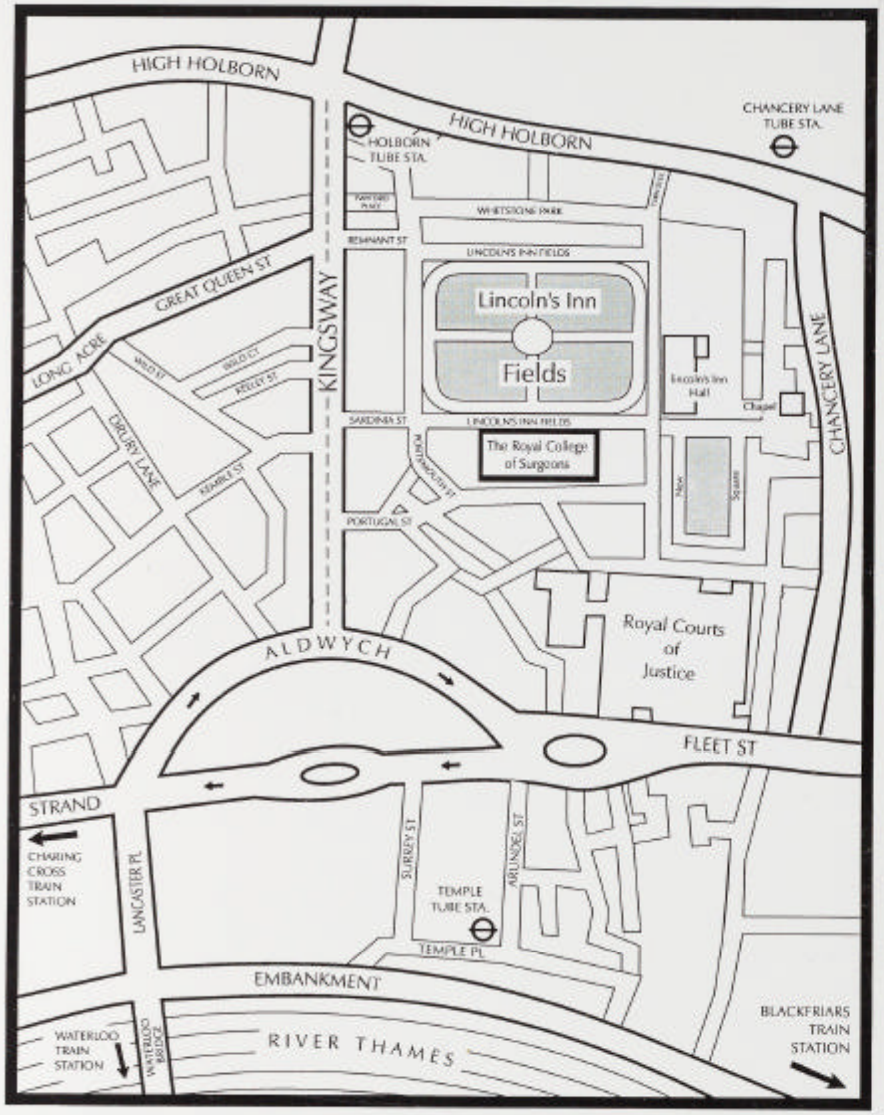

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PAPER 1

SERUM C-REACTIVE PROTEIN (CRP): A USEFUL, ECONOMICAL MARKER OF IMMUNE ACTIVATION IN CADAVERIC RENAL TRANSPLANTATION

KR Harris, NJ Digard, M Slapak and HA Lee

Wessex Regional Renal and Transplant Unit, St Mary's Hospital, Milton Road, Portsmouth, Hants PO3 6AD

The aim of this study was to investigate whether serial daily measurements of serum CRP (sCRP) in renal allograft recipients could improve the differentiation of episodes of renal dysfunction due to rejection or cyclosporin A (CYA) nephrotoxicity and could beneficially modify immunosuppression in the early post transplant period.

Methods Normal ranges for sCRP were established in 104 normal individuals, 55 patients on CAPD and 95 patients on HD. Serial daily measurements of sCRP were done on 187 renal allograft recipients during the first 90 days post transplant using an ELISA assay.

