not used for clinical management. 36 of these were from subsequently transplanted patients. The acute serum sample and historical samples with highest panel antibody reactivity (PRA) were assessed for IgG binding to CD 3 +ve donor lymphocytes. Levels of IgG binding were correlated with delayed graft function (dialysis dependent within first week post transplant), function at one month and incidence of rejection with the first month. A summary of the results is presented below.

	Sample	Flow Cytometer Crossmatch (number of transplanted patients)	
		Negative	Positive
Delayed function	Acute	12/25	6/11
	Historical	11/21	5/12
Rejection within first month	Acute	6/25	4/11
	Historical	6/21	3/12
Function at first month	Acute	21/25	8/11
	Historical	16/21	10/12

Only the incidence of rejection in the acute sample showed any level of significant difference ($p = <0.5, >0.2$) between negative and positive FACs. Positive FACs appeared to correlate with the level of PRA, and with the conventional complement dependent crossmatch. The flow cytometer crossmatch does detect low levels of IgG binding but this, in the short term, does not appear to be clinically relevant.

PAPER 17

IMPROVED IMMEDIATE FUNCTION IN CADAVERIC RENAL ALLOGRAFTS BY AGGRESSIVE DONOR MANAGEMENT AND USE OF UW SOLUTION

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Evidence exists to suggest use of Beltzer's UW perfusion solution, and better donor management (eg use of low dose dopamine, avoiding warm ischaemic time and better donor hydration) will improve immediate function (IF) in cadaveric renal allografts.

We have studied locally transplanted kidneys from 41 consecutive local donors where the kidneys were perfused with UW and the above variables were optimised (Group A) to examine the effect on IF. As controls, we studied all imported kidneys perfused with hypertonic citrate during the study period (Group B), and all local kidneys (Group C) and imported kidneys (Group D), also perfused with hypertonic citrate transplanted during the 12 months prior to the study.

	No of donors	IF1		IF2		Days to 1/2 creat. mean \pm SD	Donor urine last hour mean \pm SD
		No of kidneys	IF (%)	No of kidneys	IF (%)		
Group A	41	47	31(66%)	41	31(74%)	5.6 \pm 6	336 \pm 258
Group B	44	46	21(46%)	41	21(51%)	10.8 \pm 8.4	187 \pm 154
Group C	29	36	15(42%)	31	15(49%)	10.4 \pm 9.3	218 \pm 143
Group D	34	34	17(50%)	29	17(57%)	12.0 \pm 12.4	235 \pm 230

IF1 - all kidneys. IF2 - excluding those which never functioned.

A significant improvement in IF was found between Group A and Groups B and C ($p < 0.05$) both when all kidneys were included and after kidneys which never functioned were excluded. There was also a significant reduction between Groups A and all other groups ($p < 0.005$) in the number of days following transplantation for the serum creatinine of the recipient to be reduced to half the pre-transplant level.

These data suggest that the IF rate of cadaveric kidneys can be improved by aggressive management of the donor combined with the use of UW solution for kidney preservation.

PAPER 18

HYPERPARATHYROIDISM PROTECTS AGAINST DELAYED FUNCTION OF RENAL ALLOGRAFTS

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It has been suggested that high levels of parathormone (PTH) may cause delayed function of renal allografts [1].

We have investigated this in a prospective study using a two site immunochemiluminescent assay for intact PTH.

89 consecutive patients had PTH measured prior to transplantation. 18 patients had delayed function, defined as the requirement for dialysis or failure of serum creatinine to fall by 15% within 4 days of transplantation.

14 patients had PTH levels within the normal range (0.9-5.4 pmol/l) and 8 of them had delayed function of their graft. PTH levels were significantly lower in the delayed function group (5.58(1.9-40.2) pmol/l median and quartile deviation) than in the immediate function group (25.3(6.4-97) pmol/l median and quartile deviation) ($p < 0.002$, Mann-Whitney U test).

There was no difference in other factors thought to influence delayed function (donor and recipient age, calcium channel blocker administration and total ischaemia time). PTH is known to be a renal vasodilator and may protect against vasospasm following reperfusion of renal allografts.

Our results differ from previous findings because the assay we have used detects only intact PTH and not the inactive C-terminal fragments that accumulate in chronic renal failure.

- [1] Varghese Z, Scoble JE, Chan MK, et al. Parathyroid hormone as a causative factor of primary non-function in renal transplants. *Brit Med J* 1988;296:393.

PAPER 19

LATE PERFUSION: A PROSPECTIVE RANDOMIZED COMPARISON OF INTRA- AND EXTRA-CELLULAR PRESERVATION SOLUTIONS.

