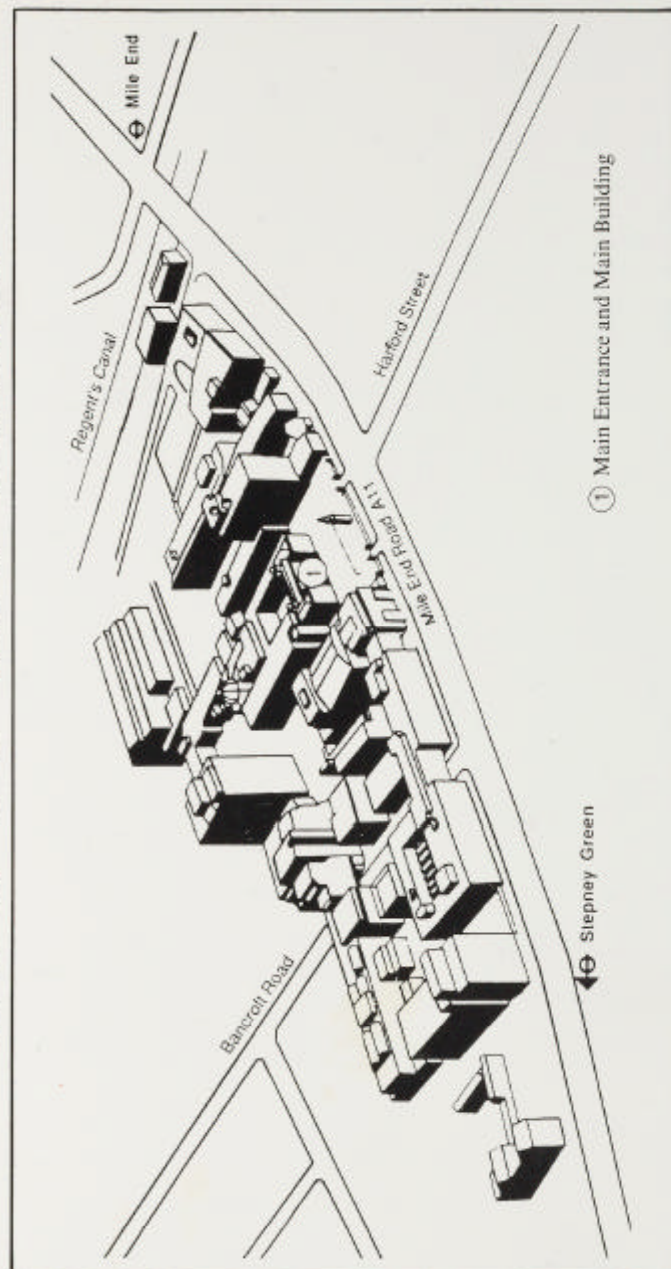


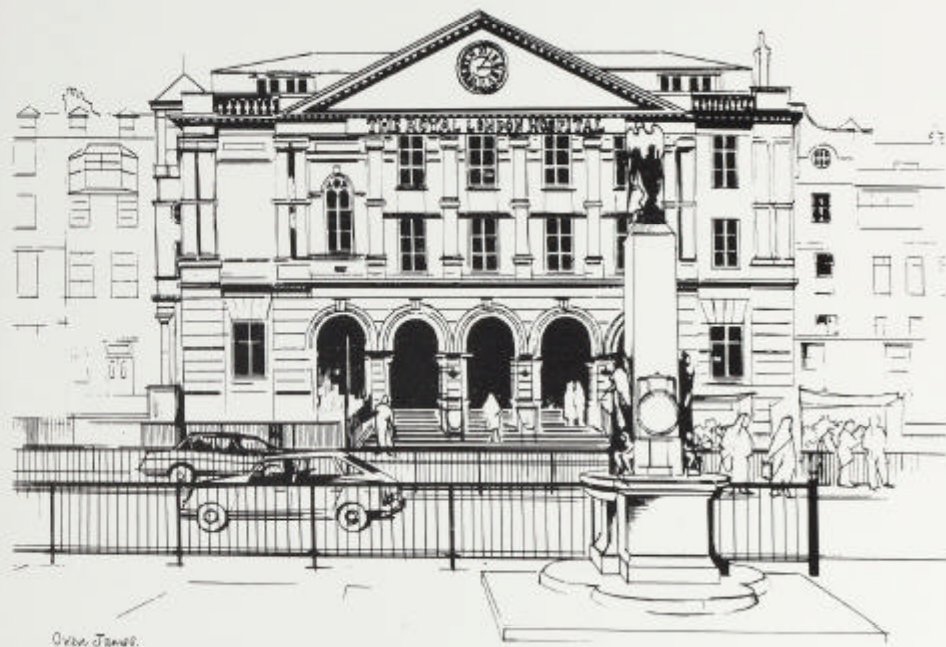
# HOW TO GET THERE



**Underground.** The College is within ten minutes walking distance of both Mile End station (Central, District and Metropolitan lines) and Stepney Green station (District and Metropolitan lines). Mile End station is east of the College on the opposite side

of the Mile End Road. Turn left out of the station and cross Burdett Road and Mile End Road at the traffic lights. Stepney Green station is to the west of the College. Turn left out of the station, cross Globe Road and continue along Mile End Road.

## THE COMBINED DUTCH & BRITISH TRANSPLANTATION SOCIETIES



THE ROYAL LONDON HOSPITAL (TRUST)

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11th & 12th October 1993

*25th ANNIVERSARY OF THE RENAL AND TISSUE  
TYPING UNITS AT THE ROYAL LONDON HOSPITAL*



## PAPER 1

### PERMISSIBLE HLA MISMATCHES FOR KIDNEY TRANSPLANTS

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Although the outcome of kidney transplantation is affected by the number of HLA mismatches, a substantial amount of grafts continue to function despite the presence of HLA mismatches. The concept, that some antigens may be "permissible" for the respective recipient offers a possible explanation of this phenomenon.

In a preliminary study, using data from living related transplants, several HLA mismatched combinations were classified as permissible or non-permissible. In the present analysis this concept is validated using data from cadaveric renal transplantation.

Cadaver grafts with one mismatch HLA-A,B,DR (N = 990) were classified according to the mismatched HLA antigen as being permissible (N = 310) or non-permissible (N = 680) for the respective recipient. The outcome of the transplantation was compared to 518 grafts with 0 HLA-A,B,DR mismatched cadaver kidney grafts.

The one year graft survival rates were found to be 87 %, 86 %, and 82 % for the 0 mismatches, one permissible and non-permissible antigen mismatched group respectively. The five year graft survival rates were 69 %, 65 %, and 56 % respectively.

Thus, mismatches with permissible HLA antigens seem to result in higher graft survival rates when compared to non-permissible ones. This difference is statistically significant ( $p < 0.05$ ). No statistical difference was found between 0 HLA-A,B,DR mismatched grafts and those with permissible mismatches, even after five years, although a slight difference in the survival rates was observed.

## PAPER 2

### DELAYED GRAFT FUNCTION: EARLY IDENTIFICATION OF PATIENTS AT RISK

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Many centres have reported that delayed graft function (DGF) reduces renal allograft survival. However, DGF is defined *retrospectively* as the requirement for dialysis in the first week following transplantation. The aim of this study was to see whether a four hour creatinine clearance ( $\text{CrCl}_4$ ) performed 20-24 hours post transplant would identify these patients.

99 first cadaveric renal transplant recipients were studied. All patients received cyclosporine therapy. Results were analysed for two groups of patients: patients with a  $\text{CrCl}_4$  less than 15ml/min (group 1) and those with a  $\text{CrCl}_4$  greater than 15ml/min (group 2).

There was no significant difference between group 1 and group 2 for age, mean number of rejection episodes, cytotoxic antibodies, HLA B or Dr mismatches. Renal allograft survival at three months for group 1 patients was only 79% compared with 98% for group 2 patients ( $p < 0.01$ ; Chi square). There were four patient deaths in group 1 compared with none in group 2 but this was not significant ( $p = 0.06$ ; Chi square). Of the 99 patients studied, 25 required dialysis in the first week post transplant. Graft survival at three months in these patients was 68% compared with 97% for the patients who did not require dialysis ( $p < 0.001$ ; Chi square).

