

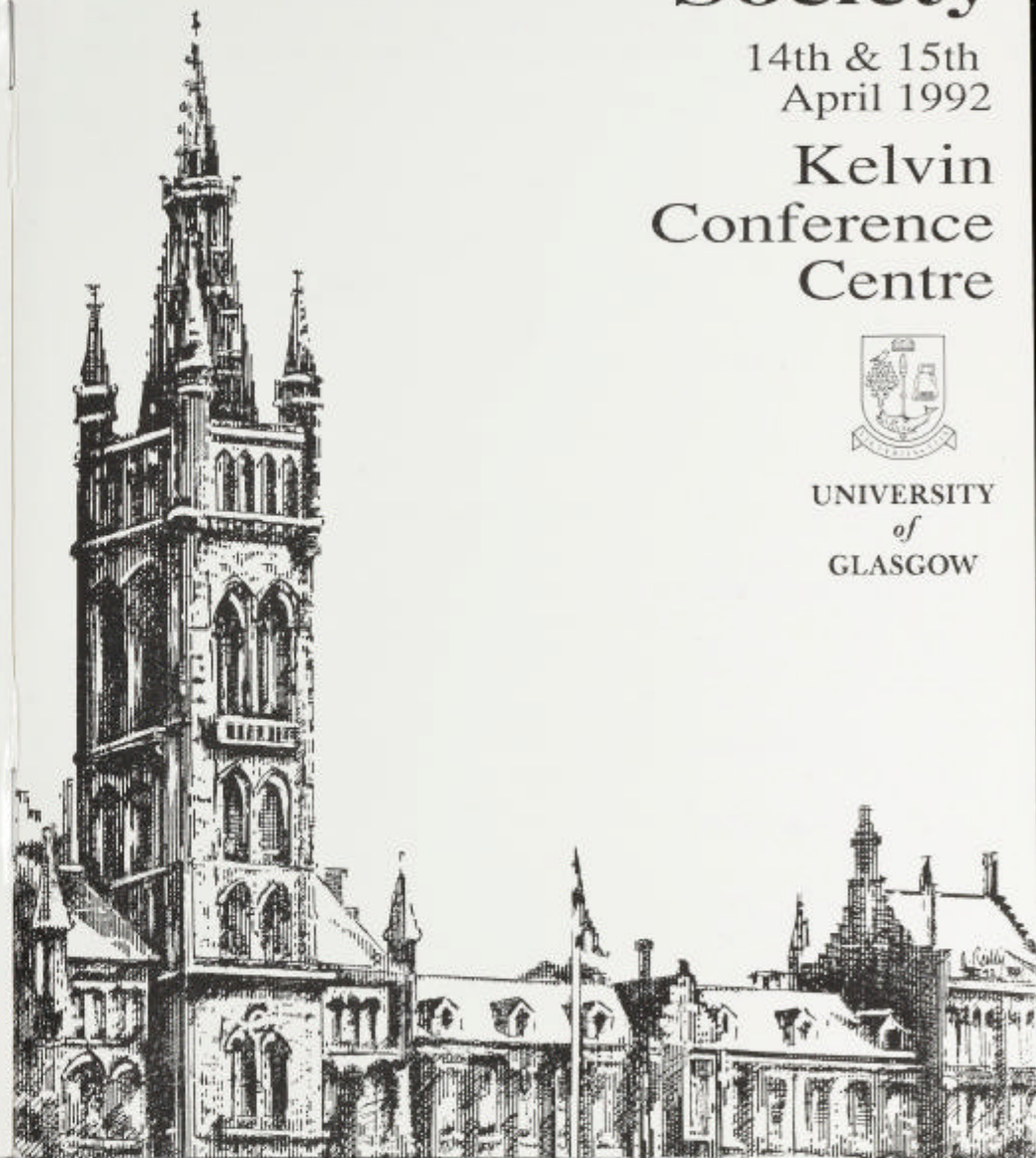
The British Transplantation Society

14th & 15th
April 1992

Kelvin
Conference
Centre



UNIVERSITY
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PAPER 1

CYCLOSPORIN-RELATED HYPERCOAGULABILITY? - A PROSPECTIVE STUDY

CM Edwards, JDT Morgan, S Harper, T Horsburgh, PK Donnelly

Renal Transplantation Unit, Leicester General Hospital, Leicester, UK

Cyclosporin (CyA) may increase thromboembolic complications following renal transplantation. We looked prospectively for laboratory (i) and clinical (ii) evidence of (CyA) related hypercoagulability.

(i) A rapid whole blood coagulation assay Thrombelastography, (TEG) was used to compare 28 consecutive renal transplant recipients receiving CyA (17 mg/kg/day) and steroid with 20 patients undergoing open cholecystectomy. TEG was measured pre-operatively on post operative days 1,3,5,7,14,42 and 6 months following transplantation. Transplant recipients were significantly more coagulable ($p < 0.001$) before operation than the cholecystectomy patients. Both groups increased in coagulability post-operatively but this resolved by day 42 in the cholecystectomy group but persisted to 6 months post-transplantation.

(ii) Sixty one consecutive first cadaver renal transplant patients were randomised pre operatively to one of three immunosuppressive regimens and all thrombotic episodes within 6 months of surgery recorded. Group A (CyA, initial dose 17 mg/kg/day), Group B (regimen A plus the vasoprotective calcium channel blocking drug (CaCB), nifedipine) Group C (CyA, initial dose 10 mg/kg/day plus azathioprine 1 mg/kg/day). All groups received identical steroid regimen. CaCBs were avoided in groups A and C. Significantly more ($p < 0.03$ Fisher's exact test) patients (16%) developed thrombotic complications (one pulmonary embolus, one deep vein thrombosis, one coronary thrombosis) in Group A, compared to zero in groups B and C.

Immunosuppression with CyA and long term effects of renal failure exacerbate post-surgical hypercoagulability. Triple immunosuppressive therapy or the use of CaCBs may offset these thrombotic tendencies.

PAPER 2

Consensus on General Medical Contra-indications to Organ Donation?

Sheila M. Gore on behalf of Raters' Dozen

MRC Biostatistics Unit, 5 Shaftesbury Road, CAMBRIDGE CB2 2BW

We investigated: is there a lack of consensus between specialties on the medical contra-indications to organ donation, and are there disagreements also within specialty?

The specialties represented were: intensive care by presidents of the Intensive Care Society, transplant co-ordination by chairpersons of the Association of Transplant Co-ordinators, kidney transplantation by presidents of the British Transplantation Society, cardiothoracic transplantation by colleagues at Papworth, and liver and corneal transplantation each by one surgeon.

With reference to the 437 confirmed brain-stem deaths with a listed general medical contra-indication from the 1989 and 1990 confidential audit, specialists were asked to score specific organs (1 = transplantation in contra-indicated, 2 = possibly suitable: requires discussion, 3 = transplantation, or 9 = not assessed). The only additional available information was the age, sex and cause of death of the deceased.

Mean age at confirmed brain-stem death was 40.9 years (sd = 22.5 years) for the 437 cases, of whom 68 (16%) were aged 14 years or younger and 144 (33%) were 55 years or older. There were 194 (44%) females. Renal specialists rated 316 (72%) cases identically and their 107 minorly disputed scores (80 contra-indicated/requires discussion, 27 requires discussion/transplantable) displayed no systematic bias between raters. In only four cases was there serious disagreement.

Between transplant co-ordinators and between intensive care specialists there was systematic disagreement in assigned kidney and corneal scores, with the same scorer in each pair being always the more liberal in interpreting the described medical contra-indication.

Transplant surgeons considered that kidneys were transplantable in 28 (6%) out of the contra-indicated 437 cases, the liver in only 5 (1%) but corneas in 209 (48%) patients. In no case did the cardiothoracic surgeons consider that heart or lungs were transplantable. Corneas apart, it is reassuring that few transplantable solid organs were missed. Raters' comments on this exercise suggest that professional training about suitability for transplantation should focus on: bacterial versus viral meningitis, donation of extra-renal organs in the context of renal failure, muscular dystrophies, previous malignancy in the context of corneal transplantation, relaxation of donor contra-indications for emergency recipients.

PAPER 3

A CONTROLLED TRIAL TO INVESTIGATE THE OPTIMUM TIME FOR INITIATING CYCLOSPORIN THERAPY IN RENAL TRANSPLANT RECIPIENTS.

Griffin P J A, Moore R H, Krishnan H, Fenn N J, Salaman J R

Department of Surgery, The Royal Infirmary, Newport Road, CARDIFF, CF2 1SZ

It is customary for patients undergoing kidney transplantation to receive their first dose of Cyclosporin either just before or during the transplant operation. The rationale for such early dosing is based on in-vitro findings that Cyclosporin is less effective in suppressing lymphocyte proliferation when given some hours after the initiation of culture. Since pre-operative dosing may jeopardise early renal function, we have studied the effect of withholding Cyclosporin for 12 hours in patients receiving triple therapy.

Consecutive adult recipients of primary cadaveric renal transplants were randomised to receive their first dose of Cyclosporin (10/mg/kg po) 6 hours prior to transplant surgery or 12 hours afterwards. All patients received Azathioprine (1.5 mg/kg iv) and Methylprednisolone (0.5 g iv) in addition during surgery. From the second day onwards, both groups received identical (triple) immunosuppression. The two groups of patients were well matched and important donor factors were also distributed equally.