Results Acceptable graft function (mean creatinine 155 μmol/L) with no rejection or infection occurred with mean sCRP of 6 μg/ml (range 0.5-19.2 μg/ml). In 30 episodes of rejection responsive to methyl prednisolone (MP), sCRP was initially significantly raised to a mean of 55.4 μg/ml (p 0.001) but fell rapidly in response to treatment to 17.6 μg/ml. In contrast, in 19 episodes of rejection unresponsive to MP, mean initial sCRP were significantly (p 0.001) higher at 114.3 μg/ml and were still at a mean of 86.1 μg/ml at the end of the treatment. 24 patients in whom renal dysfunction was associated with CYA nephrotoxicity showed no increase in CRP concentrations, mean CRP values being 4.5 μg/ml throughout the episodes. A similar pattern was seen in patients with ATN. For episodes in which no biopsy information was available the CRP concentrations were 80, 96.3 and 46.3 μg/ml, compared with CRP concentrations of 76.2, 62.9 and 36.0 μg/ml in the biopsy group (no significant difference). Clinically relevant infection was also accompanied by a rise in serum CRP. In conclusion, measurement of serial sCRP can provide economical, reproducible and acceptable evidence of immune activation to allow differentiation between renal dysfunction due to CYA nephrotoxicity or rejection and to permit appropriate modification of immunosuppressive therapy during the early post transplant period. If sCRP remains high at the end of methyl prednisolone pulse therapy and there is no evidence of infection, irrespective of renal biopsy, we suggest proceeding to ATG.

PAPER 2

HUMAN RENAL ALLOGRAFTS ARE INFILTRATED WITH MONOCYTES WITHIN 48 HOURS.

D Hughes, A McLean, M Nicholson, D Gray, PJ Morris & K I Welsh.

Nuffield Dept of Surgery, Oxford Transplant Centre, Churchil Hospital, Oxford OX3 7LJ. FAX 0865 226162,

In order to investigate the early events which occur post renal transplant we have studied, using final needle aspiration biopsy, sequential daily samples from 21 cadaver allograft recipients. A major and unexpected finding was that all grafts had discernible monocyte infiltrate at 48 hours. This infiltrate peaked at 72 - 94 hours but was absent from non-rejecting grafts by 6 days. Monocytes remain in grafts which reject, confirming previous work that has described macrophages during early active rejections. Daily monitoring for glutathione peroxidase and superoxide dismutase, supported by staining with WT14 and BerMAC3 suggest that the 48 hr monocytes have a partially active form on infiltration but that this is swiftly suppressed, possibly by SOD which peaks 24-48 hrs later than the glutathione peroxidase which mirrors the infiltrate. The pattern of infiltrate, its staining characteristics and the enzyme data were similar in all grafts to day 5. Lymphocyte infiltrate is a later and less certain event being present in all rejecting and some non-rejecting grafts. We hypothesise that it is ischaemic damage which recruits the early monocytes but that they only become irreversibly activated by, and hence additive, to damage induced by any early T cell rejection response. The early monocyte infiltrate described here is absent from kidney and heart allografts in animal models, where cold ischaemia is minimal. The infiltrate could therefore explain some of the differences observed between rodent and human allografts in terms of immunosuppressive efficacy. Staining with markers for human lymphocyte activation and IL-2 message determinations on the same daily aspirates by ourselves and others support the hypothesis. Further support stems from the observations that both haploidentical live related donor transplants and live unrelated donor transplants do better than their matching grade would suggest. In these groups cold ischaemia is minimal. We conclude that prevention of monocyte infiltrate would be beneficial for human solid organ allografts.

PAPER 3

EARLY DETECTION OF CMV INFECTION AFTER RENAL TRANSPLANTATION BY FLOW CYTOMETRY MEASUREMENT OF CD8bright / CD57bright LYMPHOCYTES IN THE BLOOD.

B.M.Shrestha, S.Couzens, S. John, C.Poynton, D.Westmoreland, R.Lord, R.H.Moore.