The performance of a simple  $\text{CrCl}_4$  at 24 hours post transplant helps to identify those patients at risk of early graft loss. Identification of these patients early in the post transplant period allows alteration in their management to improve renal allograft survival.



### PAPER 3

#### INCREASED RENAL ALLOGRAFT THROMBOSIS IN CAPD PATIENTS

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In a retrospective analysis of 202 renal transplant procedures in the years 1989-1992 we identified an excess of grafts lost from primary renovascular thrombosis in patients receiving continuous ambulatory peritoneal dialysis (CAPD) compared to haemodialysis (HD) patients (9 CAPD v 0 HD, Chi-squared = 9.63;  $p < 0.01$ ). All graft losses from thrombosis occurred within 16 days of surgery. Possible predisposing causes were identified in 3 patients. Donor age was greater in CAPD patients losing their kidneys from thrombosis compared to the overall CAPD group [mean (SD) years, 43.0(12.9) v 29.1(15.8);  $p = 0.01$ ] whereas no significant difference in packed cell volume, platelet count, antibody status, cyclosporin use, peri-operative hypotension, primary diagnosis, smoking or diabetes mellitus was found. Data from the EDTA registry for 1990-91 show that graft loss from primary renovascular thrombosis in UK-treated patients was reported in 7.1% of CAPD recipients compared with 1.8% in haemodialysis. We suggest that CAPD patients are at greater risk of graft loss from renovascular thrombosis than HD patients and may require more intensive fluid and anticoagulant treatment in the peri-operative period.

### PAPER 4

#### CONVERSION FROM CYCLOSPORINE (CyA) TO AZATHIOPRINE (Aza) AFTER CADAVERIC KIDNEY TRANSPLANTATION (KT): IN THE LONG RUN A BETTER RENAL FUNCTION, LESS HYPERTENSION AND EQUAL GRAFT SURVIVAL

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In the period 1983-1988 128 patients, who received a 1<sup>st</sup> or 2<sup>nd</sup> cadaveric kidney graft, participated in 2 prospective, randomized trials in which CyA was either continued or replaced by Aza at 3 months after KT. All patients received high doses CyA (16 mg./kg./day tapered to 10 mg./kg./day depending on CyA blood levels) and low doses of prednisone (initial dose 20 mg./day tapered to 10 mg./day). Three months after KT patients were randomly assigned to continue CyA (n=68) or were converted from CyA to Aza (n=60). In the CyA group the drug dose was tapered to 5 mg./kg./day, depending on CyA blood levels. In the converted group the prednisone was temporarily increased to 25 or 40 mg./day and was tapered off to 10 mg./day. There were no differences between the two groups in age, sex, number of KT, HLA mismatches, panel reactive antibodies, cold and warm ischemia time. The long term follow up (FU) of these patients showed an equal mortality rate in both groups (CyA: 13.2 % vs Aza: 11.7 %). Graft survival after 2, 5 and 8 years was 88.4 %, 75.4 % and 66.5 % for CyA patients and 88.3 %, 78.3 % and 76.3 % for Aza patients ( $p = 0.53$ ). Renal function was considerably better in the Aza group: after 1 year the mean creat. clearance in the converted patients was 78 ml./min. vs. 60 ml./min. in the CyA patients (difference 18 ml./min.; 95 % CI: 8.1-27.5). This difference remained the same during 8 years of FU. Less proteinuria ( $> 0.5$  g./day) was found in the Aza patients at 5 year (30 % vs. 48 % in the CyA patients;  $p < 0.05$ ). There was no difference in bloodpressure between the 2 groups before KT, at 3 months and during FU, but CyA patients needed significant more antihypertensive drugs: after 2 years 87 % versus 58 % of Aza patients. This difference did not change during FU.

We conclude that elective conversion at 3 months posttransplant can be done safely and that beneficial longterm effects were found regarding graft survival, renal function, proteinuria and hypertension.



## PAPER 5

### HETEROGENEOUS COURSE OF RENAL FUNCTION IN CYCLOSPORIN TREATED HEART TRANSPLANT RECIPIENTS.

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The renal side-effects are the major limitation of the use of cyclosporine-A (CsA) following heart transplantation (HTX). In an effort to identify those patients especially at risk for the nephrotoxic side effects of CsA, we studied 210 orthotopic heart transplant recipients who had a follow-up of at least one month. All patients received primary prophylaxis with anti T-cell agents followed by CsA at a starting dose of 8 mg/kg and low-dose steroids.

After HTX, median serum creatinine levels increased steadily over time. In 25 patients serum creatinine exceeded 300  $\mu\text{mol/L}$  at any time during follow-up. This renal impairment occurred soon after transplantation as serum creatinine was increased as early as 3 months after transplantation. Until now, 8 of these 25 patients have reached end-stage renal failure after 1.8 to 7.2 years. When excluding these 25 patients with severe renal problems, serum creatinine remained remarkably stable in the rest of the group ( $n = 185$ ) at a level of approximately 155  $\mu\text{mol/L}$ . Thus, a limited number of patients accounted for the progressive rise in creatinine, and the majority of patients appeared to tolerate prolonged treatment with CsA relatively well. No risk-factors could be identified that differentiated the 25 patients with increased susceptibility to the nephrotoxic action of CsA from those with relatively stable renal function. Serum creatinine before HTX, during the first and second weeks after HTX and creatinine one month after transplantation were not significantly different. Also, there were no differences in bloodpressure, cyclosporine dosage, or the use of calcium entry blockers in the 4 weeks after transplantation.

We conclude that in our center 12 % of the patients accounted for the progressive decrease in renal function following heart transplantation, and that this decrease appeared to occur within three months after transplantation.

## PAPER 6

### ADVERSE EFFECTS OF OKT3 THERAPY: INCREASED RISK WITH IMPAIRED RENAL FUNCTION

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OKT3 is effective for induction of immunosuppression after renal transplantation and in the treatment of steroid resistant rejection (SRR). Major adverse effects of OKT3 include infection and encephalopathy. Risk factors for development of these effects have not yet been identified.

We compared adverse effects in patients receiving OKT3 for SRR and in recipients of kidneys from non-heart beating donors (NHBD) given OKT3 as induction therapy. Of 306 consecutive cadaveric renal transplants 20 received kidneys from NHBD. 17/20 NHBD had OKT3 (2.5 or 5 mg daily for 10-14 days) as induction therapy. The other 289 received cyclosporin/prednisolone as induction. 33/306 received OKT3 (5 mg/day for 10-14 days) for SRR. OKT3 was discontinued for life threatening complications in 3/33 SRR (9.9%) and 5/17 NHBD (29.4%). Respiratory failure occurred in 2/33 SRR, 2/17 NHBD; encephalopathy occurred in 3/33 SRR, 6/17 NHBD ( $p = 0.05$ ). Deaths related to OKT3 were 1/33 (respiratory failure), 1/17 NHBD (respiratory failure plus septicaemia). Renal function (mean serum creatinine,  $\mu\text{mol/L}$ ) during OKT3 therapy was worse in NHBD; Day 0: NHBD 950 (range 413-1365), SRR 422 (range 190-1079) ( $p^{**} < 0.001$ ); Day 14: NHBD 787 (range 585-1051), SRR 284 (range 113-961) ( $p < 0.001$ ). Dialysis was required during OKT3 therapy in 17/17 NHBD, 6/33 SRR ( $p \leq 0.001$ ). 3 out of 4 patients with respiratory failure and 8 out of 9 with encephalopathy had creatinine  $> 600 \mu\text{mol/L}$ .

Uraemic toxins may combine with OKT3 induced cytokine release to mediate OKT3 adverse effects. OKT3 should be avoided in patients with severe impaired renal transplant function.

$p^*$  significant (Fisher's);  $p^{**}$  significant (Student's)



## PAPER 7

### RENAL TRANSPLANT BIOPSY: COMPARISON OF COMPLICATION AND SUCCESS RATES

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We studied three different biopsy techniques to determine the safest and most reliable for obtaining adequate samples of renal cortex: (1) Blind vertical pass (BVP) with kidney palpation and biopsy with 18G trucut needle; (2) Ultrasound guided automated biopsy (USG) using 18G biopsy gun; (3) Ultrasound guided automated biopsy with immediate examination (US+IE) of the specimen using a stereoscopic microscope.