Cyclosporin 1st dose (pts)	Pre-Tx (22)	Post-Tx (23)
Immediate function	7 (32%)	17 (74%) p<.02
S. creatinine (umol/l) 1 month	242 ± 114	169 ± 56 p<.01
Rejection episodes per patient	0.7 ± 1.1	0.4 ± 0.5 p<.005
OKT3 given (pts)		
/kidney rejected	4/1	0/0

Delayed Cyclosporin dosing resulted in significantly better immediate function, and function at one month. Although target Cyclosporin levels were achieved by the same time (6.2 days), the delayed dosed group had paradoxically less frequent and severe rejection.

PAPER 4

TOTAL ATRIOVENTRICULAR ORTHOTOPIC HEART TRANSPLANTATION: A PROSPECTIVE RANDOMISED CLINICAL TRIAL IN 56 CONSECUTIVE PATIENTS.

S Kendall, F Ciulli, PA Mullins, B Biocina, FC Wells, J Wallwork, PM Schofield, SR Large.

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

Complete cardiac atrioventricular transplantation (CCA VT) may improve cardiac function compared to the standard cardiac transplant ventricular transplantation with atrioplasty (VTA). We performed a prospective trial comparing morbidity, mortality and functional differences between the two methods. From November 1990 to December 1991, 56 consecutive patients were randomised to CCA VT (Group A, n=28, including 5 females) or VTA (Group B, n=28, including 1 female). There was no difference between the two groups in pre-transplant diagnosis (50% ischaemic heart disease and 42% cardiomyopathy in both groups), age 47 SD 8.8 vs 48 SD 8.9 years, weight 74 SD 12 vs 72 SD 13 kg, total ischaemic time 192 SD 82 vs 202 SD 73 minutes, bypass time 121 SD 33 vs 116 SD 35 minutes, post operative blood loss 670 SD 953 vs 556 SD 444 mls, duration of ventilation 30 SD 100 vs 10 SD 5.7 (p= NS), and time to discharge 26 days SD 18 and 26 SD 11 days. The mortality in Groups A and B during the study period was 3 and 4 patients respectively, from causes unrelated to the method of connection. At 4-6 months post-transplant Doppler echocardiography showed 1 Group A patient with a trace of tricuspid regurgitation whereas all patients in group B had mild to moderate tricuspid regurgitation, and mitral regurgitation in 7 patients. However, echo-contrast echocardiography did not reveal any differences. At exercise testing there were no significant difference regarding exercise tolerance and atrioventricular conduction. All patients completed stage two of the Bruce protocol and stopped at stage three. Starling curves using colloid challenge and thermo-dilution techniques were similar for both groups with mean cardiac output increases of 0.82 (0.2-1.4) and 0.63 (0.1-2) L/min respectively with an increase in pulmonary capillary wedge pressure of 6 (5-11) mmHg. CCA VT is an alternative method of transplantation with acceptable morbidity and mortality, but without improvement in function.

PAPER 5

SURVIVAL AND QUALITY OF LIFE OF CYSTIC FIBROSIS PATIENTS ACCEPTED FOR HEART-LUNG TRANSPLANTATION

Caine N, Sharples LD, Dennis C, Higenbottam TW and Wallwork J.

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

An evaluation of heart-lung transplantation (HLT) as a treatment for end-stage cystic fibrosis (CF) includes analysis of the survival and quality of life experience of CF patients accepted for HLT, with comparisons drawn from those transplanted and those not transplanted during the period of study. Results are presented from 84 patients accepted and 40 transplanted up to December 1991.

Actuarial probability of survival was calculated using the life table method. A measure of general health status, the Nottingham Health Profile (NHP), was used in interviews with patients at assessment for HLT and at 3 monthly intervals to transplantation or death. After transplant, NHPs were completed at 3, 6 and 12 months and thereafter at 6 monthly intervals. A score of 0-100 is calculated for each of 6 dimensions covering physical, social and emotional functioning; the higher the score the higher the level of dysfunction.

The actuarial probability of survival at 1 year from acceptance for transplantation was 86% for the transplant group (n=40, 10 deaths) and 36% for the non-transplant group (n=44, 29 deaths). When treated as a time dependent covariate, transplantation conferred a lower risk of death than non-transplantation (RR = 0.72). The actuarial survival rate following transplantation was 81% at 1 year with 24 patients at risk. In comparing NHP scores before and after transplantation there were marked improvements between the latest pre-transplant and the earliest after transplant responses for 25 pairs of patients in all 6 dimensions. These differences reached statistical significance in the dimensions of physical mobility and energy (P <0.001), emotional reactions (P <0.01), sleep and social isolation (P <0.05). In comparing the pretransplant NHP scores for the 2 groups of accepted patients, those transplanted and those not transplanted, their levels of dysfunction were similar at the time of assessment and at subsequent 3 monthly intervals.

In conclusion, the 1 year probability of survival following transplantation, at 81%, compares well with the overall HLT series at this centre 79% (n=121). Further, for CF patients accepted for HLT the transplant group is at less risk of dying than the non-transplant group (RR = 0.72). Early results from the health status data show no differences between the groups transplanted and not transplanted, at intervals after acceptance, but demonstrate measurable improvements soon after transplant surgery.

PAPER 6

IS CYTOMEGALOVIRUS MATCHING NECESSARY IN HEART TRANSPLANTATION

B Biocina, PA Mullins, T Wreghitt, F Ciulli, FC Wells, J Wallwork, SR Large.

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

Donor transmitted cytomegalovirus (CMV) infection is common in patients who are CMV negative prior to transplantation. We examined the mortality and morbidity in CMV mismatched heart transplant recipients in our programme. Since the introduction of triple therapy in April 1986, 273 orthotopic heart transplants (Htx) have been performed to date (July 1991). Patients were divided in groups according to their preoperative donor (D) and recipient (R) CMV status: MISMATCH (R-/D+), n=47 and COMPATIBLE (all others), n=226. 26 patients died before discharge from hospital, but no deaths were CMV related. Actuarial survival rates at 3 years after Htx were 69.7% (se 7.51) in the MISMATCH group compared to 79.5% (se 3.17) in the COMPATIBLE group (p<0.1). 1 year actuarial freedom of infection related death was 90.5% (se 4.51) in MISMATCH group compared with 96.4% (se 1.31) in the COMPATIBLE group, p<0.05. In 247 discharged patients (MISMATCH n=43, COMPATIBLE n=204) rejection and non-CMV infection were comparable in both groups. In MISMATCH group 27 (62.9%) patients required at least one readmission compared with 68 (33.3%) in COMPATIBLE group (p<0.001). Number of readmissions were higher in MISMATCH group (2/patient vs 1.68/patient in COMPATIBLE group), as well as the mean length of stay (16.6 days in MISMATCH vs 12.2 days in COMPATIBLE group, p<0.05). This totalled 24.2/1000 patient days more in hospital for MISMATCH group compared with 6.55/1000 patient days for COMPATIBLE group (p<0.001). These results indicate increased morbidity in CMV mismatched heart transplant recipients in the medium and long term, contributing to increased costs of follow up care. Heart transplant recipients should be CMV matched whenever possible.

PAPER 7

SINGLE CENTRE RESULTS OF PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY IN CARDIAC TRANSPLANT PATIENTS WITH CORONARY OCCLUSIVE DISEASE.

PA Mullins, A Chauhan, LM Shapiro, SR Large, J Wallwork, PM Schofield.

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

Percutaneous transluminal coronary angioplasty (PTCA) of proximal arterial lesions in cardiac transplant patients with coronary occlusive disease produces short-term improvement in arterial patency. The subsequent effects on prognosis and cardiac function of this form of treatment are as yet unknown. PTCA was performed in the treatment of 24 coronary artery lesions in 18 cardiac transplant patients at our centre up to September 1991. All patients had significant coronary stenoses (> 50% arterial diameter) with evidence of impairment of coronary perfusion on either exercise ECG or isotope scanning. PTCA was performed on 13 left anterior descending coronary artery lesions, 5 right coronary, 2 circumflex lesions, 2 obtuse marginal, 2 PDA artery and one diagonal artery lesion. Primary coronary angiographic success was achieved in 18 of the 25 lesions (75%). The overall time from transplantation to PTCA was 58 SD 21 months. The recurrence rate of the successfully treated lesions at 2.3 months SD 1.2 months was 2 of 17 treated (12%) and at 15.1 SD 3.2 months a further 2 lesions had recurred (24% overall recurrence rate). From the first pre-treatment film to the latest available follow up the ejection fraction measured by left ventricular angiography in the treated group fell slightly from 53.4% SD 12.5% to 51.6% SD 12.5% (p = NS). In the 5 patients in whom PTCA failed, the initial ejection fraction was 42% SD 14% but subsequent angiography data is incomplete. Of the 13 successfully treated patients one patient has died compared to the five patients in whom PTCA could not be performed where one patient has died, another patient had a cardiac retransplant and a further patient has had coronary artery bypass surgery (difference in clinical events - p < 0.05, Fishers exact test). Coronary angioplasty has an acceptable primary success rate and recurrence rate and may improve outcome in selected cardiac transplant patients with coronary occlusive disease.

PAPER 8

MANAGEMENT OF ORGAN DONORS

Wheeldon DR, Potter C, Graham TR, Johnston K, Bethune D, Large SR, Wells PC and Wallwork J.

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

Donor organ availability is the most limiting factor in organ transplantation. Overall referrals have decreased over the past three years, but a more aggressive attitude towards donor management and careful recipient selection, to optimise the use of "domino" hearts, have enabled us to sustain our levels of activity without adverse effect on 30 day mortality.