Renal Transplant Unit, Cardiff Royal Infirmary, Cardiff CF2 1SZ.

CMV remains a major cause of morbidity after renal transplantation. The emergence of newer treatments when given early in the course of disease makes it important to detect CMV infection as soon as possible.CD57 is an activation antigen on the surface of cytotoxic T-lymphocytes that, in conjunction with CD8 appears upregulated in response to CMV infection.
85 blood samples on 31 patients were analysed using a Becton Dickinson FACScan which could detect patterns of bright dual fluorescence when stained by directly conjugated antibodies. 19 patients were CMV-seronegative and 12 patients were CMV-seropositive. 9 of the seronegative patients were at high risk of developing CMV infection as they received CMV-positive kidneys.
The number of CD8 bright/ CD57 bright cells as a proportion of the total CD8 bright cells was expressed as a percentage "score". 5 patients had too low CD8 numbers (less than 200 / microlitre) to give meaningful percentages. Of the 26 evaluable patients 12 (46%)developed CMV infection as evidenced by positive immediate early antigenaemia and serological tests, and of these, 9 (35%) patients developed symptomatic CMV disease. In 2 patients this was associated with a rejection episode. 10 /12 (83%) of these patients with CMV infection had a CD8bright/CD57 bright score of >15%. In the other 14 patients who did not develop CMV infection, the score was < 15% in 10/14 (71%) patients ($X_2 = 7.79$, df =1, p=<0.01). Regardless of CMV disease the score during the first 6 months after transplantation for CMV- negative patients receiving CMV- negative kidney was <15% in 6/7 (86%) and in CMV -negative patients receiving CMV-positive kidney was > 15% in 6 / 8 (75 %) patients (X2 = 5.52, df = 1, p =< 0.02).

We conclude that the CD8 bright/CD57 bright score is of value in identifying early on a subgroup of patients who have at least a 10/14 (70%) chance of developing clinical CMV disease.These high risk patients could then reasonably be selected for CMV prophylaxis with ganciclovir. Furthur prospective studies are underway to test this hypothesis.

PAPER 4

REDUCED SURVIVAL AFTER CARDIAC TRANSPLANTATION IN PATIENTS WITH PRESUMED HAPLOTYPE A1 B8 DR3

Richens D, Farrell A#, Galbraith I#, Gallagher G*, Henderson N#, Homero F, Naik SK, Wheatley DJ

Departments of Cardiac Surgery, *Surgery and #Tissue Typing, Royal Infirmary, 10 Alexandra Parade, Glasgow G31 2ER

The extended haplotype A1 B8 DR3 has been shown to identify with a high risk of rejection after solid organ transplantation. To confirm this and to determine whether the incidence of infection was higher and actuarial survival lower the results were reviewed in a series of 46 consecutive patients who underwent cardiac transplantation at our institution over a two year period.

Results: Of the 46 patients, 11 recipients were HLA haplotype (presumed) A1 B8 DR3 and 35 had other haplotypes (control group). The 2 groups were well matched for age, diagnosis, degree of HLA mismatching and gender mismatching.

	A1 B8 DR3 (n=11)	Control (n=35)	p value
Linearized rejection rate (epis/100 pt days) 15-18 months	0.4 $\pm$ 0.54	0	0.003
Linearized infection rate (epis/100 pt days) 21-24 months	0.5 $\pm$ 0.7	0	0.005
Patients with OKT3 refractory rejection	2 (18%)	0	0.01
Actuarial survival			
1 year	81% $\pm$ 0.11	97% $\pm$ 0.02	0.075
2 year	69% $\pm$ 0.14	97% $\pm$ 0.045	0.012

Conclusions: This study demonstrates a decrease in survival following heart transplantation in patients bearing the haplotype A1 B8 DR3 associated with increased rates of late rejection, OKT3 refractory rejection and late infection.

PAPER 5

IMPORTANCE OF MINIMISING BOTH HLA-DR MISMATCH AND COLD PRESERVATION TIME IN LONG TERM CADAVERIC RENAL GRAFT SURVIVAL

CONNOLLY JK,[1] DYER PA[2], MARTIN S[2], PARROTT NR[1], JOHNSON RWG[1].