440 biopsy cores were obtained from 280 consecutive separate biopsy procedures.

Method of biopsy	No. of biopsy procedures	No. of cores	Renal tissue present	Renal cortex present	No. glomeruli per core
BVP	69	96	80	63	5.9 ± 0.9
USG	144	262	245	218	11.7 ± 1.1
US+IE	66	82	76	70	9.3 ± 1.1

BVP technique produced less cores containing renal tissue ( $p < 0.01$ ), fewer containing renal cortex ( $p < 0.01$ ) and less glomeruli per core ( $p < 0.01$ ) compared with both the ultrasound guided groups. There were no significant differences in the success rates of the USG and US+IE methods. There was a higher complication rate when only medulla was present in the biopsy than when cortex or cortex and medulla were present ( $p < 0.001$ ). There were no significant differences in complication rates between the three biopsy methods.

This study demonstrates that ultrasound guided methods are more successful than the blind pass technique. The added use of immediate examination using a stereoscopic microscope allows a 100% success rate in providing renal cortical tissue for diagnosis at a single biopsy procedure.

## PAPER 8

### DISCREPANCY BETWEEN mRNA EXPRESSION AND PRODUCTION OF Th1- AND Th2-LIKE CYTOKINES BY CULTURED GRAFT INFILTRATING CELLS AFTER CLINICAL HEART TRANSPLANTATION

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T cells have the ability to secrete various cytokines. Activated T helper type 1 (Th1) cells mainly produce IL2 and IFN $\gamma$ , while activated T helper type 2 (Th2) cells secrete IL4, IL6 and IL10. We studied the induction of cytokines in graft infiltrating cells cultured from endomyocardial biopsies (EMB) after heart transplantation.

From EMB, cell cultures were generated in the presence of 30 U IL2. Before stimulation, non cryopreserved cells were incubated without IL2 during 24 hours.  $5 \times 10^6$  EMB derived cells from different patients were extensively washed and stimulated with  $5 \times 10^4$  irradiated (60 Gy), and subsequently washed, EBV transformed donor B cells. After 20 hours of stimulation, supernatants and cell pellets were harvested in order to determine cytokine production and mRNA expression by respectively ELISA and reverse transcriptase PCR.

mRNA transcripts for IL2, IL4, IL6 and IL10 were detected in all cultures after stimulation. The Th1-like products IL2 and IFN $\gamma$  were also always induced. In contrast, the Th2-like cytokines IL4 and IL6 were found in only 50% of these supernatants. Their production seemed independent of the time after transplantation. However, in the first 6 months after transplantation EMB cultures produced more IL2 (median 805 versus 149 pg/ml) and IFN $\gamma$  (median 2338 versus 613 pg/ml) than thereafter.

These results suggest that the production of Th1-like cytokines for cultured graft infiltrating cells might correlate with immunological responsiveness early after heart transplantation.



## PAPER 9

INAPPROPRIATE RECOGNITION OF ALLOANTIGEN CAN LEAD NOT ONLY TO T CELL ANERGY, BUT ALSO THE EXPANSION OF T CELLS WITH A REGULATORY PHENOTYPE

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In view of their central role in graft rejection, CD4<sup>+</sup> T cells are attractive targets for immunotherapy. We have developed a protocol for the induction of tolerance to alloantigen whereby mice are pre-treated with donor antigen and a depleting anti-CD4 monoclonal antibody (mab) 28 days before transplantation of a cardiac allograft. This pretreatment protocol results in the indefinite survival of the primary heart grafts and acceptance of second heart or skin grafts from the alloantigen donor; grafts from a third party strain are rejected acutely. We have shown previously that tolerance induced by this strategy is dependent on the combined treatment with antigen and anti-CD4 mab; neither antigen nor antibody alone are effective. In order to explore the mechanisms underlying the development of tolerance induced by this protocol, we have examined some of the parameters that are critical for its success.

Initially, we tested the hypothesis that tolerance induction after depletion of the CD4<sup>+</sup> population was dependent on T cells newly emerging from the thymus encountering donor alloantigen under sub-optimal conditions as they repopulated the periphery. In the standard protocol recipient mice (C3H.He; H-2<sup>k</sup>) are pre-treated with anti-CD4 mab on two consecutive days (days -28 and -27) and donor alloantigen (C57BL/10; H-2<sup>b</sup>) is administered with the second dose of mab (day -27). To test our hypothesis, administration of alloantigen was delayed until 1, 7 or 14 days after mab therapy. The results obtained revealed that tolerance was only induced when antigen was delivered within 24 hours of mab treatment. Interestingly, concomitant administration of mab and alloantigen also failed to induce tolerance.

These data suggest that for the successful induction of tolerance, T cells **must not** encounter alloantigen before depletion of CD4<sup>+</sup> T cells has commenced, **but must** encounter alloantigen while mab is still present *in vivo*. Unresponsiveness is therefore unlikely to be a function of the repopulating CD4<sup>+</sup> cells. Instead, the population of CD4<sup>+</sup> T cells that escape depletion are probably the most important. We have therefore modified our hypothesis and propose that induction of tolerance to alloantigen by this treatment strategy is dependent upon the small population of CD4<sup>+</sup> T cells that escape mab induced depletion. If these T cells encounter alloantigen while mab remains on the cells surface they can develop a regulatory phenotype, and once established can expand and become capable of switching off donor reactive cells, a process that results in the development of tolerance to donor alloantigen.

## PAPER 10

GRAFT INFILTRATING cCTL AND pCTL DERIVED FROM REJECTORS HAVE HIGHER AVIDITY FOR DONOR ANTIGENS AND LOWER CsA SENSITIVITY THAN THOSE FROM NON REJECTORS.

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In a previous study we described that most donor reactive cytotoxic T cells (CTL) derived from the cardiac graft of patients with acute rejection had high avidity for donor antigens, as their function could not be inhibited by CD8 Mab. However in non rejectors CTL could be inhibited with CD8 Mab and therefore are thought to have low avidity. The experimental design of that study did not enable us to distinguish between *in vivo*, donor antigen, primed cCTL and their naive precursors (pCTL). Studies in mice have shown that *in vivo* activated CTL predominantly have high avidity for their particular antigen, whereas *in vitro* activation of pCTL results in CTL with low avidity. It is conceivable that in non rejectors pCTL is the dominant population, whereas cCTL might be the predominant cell type in the rejectors. We tested this hypothesis in a Limiting Dilution Assay (LDA) and investigated simultaneously whether pCTL and cCTL populations in those patients had a different sensitivity for cyclosporin A (CsA). Limiting dilution cultures were stimulated with autologous to measure cCTL frequencies or donor cells to assay both cCTL and pCTL. LDA were performed in absence or presence of CsA in the stimulation phase. In both groups pCTL were the predominant CTL type and were CsA sensitive. cCTL present in the graft of patients with rejections were mainly CsA resistant (see table). In the cultures from non rejectors a significant (p=0.04) part of the cCTL was inhibited by CsA. Addition of CD8 monoclonal antibody during the cytotoxicity phase of the LDA was used to differentiate between CTL populations with high avidity for donor antigens and populations with low avidity. The experiments revealed that in the graft of rejectors the relative contribution of CD8, CsA resistant cCTL with high avidity, was significant (p>0.005) higher than in the non rejectors (table). pCTL were expected to differentiate into CTL with low avidity in LDA. However in most rejectors CD8 mab had only a minor influence on the pCTL frequency estimates. These pCTL might represent an intermediate stage between the naive pCTL and mature, CsA resistant cCTL with high avidity for donor antigens.