	Referrals	Medically Unsuitable	Tx Hearts	Dominoes Generated	Mortality
1991	246	58 (24%)	66 (27%)	14	5.2%
1990	263	75 (29%)	65 (25%)	20	12.3%
1989	279	72 (26%)	67 (24%)	5	13.0%

We have carried out comprehensive haemodynamic studies of 61 multi-organ donors over the past 14 months. 14 "unsuitable" donors (on initial haemodynamic assessment) were resuscitated using a previously developed hormone pre-treatment package, and careful haemodynamic management by a cardiac anaesthetist. 11 of these hearts were successfully transplanted (2 had obvious coronary disease and a further heart was not used due to poor function). 43 donors received the hormone package whilst 18 did not, based on initial haemodynamic functional assessment.

LVSWI	No treatment(18)	Pre-Treatment(43)	Resuscitated(14)
Initial	39.4 (18.1)	29.5 (9.7)	15.4 (3.5)
Post	29.4 (10.8)	33.8 (17.3)	26.7 (11.4)
% Change	75	115	173

International Registry data indicates that 26% of heart transplant recipient deaths are due to primary graft failure. Based on the above experience we have now developed a more objective strategy for donor management and a method to assess cardiac reserve. We believe that this approach provides an opportunity for both improving the numbers of transplantable organs and for improving the quality of all organs transplanted.

PAPER 9

IN VITRO CORRELATES OF PULMONARY ALLOGRAFT REJECTION

AC Cunningham, JA Kirby, IC Colquhoun & JH Dark.

Dept. Surgery, Medical School, University of Newcastle upon Tyne, NE2 4HH.

A canine model of single lung transplantation was used to correlate clinical diagnosis of rejection (transbronchial biopsy, X ray) with immunological assays in the face of tapering doses of Cyclosporin A (CyA). Disparate combinations of donor-recipient pairs were selected on the basis of mixed leukocyte responses (MLR). Five transplants were performed in Beagles and donor responsiveness measured at weekly intervals. Peripheral blood mononuclear cells were investigated by the techniques of limiting dilution analysis and MLR. The cytotoxicity of non-adherent lavage derived cells (LC) to donor pulmonary cells (DPC) was determined by Chromium release assays. The ability of LC to proliferate in the presence of rIL2 was also assayed. IgG levels to DPC were determined by FACS analysis, and the ability of these antibodies to lyse DPC by an antibody dependent cell mediated cytotoxicity (ADCC) mechanism was investigated.

The recipients had stable grafts and were immunologically unresponsive when receiving 20-25 mg/kg CyA per day. All the recipients rejected their grafts when immunosuppression was tapered. Clinical signs of rejection were observed at CyA doses of between 17 & 9 mg/kg/day. The only consistent *in vitro* finding was a rise in DPC specific IgG levels at the time of biopsy confirmed rejection. ADCC activity was observed in the plasma samples from two transplants before the clinical diagnosis of rejection. Cytotoxicity of LC was demonstrated in 2 animals when mild rejection was confirmed. Cytotoxic precursor frequencies increased in 3 animals during severe rejection, and LC proliferation with IL2 was seen in 4 animals at this time.

No single immunological assay was effective at diagnosing pulmonary rejection in this animal model. ADCC activity preceded clinical diagnosis of rejection, and in these animals, the majority of other assays tested gave positive results at the time of biopsy confirmed rejection.

PAPER 10

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES IN PATIENTS UNDERGOING TRANSBRONCHIAL BIOPSIES 6 MONTHS FOLLOWING LUNG TRANSPLANTATION

D.S. Milne¹, A. Gascoigne², J. Wilkes¹, L. Sviland¹, A. Pearson³, T. Ashcroft¹, A.J. Malcolm¹, P. Corris².

University Departments of Pathology¹ and Child Health³, William Leech Centre for Lung Research², Royal Victoria Infirmary and Freeman Hospital, Newcastle upon Tyne.

As part of an ongoing prospective study of the histological and immunohistological features of allogeneic lung transplant biopsies, we have performed a preliminary review on biopsies taken from 22 patients approximately 6 months post-transplantation. Biopsies were separated into the following histological groups: normal (4), rejection (9), infection (2), obliterative bronchiolitis (1), multiple/non-specific features (6). Of 10 patients with no decline in pulmonary function clinically, 6 showed histological abnormalities. All of the biopsies from 12 patients with declining pulmonary function showed histological abnormalities.

Frozen tissue obtained simultaneously from all patients were stained immunohistochemically with a range of lymphocyte and class II MHC markers. Comparison between normal and rejection biopsies suggests that there may be increased expression of HLA-DP on endothelium in acute pulmonary rejection. HLA-DR was widely expressed in all biopsies. Seventeen of 18 biopsies containing lymphocyte infiltrates showed predominantly CD8-positive lymphocytes regardless of histological diagnosis. In none of the cases did natural killer cells or B lymphocytes form significant components of inflammatory infiltrates.

This study shows a good correlation between clinical and histological diagnosis and suggests that the expression of HLA-DR and predominance of CD8-positive lymphocytes may not be specifically related to acute pulmonary rejection.

PAPER 11

PILOT STUDY OF REGIONAL MULTI-ORGAN DONOR TEAM

Stewart R, Porteous C, Findlay J, Phelps J, Bradley JA, Briggs JD.

The Renal Unit, Western Infirmary, Glasgow G11 6NT, Scotland, UK.

The increasing proportion of multi-organ donors has led to delays, unsatisfactory liaison between teams and involves large numbers of personnel (multiple team). One possible solution to these problems would be to form regional (single team) multi-organ donor teams. To examine the acceptability of this and the effect on our own donor programme we compared the results of liver and kidney transplants during a two year period (1/7/89-30/6/91) when either a single local team retrieved the kidneys and liver or the conventional multiple team approach was used.

LIVER TRANSPLANT RESULTS

	Primary non function	Vascular thrombosis	Death <24hr	Survival 30/01/91	Blood replacement vol (L)
Single team	0/16	0/16	1/16	10/6(63%)	8.2±6.6
Multiple team	1/23	1/23	3/23	14/23(61%)	7.2±6.7

Analysis suggests that no detriment occurred in the results of liver transplantation with the single team approach

	No of donors	Livers retrieved single team	Livers not placed multiple team
12mth before study	31	0	6(100%)
1st 12 mth study period	49	1(9%)	10(91%)
2nd 12 mth study period	55	15(16.3%)	13(46.5%)

Although the overall incidence was low immediate graft function (IGF) in kidneys was more common when multiple teams were used (17% vs 4%). During the study period the number of donors and the proportion which were multi-organ increased ($p=0.0012$) and the number of livers offered but not placed decreased ($p=0.042$). These data suggest that use of a regional team is of no detriment to liver transplantation and may improve donor numbers, organ placement and renal IGF.

PAPER 12

CORONARY MICROVASCULAR FUNCTION IS ABNORMAL IN PATIENTS WITH CORONARY OCCLUSIVE DISEASE AFTER CARDIAC TRANSPLANTATION.

PA Mullins, A Chauhan, S Kendall, SR Large, J Wallwork, PM Schofield.

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

Coronary occlusive disease is the major long term problem following cardiac transplantation. It is a diffuse disease which also affects smaller coronary vessels and is difficult to assess angiographically. Coronary flow measurements allow investigation of vasodilatory responses in the coronary microvasculature. We investigated the hypothesis that coronary flow responses (CFR) to papaverine (a non endothelial dependent vasodilator) and acetylcholine (an endothelial dependent vasodilator) are impaired in patients with coronary occlusive disease after cardiac transplantation. CFR was assessed in 27 cardiac transplant patients with normal coronary anatomy (Group 2), and 20 patients with evidence of mild coronary occlusive disease on angiography (mean percentage stenosis diameter 23% SD 6%) in a proximal coronary vessel (Group 1), with 12 of these in the LAD (mean stenosis diameter 24% SD 8%). A Doppler flow probe was inserted into the proximal left anterior descending coronary artery (LAD) in each patient. Incremental doses of intracoronary papaverine (Pap), and glyceryltrinitrate (GTN) followed by acetylcholine (Ach) were given until maximum hyperaemia was achieved. CFR for each drug was defined as the ratio of peak to resting coronary blood velocity. CFR_{Pap} was impaired in Group 1 patients compared to Group 2 - 2.5 SD 1.0 versus 4.2 SD 1.0 ($p < 0.001$). CFR_{Ach} was impaired in Group 1 patients compared to Group 2 - 1.8 SD 0.8 versus 2.9 SD 0.9 ($p=0.04$). There was no difference in LAD diameter between groups after the vasodilatory drugs. Coronary endothelial and non-endothelial dependent microvascular responses are impaired in cardiac transplant patients with minor proximal coronary stenoses. This disturbance of cardiac microvascular function must contribute to the late morbidity and mortality seen in cardiac transplant patients with coronary occlusive disease. The disease can now be monitored by coronary flow reserve measurements.

PAPER 13

VASCULAR THROMBOSIS OF RENAL TRANSPLANTS - WHY DOES IT HAPPEN?

Krishnan H, Penn N J, Griffin P J A, Moore R, Salaman J R

Department of Surgery, The Royal Infirmary,
Newport Road, CARDIFF, CF2 1SZ

Early vascular thrombosis has become the commonest cause of kidney transplant failure in our Unit. In a 3 year period (January 1989 - December 1991) 205 kidneys were transplanted, 20 of which thrombosed. The thrombosis rate (9.8%) is twice that described by others. Immunological factors or an adverse effect of Cyclosporin have been suggested as causes. Exhaustive examination of the records of our patients have revealed the following significant findings.