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Late perfusion with hyperosmolar citrate (HOC) immediately prior to transplantation can reduce the incidence of delayed renal allograft function (1). In this prospective trial 30 kidneys from adult heart-beating donors stored in HOC were randomized to receive [A] no reflush, [B] reflush with 500 ml HOC, or [C] reflush with 500 ml phosphate buffered sucrose (PBS140). All patients were treated by a standard intra- and post-operative fluid and immunosuppression protocol. On days 1, 3 and 7 a detailed assessment of post-transplant renal function was performed by measurement of serum and urine electrolytes and osmolality, and inulin clearances. Delayed function was defined as the need for dialysis during the first week post-transplant. Results (median + interquartile range) are summarised below.

GROUP	No Reflush	HOC	PBS140	
n	10	10	10	
Storage time	21.0+4.0	19.2+6.2	22.1+4.2	hours
Delayed function	4	2	1	
Creatinine day 7	246+855	309+282	274+568	umol/l
Peak Results				
Inulin Clearance (GFR)	30.0+30.4	25.2+22.6	38.9+23.2	ml/min
Sodium Reabsorption	98.8+4.8	98.8+2.2	99.0+2.6	%
Free Water Clearance	-0.2+1.3	-0.3+0.4	-0.6+1.8	ml/min

This study suggests that late perfusion with either HOC or PBS140 immediately prior to implantation will reduce the need for dialysis but will not improve overall graft function.

Reference 1: Parrott et al. Late perfusion: A simple remedy for renal allograft primary non-function. *Transplantation* 1990; 49: 913-915.

PAPER 20

IMMUNE RESPONSES TO NON-INHERITED MATERNAL RT1A ANTIGENS IN INBRED RATS

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It is difficult to find a suitable cadaver donor kidney for highly sensitised patients (HSPs) awaiting renal transplantation. Screening of sera from such HSPs can reveal certain antigens (permissible antigens) to which the patient is not sensitised. Recent work suggests that non-inherited maternal MHC antigens (NIMA) to which the patient is exposed in fetal life may represent permissible antigens.

We assessed responses to NIMAs in inbred rats expressing the RT1^{u/c} phenotype: group 1 (haplotype RT1^{u/c}, NIMA RT1^a) the progeny of (AO x DA)₁F1 x PVG matings, group 2 (haplotype RT1^{u/c}, NIMA RT1^a) the progeny of (AO x Lew) F1 x PVG matings, group 3 (AO x PVG F1 (RT1^{u/c}) hybrids. All rats received 0.5 ml DA (RT1^a) blood transfusions (BT) on days 0, 7 and 140. Sera were obtained at weekly intervals for 6 weeks and at weeks 20 and 21 and tested against DA target cells by an indirect haemagglutination (IHA) assay and a ⁵¹Cr release complement dependent cytotoxicity (CDC) assay.

There were no significant differences between the groups in the high antibody titres to DA antigens detected using IHA ($\geq 2^8$) or CDC ($\geq 2^6$) which developed following BT. No antiidiotypic activity to cytotoxic anti-RT1^a antibodies was present in day 0 sera from group 1 (NIMA-RT1^a) animals. There was a similar, vigorous response in all groups (IHA $\geq 2^{10}$, CDC $\geq 2^{10}$) to DA BT at week 20 and there were no differences in mean proliferative response of cells from all groups in the one-way mixed lymphocyte reaction using DA lymphocytes as stimulator cells.

We conclude that in this animal model there was no evidence of humoral or cellular tolerance to NIMA.

PAPER 21

FAILURE OF NIFEDIPINE TO PROTECT RENAL FUNCTION AFTER CHRONIC EXPOSURE TO CYCLOSPORIN

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We have reported previously the beneficial effects of nifedipine on renal function in cardiac transplant patients in retrospective studies. We have now investigated prospectively, 5 cardiac transplant recipients (3 male; ages 34 to 54; time from transplant 12 to 48 months). These were the only appropriate patients in our programme (n=101) not receiving nifedipine. All were hypertensive but well-controlled on prazosin. With informed consent and local Ethical Committee approval, all 5 patients had their anti-hypertensive medication changed to nifedipine.

Renal function was assessed by measuring glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) using the clearances of ⁵¹-Cr EDTA and ¹²⁵-I hippuran respectively. These investigations were performed prior to change of therapy and at intervals of 3 and 12 months thereafter. The results are displayed below as median values.