Inhibition with	Rejectors		Non Rejectors	
	cCTL	pCTL	cCTL	pCTL
CD8	24 <sup>*</sup> 0-72 <sup>*</sup>	44 16-85	88 51-99	86 24-100
CsA	0 <sup>*</sup> 0-37 <sup>*</sup>	79 21-94	22 0-68	85 27-96
CsA and CD8	22 <sup>*</sup> 0-89 <sup>*</sup>	32 0-85	92 59-99	93 27-100

<sup>\*</sup>Median inhibition, expressed as % from the cCTL or pCTL frequency without additions, <sup>\*</sup>range. <sup>\*\*</sup>Median inhibition with CD8, expressed as % from the frequency cCTL, or pCTL resistant to CsA, <sup>\*</sup>range.



## PAPER 11

### ULTRASTRUCTURAL ANALYSIS OF CIRCULATING CYTOMEGALIC CELLS IN PATIENTS WITH AN ACTIVE CYTOMEGALOVIRUS (CMV) INFECTION

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CMV is the single most infectious complication after organ transplantation. The presence of cytomegalic inclusion cells in the peripheral blood of patients with an active CMV infection has recently been demonstrated (J. Infect. Dis. 1993;167:270-7). By immunological staining these cells were shown to express CMV antigens belonging to all three stages of the viral replication cycle, i.e. immediate-early, early, and late antigens, indicating a productive CMV infection. Furthermore, staining experiments with antibodies against different cell marker and differentiation antigens showed that these cells most probably are of endothelial origin. In the present study, circulating cytomegalic inclusion cells from three renal transplant recipients with an active CMV infection were studied by transmission electron microscopy. It was shown that numerous viral capsids were present in the nucleus of these cells, and numerous virus particles and dense bodies were present in the cytoplasm. These results demonstrate a productive CMV infection, and indicate that these cells could disseminate CMV throughout the body. In addition, the finding of a cluster of cytomegalic inclusion cells in the peripheral blood linked together by characteristic adhaerens type cell junctions is further evidence that these cells are of endothelial origin, and suggests that the endothelial damage may be extensive.

## PAPER 12

### XENOTRANSPLANTATION OF ISLETS ACROSS A STRONG SPECIES BARRIER (RABBIT TO CYNOMOLGUS MONKEY)

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Although much is known of the fate of isolated islets in xenotransplantation models where the recipients are rodents these models are of questionable relevance to islet xenotransplantation in man, since mechanisms of graft destruction such as heterophile antibody binding, direct activation of complement and or T cell mechanisms may be subtly or grossly different. We have examined the fate of islet xenografts in a recipient with direct relevance to man, the cynomolgus monkey. Rabbit islets were prepared by an intraductal collagenase technique and incubated in neat rabbit, human or cynomolgus serum for up to 6 days. Islets exposed to serum were analysed by flow cytometry for IGG and IGM binding, and scored for viability by supravital staining. For *in vivo* studies isolated islets were prepared from 4 NZW rabbits (15-34 x 10<sup>3</sup> islets 70-85% purity) and transplanted beneath the kidney capsule of normal cynomolgus monkeys after aggregation in either a rabbit or monkey blood clot. The tissue was retrieved at 1,2,3 and 4 days after transplantation and processed for light and electron microscopy. The results showed that rabbit islets bind heterophile antibody of both IGG and IGM subtype. Culture in neat human or cynomolgus monkey serum resulted in slow loss of islet viability over 3-6 days, whilst viability was maintained in rabbit serum. Destruction of rabbit islets after transplantation into cynomolgus monkeys was more rapid with visible damage within 6 hours associated with neutrophil infiltration and subsequent heavy mononuclear cell infiltration leading to total destruction by 4 days. These studies document for the first time the fate of xenogeneic islets in a primate recipient and suggest that immediate mechanisms of graft rejection, possibly antibody mediated, represent a major barrier to islet xenotransplantation in humans.



## PAPER 13

### ORGAN DONORS: ARE WE EDUCATING THE RIGHT PEOPLE?

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Renal transplantation is the most cost effective management of end stage renal failure. It has been estimated that for renal transplantation to meet current requirements, the supply of cadaver kidneys should be in excess of 40 per million population per year. Presently, UK organ donation rates of approximately 30 per million population are inadequate and there is little evidence of an improving trend.

This study consisted of a retrospective audit of the medical records of patients selected from the total deaths in 5 study hospitals in the North-West region of England during the calendar year 1992. The hospitals were regional/sub-regional referral centres for neurosurgical or intensive care facilities. The total number of deaths were generated from each of the hospitals activity database to include patients aged 10-75 years. Potential donors were selected using International Classification of Disease (ICD) codings excluding accepted medical contra-indications.

The total number of deaths in the five hospitals audited was 5200. Of these, 492 (9.5%) patients were identified as being potential donors. The hospital records were independently assessed by at least two of the authors. This revealed that 163 (33.1%) would have been realistically considered as donors if conditions had been ideal. The cause of death was cerebrovascular in 110 patients (67.5%), head injury in 34 (21%), brain tumour in 12 patients (7.4%) with seven others. 75 patients were ventilated at the time of death of which 36 (48%) became donors. 12 non-donating patients had ventilation terminated following brain stem tests and donation was refused in a further 12 cases (25%). The number of refusals plus the number of donors would indicate an enquiry rate of 64% in ventilated patients. The overwhelming reason given for non-ventilation (62/88) was that the patient had a poor prognosis with the decision being made by a consultant in 43 cases. Ventilated patients were significantly younger (mean 40.39 SD 14.56) than non-ventilated patients (mean 60.69 SD 12.28) ( $p < 0.001$ ). There was no significant difference between the length of hospitalisation of ventilated patients (mean 3.71 SD 4.17 days and 6.57 SD 10.37 days respectively).

We conclude that there remains a large and presently unused potential donor pool within our region. It would appear that the main reason for under-utilisation of this valuable resource is a failure on the part of medical staff to identify potential donors and facilitate organ donation. If 75% of the potential donors we have identified had come to donation we would exceed the present target figure of 48 cadaveric kidneys per million population.

## PAPER 14

### FIVE YEARS' EXPERIENCE OF ELECTIVE VENTILATION: THE IMPACT ON A RENAL REPLACEMENT PROGRAMME

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We report here the feasibility, results, resource implications, and long-term impact on dialysis of our elective ventilation (EV) protocol since its introduction in May 1988.

29 potential candidates for EV were notified to the transplant team, but in 5 cases (17%) ICU facilities were unavailable. The relatives refused to allow elective ventilation of 5 further cases, all of whom died rapidly. The 19 electively ventilated donors died within 24 hours of transfer to ICU, organs being retrieved from all. 36 (95%) of the 38 kidneys were transplanted with an 85% success rate despite the average donor age of 57 years.

Organs have continued to be retrieved from conventionally ventilated donors so that the total donor rate has reached a consistent level of 30 per million population (pmp) even though none of our donor hospitals has a neurosurgical unit. We have maintained a constant positive balance of trade with UKTSSA, receiving beneficially matched kidneys for our own patients. The new patient acceptance rate for dialysis is over 70 pmp, but the steady transplant rate of nearly 40 pmp has meant that the number of patients on all forms of dialysis has actually fallen over the years 1990-1993.

Elective ventilation will solve the problem of shortage of kidneys for transplantation and in addition will have a small impact on other solid organs.



## PAPER 15

### KIDNEY RETRIEVAL FROM THE NON HEART BEATING DONOR (NHBD) USING IN-SITU PERFUSION.

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In response to a gradual decline in the number of organ donors a dedicated NHBD retrieval programme was introduced in 1992. This report details our initial experience after 15 months.

A total of 36 referrals have been made, 28 from the casualty department and 8 from the medical wards. There were 13 successful retrievals, 20 kidneys being transplanted locally, 3 at other centres and 3 poorly perfused kidneys were not used. The median (range) catheter insertion time was 25 minutes (12 - 48). Catheter malposition (10) and lack of consent (10) were the major reasons for failure to retrieve organs.

16/20 (80%) of the kidneys transplanted locally functioned after a median delay of 21 (95% C.I. 14 - 35) days. The median serum creatinine was 196  $\mu\text{mol/L}$  (95% C.I. 132 - 253) at 6 months. There were 3 graft failures (1 renal vein thrombosis, 1 graft rupture, 1 irreversible ischaemic damage) and 1 patient died 9 days post operatively due to respiratory failure and septicaemia.