	<u>Function (185)</u>	<u>Thrombosed (20)</u>	
Donor's urine output media L/24 hours	3.5L	2.3L	p<.01
Kidneys damaged during removal	3.3%	35%	p<.02
Patients on CAPD	37%	45%	p=.05
Transplants performed at night (2400 - 0800)	12%	28%	p<.05
Median anastomosis time	34 mins	38 mins	p<.05

Vascular thrombosis was not seen to be associated with a high PRA or the number of arteries or veins on the kidney. It was also unassociated with the total ischaemic period, or whether the kidney was local or shipped in.

Although the grade of surgeon had no influence, these findings would point to technical rather than immunological factors as being the predominant cause of thrombosis.

Careful attention to detail during kidney removal and transplantation should reduce the frequency of this frustrating complication.

PAPER 14

THE EFFICACY OF OKT3 IN TREATMENT OF STEROID RESISTANT ACUTE RENAL ALLOGRAFT REJECTION

P.A. Rowe, K.S. Fan, J.A. Bradley, S.G. Macpherson,
R.S.C. Rodger, B.J.R. Junor, J.D. Briggs

Renal Unit, Western Infirmary, Glasgow.

The long term outcome of successful treatment with the monoclonal antibody OKT3 is uncertain. Between January 1986 and June 1991, OKT3 was used to treat acute rejection confirmed by graft biopsy and resistant to high dose steroids in 25 of 346 renal transplant recipients.

The standard immunosuppressive regimen was prednisolone 20 mg/day tapering over 12 months to a maintenance dose of 10 mg/day, and cyclosporin adjusted to achieve whole blood trough levels varying from 300 µg/L in the postoperative period to 80-100 µg/L after 12 months. Rejection occurred a median of 26 (range 4 - 521) days after transplantation. OKT3 5 mg/day for 10 days was started a median of 7 (range 3 - 29) days after commencing high dose oral prednisolone.

The median serum creatinine concentration before OKT3 was given was 663 (range 250 - 1007) µmol/L. OKT3 was successful in reversing acute rejection (defined as a fall in serum creatinine to <300 µmol/L, or a fall by >50%) in 17 cases (68%). Serious infection arose in 9 patients (36%), and caused 2 deaths; one from Klebsiella peritonitis and one from CMV.

The actuarial graft survival at 1 year after OKT3 treatment was 35%, and at 2 years 21%. There was no correlation between graft survival and the interval between steroid and OKT3 therapy. If rejection arose more than 90 days after transplantation then only 1 of 8 grafts was still functioning at 12 months, compared with 8 of 17 in whom rejection arose within 90 days of transplantation.

Although OKT3 can successfully reverse steroid resistant acute renal transplant rejection in the majority of cases, the long term survival of grafts rescued in this way is poor, particularly so if the rejection episode occurs more than 3 months after the transplant.

PAPER 15

INDUCTION OF SPECIFIC UNRESPONSIVENESS USING CELLS TRANSFECTED WITH DONOR MHC GENES - EVIDENCE FOR INDIRECT PRESENTATION OF ALLOPEPTIDES IN VIVO.

Kathryn Wood, Yvonne Bell, Daniel Shoskes and Peter Morris

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

We have shown previously that cells of recipient origin transfected with single donor MHC genes can be used to induce specific immunological unresponsiveness to fully allogeneic cardiac allografts. Our initial findings were obtained in a single strain combination, C57BL/10(H-2^b) to C3H.He(H-2^k), and have now been confirmed in this study using BALB/c (H-2^d) donors.

Transfected cells expressing either single donor class I, K^d, D^d or L^d or class II, I^A^d and I^E^d molecules, were each shown to induce unresponsiveness to BALB/c heart grafts when used to pretreat C3H recipients IV, 7 or 14 days before transplantation (median survival time controls-9d; D^d - 30d.; K^d - 23d.; L^d - 15d.; I^A^d - 17d.; I^E^d - 15d.). The capacity to prolong graft survival was shown to be due to the intrinsic immunogenicity of each molecule and the antigen load delivered during pretreatment.

To examine the mechanism responsible for the induction of specific unresponsiveness by single donor MHC antigens we followed the fate of the transfected cells in vivo. Transfectants were labelled with the fluorescent dye, DII, and tissue sections from treated mice were stained using a polymorphic monoclonal antibody specific for each donor alloantigen using immunocytochemistry. Both techniques showed that the transfected cells traffic to the marginal metallophil zone, a specialised area of the spleen reported to be involved in antigen presentation. Low density antigen presenting cells (LODACs) were isolated from the spleens of C3H recipients treated with cells transfected with either the D^d or K^b class I α chain and were found to stimulate a proliferative response in naive syngeneic C3H T cells. No proliferation was observed when the APC were isolated from the spleens of recipients treated with untransfected L cells. These data strongly suggest that indirect presentation of peptides derived from the donor alloantigen by recipient APC is taking place in vivo.

PAPER 16

T CELL RECOGNITION OF DONOR CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX PEPTIDES DURING ALLOGRAFT REJECTION

Josef FANGMANN, Rosemarie DALCHAU, Greta J SAWYER, Carol PRIESTLY and John FABRE

Division of Cell & Molecular Biology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, and The Blond McIndoe Centre, Queen Victoria Hosp., East Grinstead RH193DZ

LEW (RT1^l) recipients of DA (RT1^{av1}) skin and kidney allografts were tested for the capacity of their T lymphocytes to proliferate to three 22-24 amino acid peptides from the hypervariable regions of the RT1-A^{av1} classical class I MHC molecule. Ten days after rejecting second set DA kidney allografts, spleen cells (but, interestingly, not lymph node cells) from LEW recipients showed strong, LEW APC-dependent, CD4⁺ T cell proliferation to peptide 1 (from the α helical region of the α 1 domain). CD8⁺ T cells showed no response to peptide 1. There was no response by the spleen cells to peptide 2 (from the β sheet of the α 2 domain) or peptide 3 (from the α helical region of the α 2 domain). Immunisation of LEW rats with pure RT1-A^{av1} class I heavy chain in Freund's adjuvant gave responses identical to that seen after grafting, i.e. good CD4⁺ T cell proliferation to peptide 1, but none to peptides 2 and 3. However, immunisation of LEW rats with peptides 1, 2 and 3 in Freund's adjuvant resulted in good CD4⁺ T cell proliferative responses to each of the peptides. These data demonstrate that indirect allorecognition can be stimulated by allograft rejection, and emphasise that the physiological processing of donor antigens will influence which peptides will be important in indirect allorecognition in transplantation.

PAPER 17

CYTOKINE EXPRESSION IN ENDOMYOCARDIAL BIOPSIES FOLLOWING CARDIAC TRANSPLANTATION

D. Cunningham, M.J. Dunn, M.H. Yacoub and M.L. Rose

Department of Cardiothoracic Surgery, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK.

Diagnosis of acute rejection in heart transplant recipients is based on histological assessment of endomyocardial biopsies, the presence and extent of a mononuclear infiltrate being diagnostic of rejection. Cytokines play an essential role in the interactions of lymphocytes. However, it is not known which cytokines are important in graft rejection, whether they are produced locally within the graft, and whether their detection in the graft would assist early diagnosis of rejection. Cytokine profiles of sequential endomyocardial biopsies from a series of 20 cardiac transplant recipients are being analysed to address these questions. Using specific primers designed from published cDNA sequences, the RNA extracted from each biopsy was converted to cDNA and analysed for IL1 α , IL2, IL4, TNF α and TNF β by amplification with the polymerase chain reaction (PCR). Amplified product was quantitated using radiolabelled primers as described by Murphy et al (*Biochemistry* 1990, 29: 10351-10356). To date, of 6 patients being monitored, 5 had histological evidence of rejection in 1 or more of their biopsies and IL2 and/or IL4 were amplified from a parallel biopsy collected at the same time. In contrast, TNF α and/or TNF β mRNA was amplified from about 50% of the biopsies tested and was detected in biopsies from patients who had no histological evidence of rejection when the sample was collected.

PAPER 18

POSITIVE CORRELATION OF ANTI-ENDOTHELIAL ANTIBODIES WITH ACCELERATED CORONARY ARTERY DISEASE AFTER CARDIAC TRANSPLANTATION

M.J. Dunn, S.J. Bradd, M.L. Rose and M.H. Yacoub

Department of Cardiothoracic Surgery, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY

Accelerated coronary artery disease (CAD) is the most significant factor in the long-term survival of cardiac transplant recipients. The pathogenetic mechanisms involved in this disease are unknown, but an immune aetiology has been suggested. A technique of SDS-PAGE and Western immunoblotting has been used to detect the occurrence and specificity of anti-endothelial antibodies in serum from cardiac transplant recipients. Proteins from cultured human umbilical vein endothelial cells were separated according to Mr by SDS-PAGE and blotted onto nitrocellulose. Blots were probed with serum from 21 patients with angiographically proven CAD and 20 patients without evidence of CAD one and two years after transplantation. Both pretransplant serum and serum samples collected one and two years post-transplant were tested. Of the CAD group, 20 out of 21 patients were positive for anti-endothelial antibodies compared with 9 out of 20 patients in the non-CAD group. Furthermore, of the 20 positive patients in the CAD group, 15 had antibodies reactive with a doublet of polypeptides at 62 and 60 kDa at the time of diagnosis of CAD. Three of these patients had these antibodies prior to transplantation. In contrast, only 1 of the patients in the non-CAD group had antibodies of this specificity. This doublet was not detected when patients' serum was tested against proteins from cultured A549 epithelial cells. The size of these proteins suggest that they are not products of the major histocompatibility complex. They are currently being further characterised by protein microsequencing.