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

The introduction of cyclosporin in the 1980s and the UK "Beneficial Matching" scheme in 1989 has contributed to significant improvements in 1 yr renal graft survival. However long term loss, particularly due to chronic rejection, remains a major problem.

We have analysed the outcome of 516 primary cadaveric renal transplants performed in our single centre from 1989-1993 to determine possible risk factors for long term graft loss. Variables subjected to univariate and multivariate analysis were HLA-A, -B, -DR mismatch (mm), warm and cold ischaemic times (CIT), incidence of delayed graft function (DGF), acute rejection, local retrieval or import and donor age.

At 1 & 5 yr the overall actuarial patient and graft survival was 94.4%, 84.4% & 88.3%, 77.8% respectively. Occurrence of DGF significantly lowered graft survival at 1 & 5 yr (89.9% & 79.9% v 77.8% & 69.5%, p=0.002). However losses from 1 to 5 yr were not significantly different.

One and 5 yr actuarial survivals for grafts with CIT < 26 h and > 25 h were 88.9% & 82.0% v 85.0% & 71.4% respectively (p=0.028).

For CIT < 26 h there was a significant additional benefit in graft survival beyond 1 yr if the donor was aged less than 50 yr (p = 0.024). For CIT > 25 h the benefits of a young donor were lost.

HLA-A or -B matching showed no independent long term survival benefit. However, 0 DR mm significantly enhanced survival at 1 & 5 yr when CIT was < 26 h (92.8% & 88.3% v 84.5% & 73.9%; p=0.0009). This effect was not seen if CIT exceeded 25 h. No other variable examined had a measurable effect on outcome.

In this large single centre series we have demonstrated that a combination of prolonged CIT, rejection, DGF or donor age results in a significantly lower 5 yr graft survival. The highest survival was seen in 0 DR mm with < 26 h CIT. The benefits of DR matching were lost with increased CIT.

We suggest that to achieve full benefits from an organ sharing programme attempts must be made to reduce CIT as well as to avoid DR mm. The existing UK kidney donor exchange criteria may need revision in the light of our analyses.

PAPER 6

MIZORIBINE AS AN ALTERNATIVE TO AZATHIOPRINE IN TRIPLE THERAPY IN IMMUNOSUPPRESSANT REGIMENS IN CADAVERIC RENAL TRANSPLANTATION : TWO SUCCESSIVE STUDIES

H A Lee,, M Slapak, G Venkatraman, J Mason, N Digard and M Wise

University Department of Renal Medicine, University of Southampton
St Mary's Hospital, Portsmouth, United Kingdom. PO6 3AD

Most of the evidence for clinical efficacy comes from Japanese studies and one United Kingdom study (reference). We have already reported our preliminary results of an open, randomised Phase II study to study the tolerance and efficacy of Mizoribine versus Azathioprine in triple therapy in the first or second transplant. Initially all patients received either Azathioprine 2 mg/kg or Mizoribine 3 mg/kg with Cyclosporin A 8 mg/kg and Prednisolone 0.3 mg/kg body weight. Forty patients entered the first study (twenty into each arm) and thirty six patients into the second study. Both were conducted on exactly the same grounds.

We now present our three year follow up data. Further evidence from a further similar study of thirty six patients started in June 1993 where follow up data is available on all patients at six months. The ages, sex, tissue typing criteria and original disease distribution is the same for all patients studied with Mizoribine or Azathioprine in both studies.

The second study confirms the results obtained from the first study, showing less rejection and leucopaenic episodes with Mizoribine. In the second study there was a rejection rate of one per 5.5 patient months (765%) with Azathioprine compared to one per 11.3 patient months with Mizoribine (35% incidence). There were no leucopaenic episodes with Mizoribine (102 patient months) compared to one per 24 patient months (16.6% incidence) with Azathioprine.

Three year follow up data shows no differences in serum creatinine 163.2 umol/L (Mizoribine) versus 166.7 (Azathioprine). Four further leucopaenic episodes occurred during the second year of follow up with Azathioprine, none with Mizoribine. In the second study serum creatinine at six months was 158 +/- 92.8 with Azathioprine.