	Control	3 months	12 months	
Normalised GFR (ml/min/1.73m ²)	59	70	43	p=NS
Normalised ERPF (ml/min/1.73m ²)	224	148	200	p=NS

These results suggest that calcium channel blockade with nifedipine is not effective in improvement in renal function after chronic exposure to cyclosporin in heart transplant recipients.

PAPER 22

ANALYSIS OF ALLOGRAFT REJECTION BY GAMMA CAMERA IMAGING AFTER INJECTION OF RADIOLABELLED INTERLEUKIN 2 (IL2)

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Allograft rejection is mediated by activated T cells bearing receptors for IL2. Diagnosis of rejection by non-invasive detection of IL2 receptor positive T cell infiltrates would have clear advantages over biopsy dependent techniques. We have found in rat renal transplantation that gamma camera imaging over a graft after injection of radio-iodinated IL2 differentiates rejecting from non-rejecting organs.

IL2 was enzyme radiolabelled without loss of specific receptor binding. In preliminary experiments to investigate the kinetics of labelled IL2, an injection of ^{125}I IL2 was given to DA rats that had been transplanted 5 days previously with allogeneic or syngeneic grafts. Animals were sacrificed at intervals after injection and organs removed for counting. At 4 hours 67% of initial (peak) activity was retained by rejecting allografts compared to 14% in controls. In subsequent experiments gamma camera imaging over grafts after injection of ^{123}I IL2 was used to study uptake of tracer in vivo. Clearcut differences between rejecting allografts (n=7) and controls (n=6) were seen. At 4 hours mean retention of activity was 80% in experimental animals compared to 47% in controls (p<0.01). Retention of activity by temporarily ischaemic native kidneys was similar to controls.

We conclude that retention of IL2 by a renal allograft - perhaps by receptor binding of IL2 and internalisation by activated T cells - may be a marker of graft rejection and can be detected by external imaging. Studies are in progress to examine the specificity of IL2 retention and the potential of the technique as a clinical tool.

PAPER 23

COMPARISON OF SERUM AND CELLULAR IL-2 RECEPTOR LEVELS IN RENAL TRANSPLANT RECIPIENTS

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The expression of Interleukin 2 receptors (IL2-R) and the presence of the soluble form in serum is taken as an index of T cell activation. We have investigated the correlation between the appearance of IL2-R on infiltrating cells within renal transplant biopsies and the level of soluble receptor in the patient's serum.

Serum samples and renal transplant biopsies were taken from 12 patients. Frozen biopsy sections were immunohistologically stained using an anti-IL2-R monoclonal antibody in the APAAP technique. Soluble IL2-R in sera was estimated by an ELISA kit (T Cell Sciences). Thirteen pairs of samples were studied. Each was taken on the same day with the exception of one pair taken 3 days apart.

Pre-transplant biopsies (5/5) had no IL2-R positive cells. The corresponding levels of IL2-R in the sera ranged between 200 and 4000 u/ml, high compared to normal individuals but consistent for renal failure patients. The majority (5/8) post-transplant sera were also under 4000 u/ml with biopsies negative for IL2-R bearing cells. The remaining three sera were all over 6000 u/ml IL2-R. In two patients the corresponding biopsy had a few cells expressing IL2-R in the peritubular infiltrate. The biopsy from the third patient was negative for IL2-R positive cells. Of the two biopsies containing some positive cells, one was from a patient undergoing rejection, the other no evidence of rejection, although rejection occurred approximately one and a half weeks later. It therefore appears that a correlation exists between very high serum IL2-R levels and the presence of cells expressing IL2-R in the graft.