PAPER 19

RENAL ALLOGRAFT REJECTION: THE INDUCTION AND FUNCTION OF ADHESION MOLECULES ON CULTURED RENAL EPITHELIAL CELLS.

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

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

Activated lymphocytes may release cytokines including IFN- γ and TNF- α within graft tissues during rejection. These cytokines have been used in a study of adhesion molecule induction and function on cultured renal epithelial cells.

Human renal epithelial cells were prepared by mechanical disaggregation and fractionation of cortical tissue and were cultured with recombinant interferon- γ (IFN- γ) and/or tumour necrosis factor- α (TNF- α) at concentrations up to 1000U/ml over a time course from 0 to 120 hours. Expression of the adhesion molecules ICAM-1 and LFA-3 were quantified by flow cytometry. IFN- γ increased expression of ICAM-1 but had no effect on the expression of LFA-3. TNF- α upregulated both ICAM-1 and LFA-3 and synergised with IFN- γ to further enhance expression of both these molecules.

A ^{51}Cr -labelled lymphoid cell binding assay was used to assess the function of these adhesion molecules after stimulation by IFN- γ and TNF- α (see table).

Epithelial Cell Treatment	% Lymphoid Cell Binding (mean \pm s.d.)	n	P
Control	28.9 \pm 2.5	9	
IFN- γ (100U/ml for 48 hours)	37.5 \pm 4.6	7	<0.01
TNF- α (100U/ml for 48 hours)	38.1 \pm 4.5	5	<0.01
IFN- γ and TNF- α	45.8 \pm 4.6	4	<0.01

The efficacy with which monoclonal antibodies to ICAM-1, CD11a, CD18, LFA-3 and CD2 blocked immune cell binding was also tested using this assay system. Combination of antibodies to ICAM-1 and CD18 and to LFA-3 and CD2 were found to be most effective at inhibiting lymphoid cell binding.

A combination of IFN- γ and TNF- α are able to enhance the expression of both ICAM-1 and LFA-3 on the surface of cultured renal epithelial cells. These adhesion molecules are functional and can enhance the binding of potentially graft damaging lymphoid cells.

PAPER 20

A ROLE FOR INTERLEUKIN 4 IN INTESTINAL GRAFT-VERSUS-HOST REACTION.

Allan McI Mowat & Michael B Widmer. (Introduced by Mr J A Bradley)

Department of Immunology, University of Glasgow, Western Infirmary, Glasgow, Scotland & Immunex Corporation, Seattle WA, USA.

Intestinal damage is an important component of the acute graft-versus-host disease which remains a significant complication of allogeneic bone marrow transplantation. We have shown previously that soluble cytokines are involved in experimental models of intestinal graft-versus-host reaction (GvHR) and, if individual cytokines could be identified, they might provide targets for specific immunotherapy. Recently, it has been shown that interleukin 4 (IL4) may be an important mediator of alloreactivity *in vivo* and here we have investigated whether IL4 plays a role in intestinal GvHR. Unirradiated adult F₁ mice given parental lymphocytes develop a mild enteropathy, with increases in crypt cell proliferation, crypt length and in the density of intraepithelial lymphocytes. These alterations were abolished in a dose-dependent manner by treating mice with anti-IL4 monoclonal antibody, or with a soluble form of the murine IL4 receptor (IL4R). However, soluble IL4R had no effects on the more destructive intestinal pathology which occurs in irradiated mice with GvHR, or on systemic aspects of GvHR such as weight loss, splenomegaly and activation of NK cells. Thus, IL4 may have a selective role as a local mediator of the induction phase of immunologically mediated enteropathy and warrants investigation as a potential target for immunotherapy in GvHR and other forms of alloreactivity.

PAPER 21

THE ROLE OF NITRIC OXIDE IN THE IMMUNOPATHOGENESIS OF GRAFT-VERSUS-HOST REACTION.

Paul Garside, A.McI.Mowat, A.Severn & F.Y.Liew. (introduced by Mr J.A.Bradley)

Department of Immunology, University of Glasgow, Western Infirmary, Glasgow.

Nitric oxide (NO) synthesised from L-arginine by endothelial cells mediates a wide range of biological functions, including vascular relaxation and neurotransmission. In addition, NO is important for several effector functions of activated macrophages ($m\phi$) and recently, it has been shown that NO is involved in the immunopathogenesis of experimental diabetes. Here we have examined whether NO also plays a role in immunopathology associated with alloreactivity *in vivo*. Increased levels of both NO and NO synthase (NOS) were detected in the spleen of F_1 mice with an experimental graft-versus-host reaction (GvHR) induced by transfer of parental lymphocytes. Treatment of mice with L-N^G monomethyl-arginine (L-NMMA), a specific inhibitor of NOS, ablated all aspects of the intestinal pathology normally found in GvHR, including crypt hyperplasia, increased epithelial expression of class II MHC antigens and increased lymphocytic infiltration of the epithelium. The effects of L-NMMA were dose-dependent and were not merely due to delayed onset of the disease. L-NMMA had no effect on splenomegaly in GVHR, but reduced the concomitant activation of natural killer (NK) cells. Thus, NO is a critical effector mechanism in the tissue pathology of GvHR and NO inhibitors may have therapeutic potential for modulating a variety of forms of immunopathology.

PAPER 22

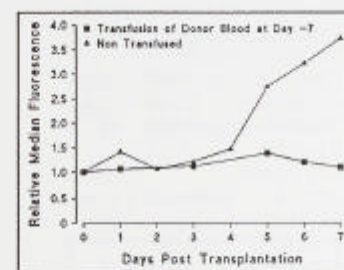
KIDNEY TRANSPLANTATION IN THE RAT: IgG ANTIBODIES SPECIFIC FOR GRAFT TUBULAR EPITHELIAL CELLS FAIL TO DEVELOP AFTER PRE-OPERATIVE DONOR BLOOD TRANSFUSION.

MR Rajasekar, JA Kirby, BK Shenton, G Proud & RMR Taylor

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

In this study the role played by graft-specific humoral immunity in 'enhancement' of rat renal allografts after pre-operative donor blood transfusion was investigated.

PVG rats in one group were transfused with 1ml of DA rat blood 7 days prior to orthotopic unilateral transplantation of DA kidneys. A second group of rats was not transfused prior to transplantation. On each day after transplantation blood was taken from rats in both groups and after clotting the serum was heat-inactivated and stored at -20°C . DA rat kidney tissue was disaggregated by passage through graded stainless steel meshes and the tubular fraction was cultured. Antibodies specific for renal epithelial cells were assayed by mixing cultured cells 1:1



with serum for 30 min and counterstaining with FITC conjugated anti-rat IgG. Non-viable cells were stained by addition of propidium iodide and the cells were analysed by flow cytometry. The median fluorescence of viable cells was normalised by comparison with cells counterstained after incubation with pre-immune serum. Representative results are shown in the figure. Antibody-dependent cell-mediated cytotoxicity (ADCC) of renal cells was

activated by serum taken 7 days after transplantation from non-transfused rats but not by serum taken from transfused animals.

Post transplant serum from non-transfused rats caused a significant increase in median fluorescence indicating development of graft cell specific antibodies. Transfused rats failed to develop IgG specific for either donor MHC or renal cell associated antigens. Graft enhancement may be due to failure of either ADCC or complement mediated cytotoxicity in animals transfused with donor blood before transplantation.

PAPER 23

NIFEDIPINE TREATMENT AND ACUTE RENAL ALLOGRAFT REJECTION

M Dennis, M Nicholson, S Smith,

University and City Hospitals, Nottingham

Since lymphocyte activation is mediated by calcium ion influx, allograft rejection may be influenced by drugs which block cellular calcium channels. This study investigated the influence of nifedipine therapy on acute rejection in 170 cadaveric renal allografts. 77 patients received oral nifedipine (10-40mg bd) during the first 12 weeks post-transplantation. There were 93 controls. Acute allograft rejection occurring in the first 3 months was diagnosed by Tru-cut biopsy and assessed (mild, moderate, severe) histologically and according to the degree of functional impairment. Variables including: warm and cold ischaemic times, HLA matching, cytotoxic antibodies, graft number, immunosuppression and nifedipine therapy were studied by stepwise logistic regression analysis. Significantly fewer and less severe rejection episodes occurred in the nifedipine group which also has significantly lower serum creatinine concentrations at 3 and 6 months than the controls (TABLE).

	Rejection Episodes		Severity of Rejection			Creatinine mean \pm SEM	
	0	≥ 1	Mild	Mod	Sev	3/12	6/12
NIFEDIPINE	49	28	11	13	7	141 ± 5	141 ± 5
CONTROLS	40	53	6	49	19	168 ± 12	161 ± 8

$$X^2=7.18; p<0.01 \quad X^2 = 12.38; p<0.01 \quad p<0.05$$

We conclude that nifedipine treatment modulates the allograft rejection process and may prove to be a useful adjunct in renal transplantation.

PAPER 24

A PLACEBO CONTROLLED STUDY OF THE EFFECT OF NIFEDIPINE ON CHRONIC CYCLOSPORIN NEPHROTOXICITY IN RENAL ALLOGRAFTS.