In the same interval we have performed 44 transplants from heart beating donors. The NHBD has therefore increased our transplant activity by 31%. With the success rate achieved to date the NHBD represents a valuable source of additional kidneys for transplantation.

C.I. = confidence interval

## PAPER 16

### COMPARISON BETWEEN THE MICROLIMPHOCYTOTOXICITY TEST AND PCR-SSP FOR HLA-DR AND HLA-DQ TYPING

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In previous studies it was demonstrated that results of HLA class II typings generated by serological methods, such as the microlymphocytotoxicity test, are not conclusive or incorrect in a substantial percentage of individuals examined. Therefore reliable alternatives for serological HLA class II typing have been sought applicable for routine clinical practice. Recently a fast and supposedly reliable HLA class II typing method has been developed which employs the polymerase chain reaction with sequence-specific primers (PCR-SSP). We have evaluated results of HLA-DR typings generated by serology and PCR-SSP, performed on 104 samples from sequential individuals. HLA-DR typings by PCR-SSP and serology were in accordance in 77 individuals examined. In the remaining group of 27 individuals, serology and PCR-SSP yielded different HLA-DR assignments in 7 individuals whereas by serology HLA-DR could not be determined from 4 individuals and in 16 individuals doubtful results were obtained. When the DR14, DR"Br", DR12 or DR16 antigens were encountered serology generated a high percentage of HLA-DR typings, 100%, 100%, 60%, and 40% respectively, which were either doubtful or different from results of PCR-SSP. In addition, on the prerequisite that results by PCR-SSP are correct serology encounters significantly more problems in HLA-DR typing of diseased compared to healthy individuals (44% vs 19%;  $P < 0.001$  chi square). We next compared results of HLA-DQ typings generated by serology and PCR-SSP in two groups of individuals; the first group consisted of the 23 individuals of whom serological DR typing was either doubtful or different from results of PCR-SSP, and the second group consisted of 20 individuals of whom serological DR typing was in accordance with results of PCR-SSP. The results indicate that HLA-DQW1, -2 and -3 typings determined by serology and PCR-SSP were discordant in only 2 cases, belonging to the first group of 23 individuals.

To examine which of the two techniques yielded correct HLA-DR typings in the 7 discrepant cases, HLA-DR alleles of these individuals are currently examined using sequencing based typing. Nevertheless, the results generated so far demonstrate a number of significant merits for the PCR-SSP compared to serology: 1) PCR-SSP did not yield any doubtful HLA-DR typing nor did it encounter technical failures 2) using PCR-SSP, DR14, DR"Br" (and DR18) can be typed conclusively, and 3) PCR-SSP results can be much easier interpreted and obtained quicker than by serology. Therefore it can be envisaged that in the near future HLA-DR typing by serology will be replaced by PCR-SSP in routine clinical practice.



## PAPER 17

### COMMITTED CYTOTOXIC T CELLS (cCTL) HAVE LOW AVIDITY FOR DONOR CLASS I AND CLASS II ANTIGENS LATE AFTER HEART TRANSPLANTATION (HTX).

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Recently we demonstrated that cCTL propagated from endomyocardial biopsies (EMB) obtained during or before rejection had high avidity for donor class I antigens, cCTL obtained in the same period from patients without rejection had low avidity. In the present study we investigated the frequency and avidity of cCTL for both HLA class I and II antigens long after transplantation, in patient with and without acute rejection (AR) in the early post-operative phase. EMB from 4 patients without acute rejection (AR-) immediately post-transplantation were taken 224 days (median, range 164-334 days) after transplantation. EMB from the 6 patients who previously experience acute rejection (AR+) were taken 205 days (median, range 128-449 days). Using Limiting Dilution Analysis, we measured the frequency of class I reactive cCTL with PHA-blasts, which share only class I antigens with the donor, as targets, both in the presence and absence of CD8 Moab. The class II reactive cCTL were measured with B-LCL, which share only class II antigens with the donor, this time in the presence and absence of CD4 Moab. Patients had at least one mismatch for class I and class II. The median frequency of cCTL against class I antigens in the AR+ group was  $172/10^6$  cells (range 17-1120/ $10^6$ ), and in the AR- group  $636/10^6$  cells (range 17-5136/ $10^6$ ). After addition of CD8, the median frequency of the cCTL in the AR+ was  $1/10^6$  cells (range 1-340/ $10^6$ ) and the AR-  $331/10^6$  cells (range 1-600/ $10^6$ ). The cCTL class II frequency of the AR+ group was  $783/10^6$  cells (median, range 43-3928/ $10^6$ ) and in the AR- group  $785/10^6$  cells (range 148-2223/ $10^6$ ). In the presence of CD4, the median frequency of cCTL in the AR+ group was  $56/10^6$  cells (range 1-5375/ $10^6$ ) and in the AR- group  $22/10^6$  cCTL (range 10-1894/ $10^6$ ). The median inhibition percentage for class I cCTL in the AR+ group was 94% (range 70-98%) and in the AR- group 94% (range 5-94%). For class II cCTL, the median inhibition in the AR+ group was 66% (range 0-98%) and in the AR- group 85% (range 15-99%). None of the patients with acute rejection episodes in the past, had high avidity cCTL for class I anymore, while high avidity for cCTL class II was found in only one of them. Apparently anti-rejection therapy is able to change the pattern of immunological activity from high to low avidity for donor antigens, resulting in long term graft survival.

## PAPER 18

### REJECTION OF TRANSPLANTED KIDNEYS IS PREDICTED BY ANALYSIS OF LEU-4, TAC, WT14, AND ICAM-1 EXPRESSION ON RENAL BIOPSIES.

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To study early intragraft events after renal transplantation renal biopsies were performed one week following renal transplantation at a time without clinical evidence of rejection in 45 patients (13 females, mean age 48 range 18-60 and 32 males, mean age 43 range 17-59 years). 35 biopsies were available for histological and immunohistochemical analysis. Immunohistochemical analyses were performed with monoclonal antibodies against leucocytes (CD45), monocytes (WT14), complement factor 3 (C3), T-cells (Leu4), T-cellreceptor  $\alpha\beta$  and  $\gamma\delta$ , tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), IL2-receptor (TAC), intercellular adhesion molecule-1 (ICAM1) and HLA-DR. The slides were scored semi-quantitatively with the observers having no knowledge of clinical or patient data. None of the studied parameters correlated to delayed graft function or graft loss. Histological analysis showed that both focal interstitial infiltrate (18/35) and tubular basement membrane disruption (13/35) correlated with a higher incidence of subsequent rejection ( $p=0.02$  for both parameters). The relevant immunohistochemical analyses showed a strong correlation between positivity for CD45 or WT14 in peritubular capillaries (PTC) ( $p<0.001$  and  $p=0.02$  respectively) and subsequent occurrence of rejection. There also was a strong correlation between the intensity of staining of ICAM-1 on PTC as well as TAC on proximal tubular cells and the number of subsequent rejection episodes ( $p=0.0005$  and  $p<0.001$  respectively).

Conclusion: In one week renal biopsies, infiltration of peritubular capillaries with mononuclear cells seems to predict the number of subsequent rejections. The finding of increased expression of IL2-R on proximal tubular cells or ICAM-1 on PTC correlates strongly with rejection, and the intensity of the staining correlates with the number of rejection episodes that will ensue ( $p<0.001$ ) in the first 3 months following renal transplantation.



## PAPER 19

### ROLE OF THE KIDNEY IN CLEARANCE OF TNF AND ITS SOLUBLE RECEPTORS.

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Tumor Necrosis Factor (TNF) can be injurious to the organism when present in excessive quantities. Circulating soluble TNF receptors (sTNFR) appear to represent a natural mechanism that protects against circulating TNF. Recent data suggest that TNF and sTNFR are removed from the circulation by the kidneys. Since kidney allograft rejection is accompanied by renal dysfunction, we investigated the effects of nephrectomy in a murine model on TNF as well as sTNFR metabolism.