PAPER 24

THE IMPORTANCE OF NON MHC MISMATCHES IN NON-SENSITIZED AND SENSITIZED CORNEAL TRANSPLANT RECIPIENTS

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The value of matching for MHC antigens to improve the survival of corneal grafts is still not well established. We have investigated the role of non-MHC antigens in corneal graft rejection using a high responder orthotopic rat model. We have simulated the human situation by backcrossing LEW(RT1^l) to (DA(RT1^a)xLEW)F₁ hybrids and using the backcross generation as recipients for DA strain grafts. As with humans the MHC disparity between each donor-recipient pair could be controlled, while non-MHC mismatches remained variable and unknown for each individual (but averaged 50%). Animals were first typed, then divided into two groups, either homozygous RT1^{l/l} or heterozygous RT1^{a/l}. Some animals in each of the two groups were sensitized by three parental DA strain skin grafts at intervals of two weeks. Prior sensitization caused more rapid rejection of corneal grafts in both MHC mismatched (p<0.001) and matched (p<0.01) animals. Animals in the two MHC mismatched groups (26/26-sensitized, 17/17-unsensitized) and most in the MHC matched groups (25/27-sensitized, 13/13-unsensitized) rejected their grafts. MHC matching resulted in less rapid rejection and a greater range of survival times, although the difference in survival in unsensitized animals between matched and mismatched groups was not significant (p<0.05-unsensitized, p<0.001-sensitized). Thus, it appears that non MHC antigens play a significant role in corneal graft rejection in this rat model. Moreover, the high rejection rate of MHC-matched grafts suggests that, as with skin, several non-MHC genes are involved. If such genes are also important in man, matching for MHC in high risk cases may be of limited benefit. Indeed, total or partial matching for MHC may render rejection on account of non-MHC disparities more likely, especially in sensitized recipients.

PAPER 25

ANTIGENIC HETEROGENEITY OF VASCULAR ENDOTHELIUM

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In view of the participation of vascular endothelial cells in rejection of vascularised organs, the possible existence of phenotypic and functional heterogeneity is an important concept for consideration.

We investigated the antigenic status of vascular endothelium from different sites of the human cardiovascular system and umbilical cord. The presence of endothelium was defined by immunoreactivity with a pan-endothelial cell marker EN4. This being established the endothelium was studied using a panel of monoclonal antibodies. These included other recognised endothelial markers (FVIII-Rag, Pal-E & 44G4) and markers of immune activation (MHC class I and class II molecules, the adhesion molecule ICAM-1 and the monocyte/endothelial marker (OKM5).

Results show that a distinct heterogeneity of antigenic expression exists between large vessel (aorta, pulmonary artery, coronary artery and umbilical vessels) and small vessel (myocardial capillary) endothelium. Capillary endothelium exhibited strong expression of immunological markers (MHC I & II, ICAM-1 & OKM5) with infrequent FVIII-Rag binding. Conversely, large vessel endothelium exhibited strong reactivity with FVIII-Rag, weak expression of immunological markers MHC I/ICAM-1, with MHC II & OKM5 being undetectable. Furthermore, endocardial endothelial cells were found to be phenotypically similar to large vessel endothelium.

These results suggest that capillary endothelium is more immunogenic than that lining large vessels. In view of this, monitoring the phenotype of capillary endothelium may prove a sensitive method of estimating local immune response within the graft during an episode of rejection.

Finally, the question arises as to whether the heterogenous expression of endothelial antigens reflects a permanent specialised state for specific functions or is an induced response maintained by factors in the immediate environment.

PAPER 26

USE OF ISOLATED HUMAN CARDIOCYTES AS A MODEL OF DAMAGE TO TRANSPLANTED HEART DURING REJECTION

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Although myocytes are clearly damaged during rejection of cardiac allografts, there has not been a human model suitable for analysing the mechanism of damage to these cells (ie effect of cytokines and effector cells). In this study we use human myocytes isolated from the adult heart, and show that they can be damaged *in vitro* by allogeneic lymphocytes. Single cardiac myocytes were isolated by collagenase and pronase digestion of human ventricle obtained from explanted adult hearts at time of transplantation. Allogeneic and autologous peripheral blood lymphocytes were obtained from normal volunteers and patients. Myocytes (50-100 per well) were co-incubated with allogeneic and autologous lymphocytes (10^6 cells/ml), K562 NK sensitive cell line (5×10^5 /ml) and 3T3 fibroblast cell line in 96-well microtitre plates. Experiments were performed using RPMI 1640 culture medium containing 10% AB serum, 2mM l-glutamine and 150U/ml Penicillin/Streptomycin. Cell death in the cardiac myocytes was indicated by a rapid change from a rod shaped to a round morphology, with hypercontracture of the sarcomeres. Without the addition of lymphocytes, $81.0 \pm 4.5\%$ of the original rod-shaped cells remained after 1h, $68.7 \pm 7.5\%$ after 2h and $56.7 \pm 4.6\%$ after 3h (mean \pm sem, n=6). In four out of five cases addition of lymphocytes to the myocytes resulted in pronounced increases in cell death. At 2h, $29.6 \pm 7.5\%$ of the original rods were left in the lymphocyte containing wells compared with $72.2 \pm 3.3\%$ in their matched controls (n = 4, p < 0.01). Preliminary experiments indicate that the cytotoxic effect is similar for both allogeneic unsensitised, and lymphocytes sensitised in a mixed lymphocyte reaction against unrelated cells mismatched at the major histocompatibility complex loci. Autologous lymphocytes, fibroblasts and non-related cell lines did not show this cytotoxic effect. Autologous sera, normal sera and sera from patients undergoing a rejection episode were not cytotoxic. We propose that this is an excellent model in which to analyse the mechanism of damage to myocytes during rejection.