M Wilkie, J Beer, *A Dawney, MJ Raftery, FP Marsh.

Depts Nephrology, Royal London and *St Bartholemew's Hospitals, London. UK.

Nifedipine 10mg bd for 4 weeks was administered to 22 stable renal allograft recipients in a placebo controlled, double blinded randomized cross-over study. 15 (grp A) 12-39 months post transplant, were immunosuppressed with CyA (mean dose 4.2 mg/kg), prednisolone (pred) and azathioprine (aza). 7 (grp B) 84-216 months post transplant received pred and aza alone.

Measured variables after nifedipine and placebo administration were ^{51}Cr EDTA GFR, ^{131}I iodohippurate RBF (mls/min/1.73m²); renal vascular resistance (RVR 10¹⁰dyne.s.cm⁻⁵/1.73m²); mean arterial pressure (MAP mmHg); whole blood CyA (ng/ml); and urinary proteins - albumin (mg), N-acetyl glucosaminidase (NAG μmol), Tamm Horsfall glycoprotein (THG mg), retinol binding protein (RBP μg).

Results after placebo and nifedipine are shown in the table.

	group A		group B	
	placebo	Nifedipine	placebo	Nifedipine
GFR	65 \pm 21	64 \pm 19	89 \pm 32	85 \pm 27
RBF	425 \pm 142	424 \pm 137	505 \pm 204	501 \pm 143
RVR	0.28 \pm 0.1	0.29 \pm 0.1	0.24 \pm 0.2	0.21 \pm 0.1
MAP	108 \pm 11	109 \pm 11	99 \pm 11	97 \pm 8
CyA	154 \pm 49	150 \pm 38		
	urinary proteins / mg urinary creatinine			
Albumin	4.9 \pm 8	4.5 \pm 9	19.0 \pm 43	29.5 \pm 67
NAG	36.0 \pm 25	35.8 \pm 20.9	32.9 \pm 19	36.1 \pm 24
THG	1.4 \pm 0.8	1.5 \pm 0.7	4.0 \pm 4.1	3.0 \pm 2.5
RBP	180 \pm 495	435 \pm 1021	65 \pm 126	46 \pm 67

Nifedipine at this dose made no difference to GFR, renal blood flow and vascular resistance, or to indicators of tubular dysfunction.

Conclusion.

Contrary to the findings of an open study of nifedipine 20 mg bd¹, in this double-blinded study nifedipine 10 mg bd for 4 weeks did not alter renal allograft function in longterm stable recipients and specifically did not improve function in a cohort of patients who were likely to have renal dysfunction as a consequence of CyA treatment.

1. McNally PG, Walls J, Feehally J. 1990 Nephrol Dial Transplant:5:962-8.

PAPER 25

ACTIVE IMMUNISATION WITH PURE DA CLASS II MHC ALLOANTIGENS SUPPRESSES THE ANTIBODY RESPONSE TO CLASS I MHC ANTIGENS FOLLOWING A DA KIDNEY ALLOGRAFT

C. Kelly, R. Dalchau, G. Sawyer, C. Priestley and J. Fabre

Blonde McIndoe Centre, Queen Victoria Hospital, East Grinstead, West Sussex, RH19 3DZ

Removal of interstitial dendritic cells from grafts, even if it does not result in graft survival, can lead to almost total suppression of the antibody response to the graft. This suggests that such cells might be the major stimulus to primary alloantibody production after organ transplantation. With this in mind, we examined the effect of anti-donor class II immunisation on the fate of a subsequent renal allograft, in the DA-to-LEWIS and DA-to-PVG strain combinations.

The membrane bound form of RT1-B class II MHC antigens of the DA strain was purified from detergent extracts of DA spleens by a combination of MRC-0X6 mAb affinity, lentil lectin affinity and gel filtration chromatography. PVG and LEWIS strain rats were immunised by subcutaneous injections of the pure DA class II MHC antigens in Freund's adjuvant, in doses equivalent to 10^8 nucleated DA spleen cells.

Although such treatment prior to transplantation did not influence kidney graft function or survival, recipients sensitised to the class II antigens had very substantially reduced antibody responses to class I antigens. There was also evidence of mild but definite improvement in graft tissue preservation at the 7 day biopsy, this being most obvious in the PVG strain. We propose that the anti-class II response induced by the pre-immunisation, especially the antibody response, neutralises the donor interstitial dendritic cells and that this is responsible for the diminished antibody response to class I.

These data are consistent with the idea that the dendritic cell is the major stimulus for the anti-class I antibody response. We propose that the stimulation of the recipient T and B cells is dependent on the direct recognition pathway and present a three cell cluster hypothesis of B cell, T cell and interstitial dendritic cell to explain the nature of such a response.

PAPER 26

DR MATCHING, HLA SENSITISATION AND RENAL ALLOGRAFT FUNCTION.

Taylor CJ, Bayne AM, Welsh KI, Morris PJ

Transplant Immunology, Transplant Centre, Churchill Hospital, Oxford

Since the introduction of triple therapy (CyA, Aza and steroid) 454 cadaver renal transplants have been carried out in our centre. We have now re-analysed factors which have been reported by ourselves or others to influence graft function and outcome. DR matching and sensitisation proved to have strong and inter-related effects. Rejection treatments including OKT3 and ATG, grams of MP, creatinine levels from 3 months to 3 years, post transplant dialysis and graft failure were all greater in the mismatched groups. Increased numbers of rejection episodes proved the most powerful indicator of DR mismatching. Sensitisation, as measured by pregnancy, panel reactivity or previous graft failure also increased the average number of rejections seen per patient. For example unsensitised male recipients tended to be rejection free if they received a DR matched graft (Table 1a). In contrast retransplants into sensitised recipients were seldom rejection free (Table 1b) but DR matching still played a significant role in terms of ultimate graft outcome (eg 92% of DR matched compared to 76% of DR mismatched were successful at 3 months).

Table 1. Recipients of cadaver renal allografts on triple therapy

DR mismatch	1a. unsensitised males. 1st cadaver grafts no. of rejections				n	DR mismatch	1b 2nd & subsequent grafts no. of rejections				n
	0	1	>1				0	1	>1		
0	67%	30%	4%	27		0	6%	33%	60%	48	
1	32%	24%	44%	62		1	19%	24%	57%	37	
2	7%	46%	46%	28		2	6%	25%	69%	16	

We conclude that DR matching is cost efficient both in human and financial terms with our current immunosuppressive regime. Although sensitisation *per se* also plays a major role, other parameters such as cold ischaemia time, blood transfusion, HLA A, B, C or DQ mismatch appeared to have no short term effect. A simple hypothesis to explain the data is that DR mismatches and previous MHC sensitisation are additive in increasing the magnitude of the rejection response.

PAPER 27

A PROSPECTIVE RANDOMISED COMPARISON OF UW OR HOC VASCULAR PERFUSION ON HUMAN ISLET ISOLATION.

G Robertson, H Contractor, D Chadwick, R Chamberlain, S Swift, J Davies, R James, N London.

Department of Surgery, Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX.

It is well established that vascular perfusion with University of Wisconsin solution (UW) optimises human islet purification from pancreata with long cold ischaemic times (>6 hours). This study aimed to examine whether it offers any benefits during shorter periods of cold ischaemia.

Seven pancreata were prospectively randomised to perfusion with hyperosmolar citrate (HOC), and 7 with UW. After a cold ischaemic time of <6 hours, an identical technique was used for digestion of each pancreas, although a higher concentration of collagenase (4mg/ml compared with 3mg/ml) was needed for UW perfused pancreata.

The median number of 150µm islet equivalents in the pancreatic digest was 3,909/g (range 2,335-18,653) following UW perfusion, and 5,919/g (605-9,572) for HOC. The percentage of cleaved islets was 87% (33-96) UW, and 84% (53-90) HOC. The percentage of amylase contaminating a 60% islet yield was 22% (1-60) UW, and 25% (5-47) HOC.

No differences were statistically significant. We conclude that there is no benefit from UW perfusion in pancreata with short cold ischaemic times. In view of the relative cost of the UW and the extra collagenase required, pancreata with short cold ischaemic times should be perfused with HOC.

PAPER 28

EXPRESSION OF ADHESION MOLECULES IN CADAVERIC KIDNEY ALLOGRAFTS.

P Gibbs, LM Berkley, EM Bolton, JA Bradley.

University Department of Surgery, Western Infirmary, Glasgow G11 6NT.

The extent to which leukocytes are recruited from the recipient circulation into an organ allograft may depend upon the expression, by vascular endothelium and other cell types, of a range of adhesion molecules. Expression of ICAM-1, VCAM-1, PECAM and ELAM-1 in biopsies of cadaveric donor kidneys prior to and following transplantation was assessed by immunocytochemical staining of cryostat tissue sections.

45 biopsies from 42 patients with renal allograft dysfunction were studied. Clinical diagnoses included acute rejection (n=19), chronic rejection (n=6), ATN (n=3), CyA toxicity (n=5) and non-immunological (n=13).

Expression of ELAM was minimal, confined to a few intertubular capillaries in pre-transplant kidneys and was only occasionally induced following transplantation. In contrast, PECAM was uniformly expressed on capillary endothelium before and after transplantation and was not induced during acute rejection. In pre-transplant kidneys ICAM was widely present on capillary endothelium and variably expressed on proximal tubules and glomerular epithelium, while VCAM was consistently detected on Bowman's capsule and occasionally capillary endothelium. There was a striking association between heavy leukocyte infiltration with induced MHC class II and induction of both VCAM and ICAM in 16/19 patients with acute rejection and 3/6 patients with chronic rejection. ICAM was induced on proximal tubules and infiltrating cells, while VCAM was increased on proximal tubules and glomerular and capillary endothelium, but little induction was seen in patients with non-immunological causes of dysfunction.