#### Methods:

- I. Mice underwent bilateral nephrectomy (BN) or sham operation (SR); TNF and sTNFR levels in the serum were measured after 0.5, 1, 2, 4, 8 and 12 hours.
- II. 3 hours after BN or SH operation, endotoxin (an inducer of TNF and sTNFR) was given to investigate TNF and sTNFR metabolism; samples were taken at designated times.

#### Results:

- I. 2 hours after BN, TNF was detectable (200 pg/ml) and increased thereafter, while TNF in SH mice was not detectable. sTNFR increased 30 min. after BN and reached plateau levels after 4 - 8 hours, while sTNFR levels in SH mice revealed one minor peak after 30 min.
- II. Endotoxin injection induced a rapid increase in circulating TNF and sTNFR levels. In BN mice however, TNF and sTNFR levels remained significantly longer in the circulation than in SH mice (<0.05).

#### Conclusion:

The data presented show that TNF and sTNFR are removed from the circulation by the kidneys. Thus renal dysfunction, present after kidney allograft rejection, affects TNF and sTNFR clearance, leading to increased levels of TNF coupled to sTNFR in the circulation. The role of the kidney in clearance of TNF and its soluble receptors in the process of kidney allograft rejection will be discussed.

## PAPER 20

### MYOBLAST TRANSPLANTATION: DO MYOBLASTS INDUCE TOLERANCE?

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It is not clear if muscle cells can act as antigen presenting cells (APCs). Although it has been suggested that human myoblasts can stimulate T cells in response to antigen (1), others have demonstrated that cardiac myocytes cannot stimulate a primary alloresponse (2), which might be predicted from observations that non-professional APCs tend to induce tolerance rather than T cell activation (3-5). How skeletal myoblasts behave as APCs has clear implication for their potential therapeutic role in transplantation for congenital myopathies, or as vectors for the introduction of new genes, the products of which may be recognised by the immune system as neoantigens.

TE671 is a rhabdomyosarcoma-derived cell line, the origin of which has been confirmed by the presence of numerous phenotypic features of normal myoblasts. We used TE671, transfected to express surface HLA-DR1, as our model of myoblasts and found that these cells could reconstitute a phytohaemagglutinin (PHA) response by purified T cells. However, they were unable to stimulate 6 out of 6 anti-DR1-allospecific T cell clones or stimulate a primary alloresponse. Similarly, they could not stimulate DR1-restricted haemagglutinin-specific T cell clones when pulsed with the relevant haemagglutinin peptide. Following overnight preincubation with TE671-DR1, these clones failed to respond to conventional stimulation the following day with DR1+ B lymphoblastoid cells, implying that these muscle cells have the ability to tolerise T cells.

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## PAPER 21

### PROLONGATION OF MURINE CARDIAC ALLOGRAFTS AFTER TREATMENT WITH ANTI-CD4 ANTIBODY AND INTRATHYMIC ALLOANTIGEN

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Recently, several studies have reported the induction of donor-specific tolerance to islet cells, hearts, livers and kidneys in rats after the intrathymic (i.t.) injection of alloantigens in antilymphocyte serum (ALS) treated recipients. One group has also reported the prolongation of skin grafts in ALS treated mice after the i.t. inoculation of donor-specific spleen cells (SCs). We wished to test whether this phenomenon could be reproduced in the mouse using anti-CD4 monoclonal antibody (Mab).

Methods: C3H/He (H-2<sup>K</sup>) mice were given 50mcg of YTA3.1 anti-CD4 Mab intravenously (i.v.) on the day before and the day of heterotopic cardiac allograft transplantation. Balb/c (H-2<sup>d</sup>) mice were used as donors. Seven days later, the C3H/He mice were injected i.t. with  $5 \times 10^7$  untreated Balb/c SCs (group 1). Control groups received either no treatment at all (group 2), anti-CD4 alone (group 3), anti-CD4 and  $5 \times 10^7$  Balb/c SCs i.v. (group 4), or anti-CD4 and  $5 \times 10^7$  C3H/He SCs i.t. (group 5). Allograft survival was monitored by regular palpation and electrocardiography.

Results: Survival in days	MST	n	(MST= median survival time)
Group 1 38,58,>100 x 4	>100	6	
Group 2 8 x 6	8	6	
Group 3 14,18,23,26,58,60	25	6	
Group 4 15,18,24,24,43	24	5	
Group 5 22,22,25	22	3	

Conclusion: Prolonged cardiac allograft survival can be induced one week after transplantation under the cover of anti-CD4 by the direct i.t. injection of alloantigens. This prolonged graft survival can be induced against a full MHC class I and Class II difference. This is the first report of this phenomenon using a vascularised graft and anti-CD4 antibody in the mouse. This model should be a useful approach to further explore the mechanisms by which thymic injection of alloantigen is able to induce tolerance.

## PAPER 22

### THE EFFECT OF PRETRANSPLANT BLOOD TRANSFUSIONS ON THE IMMUNE SYSTEM.

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The use of deliberate pretransplant blood transfusions to improve graft survival rates is common in various transplant centers. The mechanism of the beneficial effect of transfusions is not understood. To gain insight into the immunological events caused by blood transfusion, we performed various studies.

One retrospective study among kidney transplant patients (N=62) and two prospective clinical studies among kidney (N=80) and heart transplant candidates (N=80) were performed. We studied patient and graft survival, and leucocyte antibody formation. We performed cell mediated lympholysis tests, mixed lymphocyte cultures, inhibition experiments by post transfusion leucocytes, and monitored leucocyte subpopulations.

The data show that the sharing of HLA-DR antigens (especially the more precisely defined HLA-DR subtypes) between blood donor and transfusion recipient greatly influences the immunological consequences of blood transfusion. HLA-DR mismatched blood transfusions lead to decreased patient survival ( $p=0.02$ ), increased graft rejection ( $p=0.003$ ), increased sensitisation ( $p<0.001$ ), increased cell mediated cytotoxicity ( $p<0.001$ ) and increased T cell proliferation ( $p<0.001$ ). HLA-DR matched blood transfusions result in improved patient and graft survival rates, no humoral or cellular immunisation and a decrease in the CD4/CD8 ratio and the number of CD4+ T helper cells. In view of these serious clinical and immunological consequences we suggest that an international registry should be created to compile the results of HLA-DR typed blood transfusions.



## PAPER 23

### TOLERIZING AND IMMUNIZING EFFECTS OF BLOOD TRANSFUSION

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Previously, we have shown that blood transfusion (BT) can have a tolerizing effect on the T cell repertoire. Patients receiving a BT from a donor who shares one HLA haplotype with the patient (HLA-sharing BT) develop CTL non-responsiveness against cells of the BT donor.

The present study demonstrates that HLA-sharing BT also results in a selective decrease in the usage of T cell receptor (TCR) V $\beta$  families. In contrast, patients receiving a non-HLA-sharing BT remain CTL responders and do not show any change in TCR V $\beta$  usage following BT. In the latter group of patients the fine CTL specificity was analysed. CTL precursor (CTLp) frequencies were measured in split-well analysis and blocking studies of CTL-target cell interaction were performed with anti-CD8 monoclonal antibodies.

The results demonstrate that non-HLA-sharing BT influences the T cell repertoire according to the immunogenicity of the mismatched class I antigens and induces high affinity CTL against dominant class I mismatched antigens. Such HLA class I antigens should be regarded as non-acceptable mismatches in subsequent organ transplantation.

## PAPER 24

### CD8+ T-CELLS INHIBIT DONOR-ANTI-HOST REACTIVITY AFTER PRE-TRANSPLANT BLOOD TRANSFUSIONS.

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Administration of HLA-DR-matched blood transfusions before transplantation can have a favourable effect on graft survival. The mechanism of this effect is still not understood. We questioned whether after blood transfusion T-cells develop that actively inhibit the immune response. We generated T-cell clones derived from post-transfusion PBL (peripheral blood leucocytes) from a recipient of an HLA-DR matched blood transfusion. These clones were tested for inhibition of the Mixed Lymphocyte Reaction. Two out of five CD8+ clones inhibited the MLR. None of the eight CD4+ clones had a suppressive effect. Unexpectedly the CD8+ T-cell clones inhibited the response of blood donor against recipient. Panel studies revealed that the effect was specific and appeared to be HLA-DR restricted. This patient was followed in time. Reactivity of blood donor against hosts gradually decreased after blood transfusion. The loss of donor-anti-host reactivity was confirmed in sixty other patient-donor combinations. The decrease of the response was significantly correlated with time ( $p < 0.001$ ). These data indicate that after transfusion an active immunological mechanism is involved in the protection of the recipient against host-specific chimaeric T cells of the blood transfusion donor.