PAPER 27

A NEED FOR TRIPLE THERAPY?

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Cyclosporin A immunosuppression in renal transplant patients is known to give improved graft survival over combination therapy with prednisolone and azathioprine. Similar trials of cyclosporin versus cyclosporin, prednisolone and azathioprine "triple" therapy have yet to be published. In this study of 318 consecutive renal transplants from January 1988 the rejection rates and graft survival are compared in patients treated with cyclosporin monotherapy and those treated with triple therapy for primary delayed function.

199 patients with primary function received cyclosporin at 15mg/kg. 56 patients received triple therapy with cyclosporin 5mg/kg, azathioprine 2.5mg/kg and prednisolone 20mg. 63 patients were excluded (children and patients on different initial therapy). Their demography in terms of age, sex, aetiology and HLA matching were similar.

	Triple therapy	Cyclosporin
No of patients	56	199
Patients rejecting by 1 month	12 (21.4%)	118 (59.3%) p 0.0001
Patients rejecting by 3 months	13 (23.4%)	129 (64.8%) p 0.0001
No of rejections	15 (mn 1.15)	232 (mn 1.8)
Actual graft loss	3 (5.4%)	21 (10.6%) p=NS

Initial immunosuppression with triple therapy produces significantly less rejection compared to cyclosporin monotherapy and appears to decrease graft loss.

This work has not been previously published or presented.

PAPER 28

CANCER IN THE CYCLOSPORIN ERA

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An increased incidence of cancer in transplanted patients following long term immunosuppressive therapy is a well known and accepted fact of life. Since the introduction of Cyclosporin (Cy) and its widespread use over the last decade there has without doubt been an increase in the incidence of tumours such as squamous carcinoma of the skin, lymphomas and kaposi sarcoma. These tumours are also said to be occurring earlier. We have been using Cy since 1982 and have noticed an increase in both skin and deep tumours with an earlier time of occurrence. The time in years of appearance of tumours in our transplant patients is as follows:

	Aza/Pred	Cyclosporin
Nos. of transplants	464	505
Deep tumours	5.75 ± 2.6 (8)	2.5 ± 0.9 (9)
Skin tumours	12.1 ± 6.3 (8)	2.7 ± 1.3 (10)

Nos. of tumours in brackets.

In the Cyclosporin group all patients who developed tumours had been treated with Azathioprine, Prednisolone, ATG or OKT³ in addition to Cy. No patient commenced and remaining on Cy monotherapy has developed a malignancy of any sort. We conclude therefore that the Cy has undoubtedly led to an increase in malignant disease, but probably due to overall increase in immunosuppressive therapy rather than Cy per se.

PAPER 29

ASYSTOLIC KIDNEY DONORS - IS IT WORTH IT?

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In an effort to increase the number of available kidneys for transplantation, we decided to re-examine the viability of removing kidneys from asystolic donors.

Since September, 1988 we have recovered kidneys from 23 asystolic donors (7 female, 16 male). The donor age ranged from 12-80 years (mean 39). 11 pairs of kidneys were recovered using a monoblock bench perfusion technique and 12 using an in-situ perfusion technique.

A total of 43 were transplanted (3 were unsuitable for transplantation for technical reasons).

The warm ischaemic time ranged from 5-65 mins (mean 31 mins). The cold ischaemic time from 12-36 hrs (mean 22.5hrs).

10 (23%) kidneys functioned immediately, 23 (53%) kidneys had delayed function needing dialysis for between 5 and 83 days (mean 21 days) but all functioned eventually.

10 (23%) kidneys never functioned and graft nephrectomies were performed within 2 months of transplantation. Of these, 5 grafts infarcted, 2 grafts suffered severe rejection, 1 graft had vascular surgical problems, 1 graft had a biopsy induced haemorrhage and 1 patient died following myocardial infarction.

33 (76%) of all the kidneys transplanted are still functioning at the time of writing. Creatinine results-

at 1 month (33 pts) ranged from 79-1291 (mean 345)
at 3 months (27 pts) ranged from 62-800 (mean 198)
at 6 months (16 pts) ranged from 54-379 (mean 170)
at 1 year (13 pts) ranged from 57-250 (mean 154)

We believe that these figures are very encouraging and we will continue to actively procure kidneys from asystolic donors.