We have observed marked variability in the expression of adhesion molecules in cadaveric kidney allografts. In particular, ICAM and VCAM are strongly induced in association with cellular infiltration suggesting that their expression may be an important factor in the pathogenesis of organ allograft rejection.

PAPER 29

PAEDIATRIC RENAL TRANSPLANTATION: UPDATE ON RESULTS

J. Thorogood, G.G. Persijn, P. de Lange and J.J. van Rood

Eurotransplant Foundation and University Department of Immunohaematology and Blood Bank, Leiden, The Netherlands

We had previously observed an increased risk of graft failure for paediatric recipients following first renal transplantation between 1984 and 1987. We wished to investigate paediatric allograft survival for 1988-1990 and relate this to results for adults within both time periods.

The data comprised 12822 first transplants from unrelated, non-living donors, transplanted between 1 January 1984 and 31 December 1990, within 52 European transplant centres. All patients received cyclosporine, either alone or in combination with other immunosuppressive therapy. The age range was 0-76 years. For comparability of follow-up time, we limited our analysis to 3 years posttransplant. 459 recipients were transplanted in the age range 0-15 compared with 12363 in the age range 16-76. For 1984-1987, 1-, 3-year graft survival was 77%, 65% for children and 83%, 72% for adults, respectively ($p=0.005$, logrank test). For 1988-1990, these figures increased to 80%, 74% for children and 86%, 79% for adults ($p=0.062$). A Cox proportional hazards analysis revealed a significant improvement in graft survival over the years ($p=0.0001$) but also a significant difference between children and adults, not accounted for by the improvement over the years. The relative risk of graft failure for children was 1.35 (95% confidence interval 1.14 to 1.61, $p=0.001$) compared with adults. Results by donor age groups are shown in Table 1 for all children, revealing better prognosis with donors over 10 years.

We conclude that first renal allograft survival has improved since 1984-1987, but for children transplanted 1988-1990 remains significantly lower than for adults.

TABLE 1: 1-year graft survival and number of patients, for children aged 0-15, by donor age and period

Donor age	0-5 (N)	6-10 (N)	11-15 (N)	16-58 (N)
1984-1987	62% (93)	76% (59)	87% (30)	92% (77)
1988-1990	69% (70)	81% (63)	91% (22)	91% (45)

PAPER 30

THE USE OF ENALAPRIL IN POST RENAL TRANSPLANT ERYTHROCYTOSIS

P. CONLON, J. FARRELL, M. CARHODY, J. DONOHUE, JJ. WALSH.

DEPT. OF NEPHROLOGY, BEAUMONT HOSPITAL, DUBLIN, IRELAND.

Following successful renal transplantation between 6 and 15% of recipients will develop erythrocytosis. The importance of this as a clinical problem is that these patients are at increased risk of thromboembolic events. Traditional therapies for this condition has involved repeated phlebotomy or bilateral native nephrectomy. A number of mechanisms have been proposed for this phenomena, most of which have involved abnormally high production of erythropoietin by the native kidneys. Enalapril has been shown in a number of settings to reduce circulating EPO levels and to exacerbate anaemia in dialysis patients.

Nine renal transplants with a haematocrit of 0.51 or greater on two consecutive occasions were enrolled in a study in which existing anti-hypertensive medication was substituted for Enalapril 2.5mg. Baseline measurements of haematocrit and serum EPO were made. Patients were followed at two weekly intervals for 6 months.

Mean haematocrit fell from 0.52 to 0.45 after 8 weeks and remained at that level for the subsequent 12 weeks ($P<0.05$), serum EPO also fell from 61.12u/ml to 18.25u/ml ($P<0.05$) after 4 weeks. The mean blood pressure, serum K or serum creatinine did not change during the 6 month period of observation.

It would thus appear that Enalapril is a safe and effective treatment for post renal transplant erythrocytosis.

PAPER 31

THE VALUE OF FLOW CYTOMETRIC MONITORING OF POST-TRANSPLANT ANTIBODY STATUS IN RENAL TRANSPLANTATION.

B.K.Shenton, K. Al-Husseini, Alison Bell, D.Talbot, K.Clark, K.M.Rigg, J.R.Forsythe, G.Proud, R.M.R.Taylor.

Department of Surgery, The University, Newcastle Upon Tyne. NE2 4HH

Over the last few years many studies have shown the prognostic value of pretransplant flow cytometric crossmatching in renal transplantation. However the possible importance of antibodies in the immediate post transplant period has received little attention and the aim of the present study was to examine the relationship between the presence of IgG antibodies binding to donor lymphocytes and the rejection course of the graft. The patient group comprised 24 renal transplants and sera were collected both before and for the first 14 days after transplantation. All sera were stored at -20°C. until tested. Donor cells were separated at the time of transplant and stored in liquid nitrogen until required. Samples were tested with the normal FACS crossmatch method. To quantify results the median fluorescence intensity of all tests were compared to that of control AB sera. Results are shown in the table below.

STUDY GROUP	T or B cell directed T IgG	T Cell Mean % Rise	B Cell Mean % Rise
No Rejection (2)	nil	1%	5%
Mild rejection (10)	100%	25±13.0%	34±32.7%
Severe Rejection (7)	100%	220±89%	269±45.0%
Primary Non Function (5)	80%	39±19%	167±88.0%

As can be seen all patients showing either mild or severe rejection exhibited antibodies directed against donor cells after transplantation. Patients with no rejection showed T and B cell rises of only 1% and 5% respectively. Mild rejection was associated with higher rises than in the no rejection group but these frequently occurred after the diagnosis of clinical rejection had been made. (Mean 3 days (T cell) or 2 days (B cell) post). Patients with severe (steroid resistant) rejection exhibited much higher levels of donor directed IgG and its presence was always before the onset of clinical rejection (Mean 6days (T cell) or 3.25 days (B cell) pre). Of particular interest are the group of primary non-function grafts with 80% of patients showing the presence of donor directed antibodies and examples of both the mild and severe antibody patterns being observed. This study shows that the highly sensitive FACS crossmatch technique can detect the presence of antibodies in renal patients following transplantation. The correlation of the presence of antibodies with the rejection profile of patients suggest they may have a more important role in the rejection process than is thought at the present time. Such findings have important consequences for therapeutic regimes used in the treatment of clinical rejection.

PAPER 32

DONOR RELATED BACTERIAL INFECTION AFTER HEART-LUNG TRANSPLANTATION.

F.Ciulli, M.Tamm, C.Dennis, P.Mullins, S.Kendall, B.Biocina, S.R.Large, F.C.Wells, J.Wallwork

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

Transmission of microorganisms by donor lungs is a potentially important source of infection. We investigated our experience with donor related infections in 121 heart-lung transplant (HLTx) patients operated on between April 1984 and December 1991. Routine perioperative antibiotic prophylaxis consisted of flucloxacillin and a third generation cephalosporin, administered for 48 hours. In patients with cystic fibrosis, nebulized aminoglycoside and colistin were administered in the post-operative period and continued until the time of intermediate discharge. In the case of positive donor tracheal cultures, appropriate antibiotics were administered for 10 days. Hospital mortality (≤ 30 days) was 8.3% (10/121). The actuarial survival at 1, 3 and 5 years was respectively 79% (± 4), 64% (± 5.04) and 54% (± 7). 53 (44%) of donor tracheas produced positive cultures. The organisms frequently isolated from donor tracheas in this series were: S.aureus (47%), H.influenzae (25%), Pseudomonas species (17%) and Candida albicans (11%). 3 of the 72 patients (4%) receiving organs from donors who were mechanically ventilated for less than 48 hours died within 30 days of transplantation compared to 7 of the 47 patients (15%) who received organs from donors ventilated for more than 48 hours ($p=0.05$, Fisher's Exact Test). The prevalence of positive donor tracheal cultures in the patients receiving lungs ventilated for more than 48 hours (26/47 pts) was not significantly higher than the prevalence for those ventilated for less than 48 hours (25/72 pts, $p > 0.05$ Fisher's Exact Test). The prevalence of early infection (≤ 30 days) was 12% (14/121) and the linear infection rate was 0.44/100 patient days. 7 of the 14 cases developing infection had positive donor tracheal cultures. Only 1 patient (2%) with positive donor tracheal cultures developed a respiratory infection caused by the same organism. The mortality associated with early respiratory infection was 3% (4/121). Donor transmitted respiratory tract infection has not been a major clinical problem and ventilator times above 48 hours have not significantly increased infection rates although early survival may be affected. We consider appropriate donor management and chemoprophylaxis in the perioperative period to be the key to low early infection rate in our patients.

PAPER 33

PAEDIATRIC DONORS, PAEDIATRIC RECIPIENTS. A POLICY REEVALUATION IN RENAL TRANSPLANTATION.

KS Wynne, MW Brown, SH Kashi, SA Sadek, GR Giles.

Academic Unit of Surgery, Department of Clinical Medicine,
St James University Hospital, Leeds, UK.

Current policy under UKTS guide-lines, is to preferentially transplant kidneys from paediatric donors (<16 years) to similarly aged recipients. Analysis of results of 81 transplants where the age of the donor or recipient was less than 16 years, suggests that this policy may require reexamination.