## PAPER 25

### REJECTION OF PIG HEARTS BY HUMAN BLOOD IN A WORKING HEART MODEL IS MEDIATED BY THE CLASSICAL PATHWAY OF COMPLEMENT.

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The transplantation of pig hearts into man may offer an attractive solution to the problem of donor organ shortage. This represents a discordant xenograft combination in which the transplanted organ is hyperacutely rejected. An *ex-vivo* working heart perfusion model has been used to study the rejection of pig hearts by human blood.

Hearts were removed from young pigs (3 kgs) and perfused with pig blood or human blood. Blood was unmodified (n=10), heat treated (50°C for 20 mins : n=10), treated with cobra venom factor (n=10) or antibody depleted (n=6). Serial blood samples were analysed using standard haemolytic complement and antibody assays. Tissue was examined with conventional histology and immunohistochemistry. The stroke work performed by the heart was monitored throughout the experiment.

Pig hearts perfused with unmodified human blood performed less well and stopped working sooner than pig hearts perfused with pig blood (median survival 47 vs. 158 mins). Decomplementation of blood by heat treatment produced improved performance for hearts perfused with pig and human blood (median survival >4 hrs). Decomplementation of human blood by cobra venom factor improved stroke work performance and increased the period of survival (median survival >4 hrs). Hearts perfused by human blood from which antibody had been absorbed performed greater stroke work and survived longer (median survival 210mins) than those perfused with unmodified human blood. Quantitative differences were demonstrated in tissue complement fixation between the groups.

The survival and performance of hearts perfused with human blood may be significantly improved by decompensation. Hearts perfused with antibody depleted blood also perform better. Although alternative pathway of complement activation is important in hyperacute xenograft rejection for some species combinations, it appears that in the pig to human combination the classical pathway of complement activation is important for hyperacute rejection.

## PAPER 26

### IgG ANTIBODIES AGAINST AN HLA ANTIGEN ARE ASSOCIATED WITH ACTIVATED CYTOTOXIC T CELLS AGAINST THIS ANTIGEN, IgM ARE NOT.

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Pre-existing alloantibodies against the mismatched HLA-A and HLA-B antigens of the donor, when present in current sera, are believed to be detrimental for kidney graft survival. When these antibodies are only present in historical sera, their immunoglobulin class has reported to be important with respect to the expected graft survival, IgG antibodies being associated with poor graft prognosis and IgM with a reasonable graft survival.

In the present study we have tested whether the immunoglobulin class of anti-HLA antibodies is reflected in the activation state of cytotoxic T lymphocytes (CTLs) directed against these HLA antigens as measured by their *in vitro* resistance or sensitivity to Cyclosporine A (CsA). The results indicate that the presence of IgG anti-HLA antibodies is associated with the presence of activated CTLs (CsA-resistant), whereas in case of IgM antibodies mainly naive CTLs (CsA-sensitive) are found. This observation may explain the different prognosis of historical positive crossmatches due to IgG versus IgM alloantibodies.



## PAPER 27

### FORMATION OF ANTI-ENDOTHELIAL ANTIBODIES BY PATIENTS WITH CHRONIC REJECTION FOLLOWING RENAL TRANSPLANTATION

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Chronic rejection after renal transplantation remains a serious complication, the immune mechanisms of which are poorly understood. Here we have used a technique of SDS-PAGE and Western blotting to investigate the possible involvement of anti-endothelial antibodies in this disease. Three groups of patients were studied retrospectively; 11 renal transplant patients with chronic rejection (10 months - 22 years post op), 16 stable renal transplant patients (1 - 18 years post-op) and 13 patients with chronic renal failure who had not been transplanted. Serum samples were taken within twelve months of the time of initial diagnosis of chronic rejection (diagnosed at 4 months - 16 years post transplantation). Chronic rejection was diagnosed by creatinine levels and examination of renal biopsies. Serum samples were tested for antibody reactivity against human umbilical vein cells using Western blotting. Reactivity against a specific band of endothelial peptides of molecular weight 56 and 58 kDa was found in 6/11 patients with chronic rejection, 1/16 stable patients and 2/12 non-transplanted patients with chronic renal failure. Anti-endothelial antibodies of any reactivity were found in 9/11 patients with chronic rejection, 12/16 stable transplant patients and 9/12 patients with chronic failure.

In conclusion, there appears to be an association between antibodies against a particular doublet of endothelial peptides (at 56 and 58 kDa) and development of chronic rejection. Prospective studies now need to be done to investigate when these antibodies first appear and whether their production is also associated with acute rejection episodes.

## PAPER 28

### IMMUNOGENICITY OF FRESH AND CRYOPRESERVED HUMAN CARDIAC VALVE ALLOGRAFTS.

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To reconstruct ventricular outflow tracts, mechanical valves, xenografts and human cardiac valves are used. In adults, aortic valve allograft calcification is frequently observed, while early destruction and insufficiency is commonly seen in children. Failure of these allografts could have an immunologic basis because valve allografts are randomly allocated to patients without reference to their HLA and ABO status. Indeed, studies in animals showed higher reactivity against valve allografts when donor and recipient were not matched for MHC. To determine the cellular immunogenicity of human valve allografts, we tested 2 mm diameter pieces of fresh and cryopreserved pulmonary and aortic leaflets in a lymphocyte proliferation assay. Ten irradiated (30 Gy) pieces of each valve were incubated with peripheral blood lymphocytes, mismatched for HLA antigens. After 7 days <sup>3</sup>H-thymidine incorporation was measured. Proliferation was expressed as stimulation index (SI). Significant lymphocyte proliferation was observed with a maximum SI of 70 (median, range 1.5-284) for the fresh and 8 (median, range 6-115) for the cryopreserved valves. This difference proved to be statistically significant ( $p < 0.05$ , Wilcoxon). Fresh leaflets also induced higher median SI: 11 versus 2.4 ( $p < 0.05$ , Wilcoxon) compared to cryopreserved allografts. Although the latter induced significantly less lymphoproliferation, they nevertheless remained able to stimulate immune competent cells in vitro. This could be an explanation for the early degeneration of aortic valve allografts observed in the clinic.



## PAPER 29

### THE SMALL BOWEL TRANSPLANT : DOES IT WORK?

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The absorptive capabilities of transplanted small bowel are uncertain. Long chain fatty acids are absorbed exclusively via lymphatics which take 14-20 days to reconnect and up to 4 weeks to be anatomically normal. A model of jejunoileal autotransplantation was designed to eliminate rejection, ischaemia and reperfusion, while keeping portal venous drainage. A 5mmol solution of oleic acid in isosmolar solution with bile salts was used to assess absorption in jejunum and ileum. There were 6 dogs in each of 4 groups: Group 1- jejunal control, Group 2- jejunal autotransplant, Group 3- ileal control, Group 4- ileal autotransplant; each had an isolated 80cm loop perfused with the warmed solution for 3 hours at 3ml/min on 3 occasions at Week 2 and 9. Loop effluent was analysed for volume, sodium, chloride and oleic acid and transit time was assessed at 1 and 2 hours with a bolus of marker. Oleic acid absorption in the jejunum was not impaired by autotransplantation early on at Week 2 (Table) or late at Week 9.

	Group 1	Group 2	
Volume (%/15mins)	-1.0 (5.5)	4.7 (10.5)	(p=0.2)
Sodium (%/15mins)	-1.8 (6.8)	4.4 (11.2)	(p=0.2)
Chloride(%/15mins)	-0.2 (8.4)	2.3 (11.4)	(p=0.6)
Oleic acid(%/15mins)	44.5(9.4)	50.3(15.9)	(p=0.4)
Transit time (mins)	3.7 (1.1)	4.7 (1.8)	(p<0.2)

Results expressed as mean (standard deviation).