	1 year graft survival	% Technical Complications	% Graft Loss (Rejection)	Mismatches Mean(SD)*
Group 1 (n=26) Paed donor, Paed recip.	73%	11.5%	19.2%	3.04(0.92)
Group 2 (n=13) Adult donor, Paed recip.	85%	0%	15.3%	2.23(0.73)
Group 3 (n=42) Paed donor, Adult recip.	79%	9.5%	14.2%	2.31(1.24)

* Group 1 vs Group 2, $p=0.007$ Group 1 vs Group 3, $p=0.015$

No significant difference in graft survival was demonstrated between any of the groups. Recipients of grafts from paediatric donors (group 1 and 3) suffered a higher rate of technical complication. In one sub-group (donor < 5 years, recipients < 20kg) two out of four patients lost grafts due to early thrombosis.

The incidence of rejection episodes was similar in all three groups. Interestingly, paediatric recipients of adult kidneys who had a rejection episode were less likely to lose their graft (as a consequence of rejection) as compared to paediatric recipients of paediatric kidneys.

Presumably due to a smaller donor and recipient pool, the extent of mismatches was significantly higher in paediatric recipients of paediatric kidneys.

The present results suggest that paediatric recipients should be considered in the same pool as adults, and that waiting for a paediatric donor will not improve results. Equally paediatric kidneys could be offered to paediatric or adult donors depending upon HLA compatibility.

PAPER 34

1160 LIVING RELATED DONOR TRANSPLANTS (LRT'S) - PERSONAL EXPERIENCE

Drs A Pandey, G Gopalkrishnan, C K Jacob, J C M Shastri
(Mr Martin Wise MD FRCS Consultant Surgeon, Wessex Regional
Transplant Unit, St Mary's Hospital, Portsmouth, Hants PO3 6AD)

Christian Medical College & Hospital
Vellore, India

A review of 1160 LRT's performed at the Christian Medical College Hospital, Vellore, India between 1971-1991 shows the main causes of ESRF in Recipients to be Chronic Glomerulonephritis (44%) and Chronic Pyelonephritis (38%).

Transplants were done mainly based on ABO compatibility, as HLA, A and B typing not being available until the mid 80's. Results show 1 year graft survival of 86% on conventional immunosuppression and 94% when Cyclosporin added. Five year graft survival is 58%. All rejection episodes were treated with steroids and Chronic rejection was the main cause of graft loss.

Late complications in Recipients included renal artery stenosis (3.6%), Tuberculosis (3%), Avascular necrosis of Femoral Head (1.8%) and ureteric obstruction (0.8%).

Complications in donors included Hypertension (0.5%) and Proteinuria (0.3%). Three donors died in their 70's (10-12 years after donation) due to unrelated causes.

This experience emphasises that to treat more patients in future, pre-emptive LRT's is the ideal solution in a country where under joint family system, kidney donation is acceptable, thus reducing the cost of the dialysis and transplantation programme.

Could LRT compensate for the shortfall in cadaver kidneys in the UK if more efforts were directed to procure living donors.

PAPER 35

CONVERSION FROM CYCLOSPORIN TO AZATHIOPRINE: SIX YEARS EXPERIENCE OF A PROSPECTIVE RANDOMISED TRIAL

Mary A Watson on behalf of the Glasgow Transplant Group

Renal Unit and University Department of Surgery, Western Infirmary, Glasgow.

Renal allograft recipients with stable renal function were asked to participate in a conversion study one year after transplantation. At trial entry, immunosuppression consisted of cyclosporin in a mean daily dose of 3 mg/kg and prednisolone 10 mg/day. The patients were randomised either to continue cyclosporin (104 patients) or convert to azathioprine, initially 3 mg/kg (96 patients). Any significant renal dysfunction occurring subsequently was investigated by biopsy and the patient was withdrawn from the trial.

In the cyclosporin group, 23 patients have been withdrawn. The biopsy showed acute rejection in 9 and changes compatible with chronic rejection and/or cyclosporin nephrotoxicity in 11. In the azathioprine group, 22 patients have been withdrawn, 16 with acute rejection and 6 with intolerance to the drug. Nine grafts have failed in each group with 5 deaths in those taking cyclosporin and 4 in those on azathioprine.

No significant change in the level of renal function has so far occurred in either group but antihypertensive therapy has decreased in the azathioprine group.

The results of this study at present suggest no overall detriment in changing from cyclosporin to azathioprine at one year while there is the benefit of reduced cost and the possible advantage of less immunosuppressive drug toxicity.

PAPER 36

IMPROVED LONG TERM RENAL ALLOGRAFT FUNCTION WHEN KIDNEYS ARE PERFUSED WITH UW

Porteous C, Stewart R, Findlay J, Bradley JA, Briggs JD.

University Department of Surgery, Western Infirmary, Glasgow G11 6NT, Scotland, UK

Better immediate graft function (IGF) for kidneys preserved with UW solution has been reported but as yet the long term effects are unknown. To address this, we analysed the graft survival (at 18 months) and the serum creatinine (at 1,3,12 and 18 months) for two non randomised groups of recipients. The first group - the citrate group - received hypertonic citrate perfused kidneys from local donors between 1/7/87 and 30/6/89 and the second - the UW group - received UW perfused kidneys from local donors between 1/7/89 and 30/6/91. The minimum follow up period was 30 months for the citrate group and 6 months for the UW group. Patients who died with a functioning kidney (5 citrate group and 4 UW group) were treated as a graft failure.

	No. of transplants	Median serum creatinine ($\mu\text{mol/L}$) at months post-transplant				Graft survival 18 months
		1	3	12	18	
Citrate	78	183	152	140	142	65%
UW group	104	144	124	118	119	85%
p=		0.002	0.005	0.02	0.03	0.01
(Bonferroni corrected)		(0.01)	(0.02)	(0.02)	(0.08)	(0.13)

Perfusion with UW in this non randomised trial led to significantly improved in IGF (71% IGF UW group vs. 56% IGF citrate group) ($p = 0.03$) improvement in the proportion of kidneys which never functioned (5% UW Group vs. 12% citrate group) ($p = 0.09$) and significantly better graft survival at 18 months and graft function in the first few months. Use of a Cox proportional hazard model also suggested benefit for graft survival with a point estimate of 2.4 for the relative hazard with a 95% confidence interval of 1.27 - 4.54. In conclusion preservation with UW solution improved immediate and long term renal allograft function.

PAPER 37

IS RENAL TRANSPLANTATION FOR DIABETIC NEPHROPATHY THE BEST OPTION?

MZ Chawdhery, KR Harris, M Wise, M Slapak.

Wessex Regional Transplant Unit, St Mary's Hospital, Portsmouth, PO3 6AD.

108 diabetic patients in end stage renal failure were taken onto the renal replacement programme between 1982 and 1991 for treatment. 50 patients were supported on haemodialysis (HD) or Continuous Ambulatory Peritoneal Dialysis (CAPD) prior to receiving 56 kidney grafts (Group A), whilst 58 patients received HD or CAPD only (Group B). Actual patient survival at one, two, three, four and five years was 88%, 78%, 68% and 49% for Group A, and 59%, 24%, 24%, 8% and 8% for Group B. The difference between the two groups is significant and remains so even when patients over the age of 60 years at the start of treatment are excluded from the analysis. Actual one year graft survival for Group A was 68% compared with 65% in a cohort of 455 non-diabetic patients (Group C) receiving a renal transplant during the same period, (1982-1991), whilst actual one year patient survival was 92% for Group A and 91% for Group C respectively.

We conclude that early renal transplantation is the best treatment for diabetic patients in end stage renal failure.

PAPER 38

SEQUENTIAL THERAPY IN HIGH RISK PATIENTS

K R Clark, J L R Forsythe, B K Shenton, G Proud and R M R Taylor

Transplant Unit, Royal Victoria Infirmary,
Queen Victoria Road, Newcastle Upon Tyne. NE1 4LP

The use of antithymocyte globulin (ATG) as part of sequential therapy following renal transplantation is becoming increasingly popular. However, the increased risk of infection eg with cytomegalovirus (CMV) and the increased cost has prevented its widespread use. We compared a group of patients treated with cyclosporin and prednisolone (group 1, n=25) with a group of patients treated with sequential therapy using Merieux rabbit ATG (group 2, n=14). Both groups contained patients who were either a poor HLA match (group 1 n=14; group 2 n=6) or patients who had a historical positive/current negative T cell cross match prior to transplantation (group 1 n=11; group 2 n=8). Cyclosporin was commenced at 10 mg/kg per day in group 1 patients. ATG was given over 10 days with commencement of cyclosporin on day 7. Doses of ATG were adjusted on a daily basis to keep the absolute T cell count < 50 cells/ul. The absolute T cell count was calculated from flow cytometric analysis of CD3⁺ cells in the peripheral blood and the absolute lymphocyte count. There was no difference between group 1 and group 2 for the major variables that affect graft outcome.

	<u>Group 1</u> (n=25)	<u>Group 2</u> (n=14)	
1 year graft survival	76%	93%	p=0.16 (Fisher's)
No. of patients experiencing rejection	21	4	p<0.002 (Fisher's)
Serious viral infection	2(CMV)	0	p=0.40 (Fisher's)

For a 70 kg man the cost of a 10 day course of cyclosporin is £176 compared with £880 for a 10 day monitored course of ATG.

Sequential therapy using ATG and sensitive daily monitoring reduces the number of rejection episodes without increasing the risk of serious viral infection. Overall it provides an excellent 1 year graft survival at a reasonable cost even in high risk patients.