Oleic acid absorption in the ileum is also unaffected by autotransplantation at both time points (data not shown).

Long chain fatty acids can be absorbed normally by the small bowel at the earliest stages in the post-transplant period, allowing small bowel transplant patients early resumption of enteral nutrition.

## PAPER 30

### SUCCESSFUL TRANSPLANTATION OF MARGINALLY ACCEPTABLE DONOR LIVERS

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Organ shortage frequently results in emergency liver transplantation or deaths on waiting lists. These pressures have compelled us to widen our definition of the suitable liver donor. Of 212 liver transplants carried out between March 1991 and September 1992, 29(14%) livers were retrieved from donors felt to be of suboptimal quality for the following reasons: alcoholism(7), paracetamol overdose(2), grossly abnormal liver tests(11), sepsis(4), advanced cardiovascular disease(6), intraoperative arrest(2), suspected steatosis(4) and other(4). There were 9 local donors and 10/15 livers were previously declined by other centres on medical grounds.

After careful consideration, 15 were used for routine recipients, 9 for urgent and 5 for patients with fulminant failure. All 29 grafts showed satisfactory early function, but with significantly greater day 1 AST(p=.004) and peak AST(p=0.0008). Graft survival at 12 months were similar (72% vs 73% from "good" donors). There were 8 deaths - 1 fulminant, 4 urgent and 3 routine recipients. Preliminary reports from a questionnaire outlining details of these donors to all European transplant centres showed that a median of 23%(0-60%) of these donors would have been refused.

These results suggest that a reassessment of liver donor criteria may be appropriate, in order to maximise donor utilisation.



## PAPER 31

### IN VIVO EFFECTS OF IgA AND IgG2a ANTI-CD3 ISOTYPE SWITCH VARIANTS: A PROSPECTIVE DOUBLE-BLIND STUDY IN RENAL TRANSPLANT RECIPIENTS.

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Side-effects after the first administration of OKT3, a murine anti-CD3 monoclonal antibody of the IgG2a class, are attributed to the release of cytokines as a result of transient activation of T cells. T cell stimulation by OKT3 is supposed to be dependent of interaction with Fc receptors on human monocytes. As human monocytes do not possess Fc receptors for murine IgA, it is likely that an anti-CD3 monoclonal antibody of the IgA class (T3.A) causes less side-effects and cytokine release as compared to its IgG2a switch variant (T3.G2a). To test this hypothesis we treated 20 renal transplant patients in a prospective double-blind study with either T3.G2a or T3.A induction therapy: 0.5 mg anti-CD3 twice daily during 10 days. Compared to T3.G2a, T3.A caused significantly less side-effects and hardly any cytokine release. Also, complement- and neutrophil activation products only increased after T3.G2a and not after T3.A. Rejection incidence was comparable in both groups. Both T3.A and T3.G2a resulted in a complete depletion of CD3+ cells, but after T3.A CD3 depletion was of shorter duration. Finally, in contrast to T3.G2a, T3.A did not affect coagulation and fibrinolysis.

In conclusion, T3.A class causes hardly any cytokine release and less side-effects as compared to T3.G2a. Provided T3.A is immunosuppressive, it is superior to OKT3.

## PAPER 32

### TREATMENT OF PERSISTENT ACUTE REJECTION IN HEPATIC ALLOGRAFTS IN AN ERA OF FK506

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The incidence of acute rejection and its response to therapy was reviewed in 326 adult (primary) liver graft recipients on immunosuppression with Cyclosporin A, Azathioprine and Prednisolone who survived to have a protocol biopsy at 7 days post transplant. 252 (77%) patients showed evidence of acute rejection (graded mild in 94, moderate in 87 and severe in 71) on histology. 226 with consistent clinical/laboratory features were treated with high dose prednisolone (200 mg x 3 days). Resolution was seen in 147 (65%) patients.

The remaining patients (n=78) were classified as having persistent acute rejection. Of these, 72 were treated with further 1 or 2 doses (n=19) or a second 3 day course of prednisolone (n=53). 6 were converted to FK506 (n=2) or received OKT3 (n=4). 58 (81%) of the patients who received more steroids for persistent acute rejection responded while only 1 (treated with OKT3 followed by FK506) out of 6 receiving OKT3 and/or FK506 responded. Of the 14 non-responders to further steroids, 5 were salvaged with OKT3 and/or FK. Overall rate of graft loss secondary to intractable rejection was 4% (14 out of 326). The severity of rejection on day 7 biopsy was not related to later graft loss to irreversible rejection.

Our results show that acute rejection resistant to initial steroid treatment can be managed with further steroid therapy in the majority of cases. Despite FK/OKT3 being used sparingly, the graft loss rate was low. At present, we believe that if acute rejection fails to respond to a single course of steroids, re-treatment with steroids rather than early conversion to OKT3 or FK506 remains the therapy of choice.



### PAPER 33

#### VASCULAR REJECTION (VR) REJECTION AFTER KIDNEY TRANSPLANTATION OCCURS EARLY AND IS A MAJOR DETERMINANT OF BOTH SHORT-TERM AND LONG-TERM SURVIVAL.

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Vascular rejection is thought to be the major cause of graft loss after the first year after kidney transplantation. To investigate the time of onset of vascular rejection we studied all consecutive patients (n=482) receiving a post mortal kidney between 1983 and April 1991 in our center. We report findings from biopsies taken during the first three months after transplantation, consequences for long term graft survival and risk factors for developing a specific type of rejection. Ninety-three patients lost their graft due to rejection during a 9-year follow-up period. One year graft survival was 88.9% for 241 patients without rejection, 87.4% for 111 patients with interstitial rejection (IR) and 50% for 84 patients with VR. Five-year graft survival for these groups was 74.4%, 72.1% and 35.8% respectively. The relative risk of graft loss was 4.35 (95% CI 2.89-6.55) for VR and 1.20 (95% CI 0.75-1.92) for IR compared to patients without rejection during the first 3 months. Risks were calculated with the Cox proportional hazards regression model with VR and IR as time dependent co-variables. Yearly graft loss due to chronic rejection was 1.97%/yr in patients without early rejection, 2%/yr in patients with IR and 7.74% in patients with VR. Major risk factors for developing vascular rejection were use of azathioprine without cyclosporine, number of HLA-DR mismatches, prolonged cold ischemia time and previous transplantations. Risk factors for IR were use of azathioprine without cyclosporine, recipient age and HLA-DR mismatches. We conclude that VR occurs within a short time period following renal transplantation (within 3 months), and is a very strong predictor of graft loss while IR has a minor negative effect on graft survival.

### PAPER 34

#### THE EFFICACY, SAFETY AND LONG-TERM RESULTS OF ANTI-CD3 ANTIBODY THERAPY IN RENAL TRANSPLANTATION

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Little data exists on the long-term outcome of patients treated with antibodies against CD3 cells (ATG and OKT\*3). We report here our experience with 39 patients so treated from 1987 to 1993.

Thirty-three patients received treatment for steroid-resistant rejection and the remaining 6 were treated prophylactically because of either having lost a previous graft with early steroid-resistant rejection, or having >85% cytotoxic antibodies. Twenty-nine were given ATG and 10 OKT\*3. Eight grafts were lost, largely due to irreversible rejection. Five patients died, all but one from overwhelming sepsis, in a period when the dose of antibody was not adjusted according to CD3 counts. The remaining 26 patients were successfully treated.

In the past year we have monitored daily CD3 counts and reduced the dose of antibody to the minimum necessary to lower the count to  $<50 \times 10^6/l$ . This has allowed an average dose reduction of ATG from 2.5 to 1.5mg/kg/day, with consequent increased safety and lower cost.

Long-term renal function has been very satisfactory: 14 patients have serum creatinines  $<150 \mu\text{mol/l}$ , 7 others are in the range 150-250, while only 5 have creatinines  $>250$ . Even more important, the long term stability of renal function has been remarkably good: at up to 6 years all but 4 patients have better graft function than at 1 month after the end of antibody therapy, and no kidney that has functioned at 3 months has yet been lost.