In conclusion Mizoribine is a well tolerated effective alternative to Azathioprine with less myelosuppresive effects associated with fewer rejection episodes, virtually no side effects and can be given with Allopurinol where hyperuracaemia needs to be treated.

PAPER 7

CYTOMEGALOVIRUS DISEASE INVOLVING GASTROINTESTINAL TRACT IN RENAL TRANSPLANT RECIPIENTS

B.M.Shrestha, D.Parton, A.Gray,C. Darby,C.Fergusson, D.Griffiths, D.Westmoreland, R.Lord, R.H.Moore.

Renal Transplant Unit, Cardiff Royal Infirmary, Cardiff CF2 1SZ.

Cytomegalovirus (CMV) infection of the gastrointestinal tract is a major cause of morbidity and mortality in immunocompromised patients. Infection with CMV can involve the upper or lower gastrointestinal tract (GIT) producing ulceration with massive bleeding, perforation, or both.
During the period of 5 years, between June 1,1989 and May 30, 1994, 340 kidney and 13 combined kidney and pancreas transplants were performed. 117 (33%) patients had CMV infection, as evidenced by seroconversion or four-fold increase in pretransplant IgG level , and 54 (15.2%) patients developed symptomatic CMV disease. 7 of these 54 (13%) patients were found to have tissue invasive GI -CMV disease involving oesophagus,stomach, duodenum, and colon which were proved by endoscopy and biopsy.The presenting features were pyrexia,abdominal pain, diarrhoea and weight loss.1 patient presented with pseudo-intestinal obstruction which was managed conservatively. Acute pancreatitis was associated with CMV duodenitis in one patient which culminated in graft loss. Immediate early antigenaemia test was positive in 3 patients and IgM was detected in all 7 patients. 6 patients were treated with ganciclovir for a mean period of 12 days. 3 (3/ 7=43%) of these patients required retreatment for relapses with ganciclovir for 10 - 14 days (mean = 11.3 days) , whereas relapse was seen in 1 patient (1/47= 2.1%) without GIT involvement ($X2 = 14.73$, $df = 1$, $p = < 0.001$) . The recurrent CMV disease was more severe (mean score: 15 in relapses vs. 7 in the first episode) and was associated with graft loss in 1 patient.

In conclusion, patients presenting with GI symptoms should undergo endoscopic examination and biopsy as a routine to diagnose tissue- invasive CMV disease. Since relapse is common (43%) and the recurrent disease is more severe in the presence of GI tract involvement, a longer duration of treatment with ganciclovir and follow-up is indicated.

PAPER 8

FACTORS INFLUENCING OUTCOME OF UNRELATED DONOR MARROW TRANSPLANTS

J M Hows[1], T R Downie[1], B A Bradley[1], G J Laundy[1], S M Gore[2], M R Howard

[1]University of Bristol Department of Transplant Sciences, Southmead Health Services, BRISTOL BS10 5NB. [2]MRC Biostatistics Unit Cambridge CB2 2QQ.

Three hundred and five unrelated donor bone marrow transplants (UD-BMT) were prospectively compared with a cohort of HLA identical sibling BMT (ID-BMT) matched for centre, diagnosis, stage of disease and recipient age. Results were analysed unifactorially and by multifactorial proportional hazards regression analysis. Diagnosis in the UD:ID cohorts were CML 52% vs 41%, AL 28% vs 38%, marrow failure 14% vs 11% and other 6% vs 10%. 60% of UD-BMT were for patients with standard risk disease vs 70% for ID-BMT. Mean recipient age at UD vs ID-BMT was 24y vs 30y. 241 of 305 UD-BMT pairs were matched or probably matched for HLA-A, B, DR, whereas 45 pairs were mismatched or probably mismatched. Unifactorial analysis at 100d showed engraftment in UD vs ID-BMT was 87% vs 99% ($p=0.01$), and probability of acute graft versus host disease (AGVHD) 46% vs 38% ($p=0.001$). Actuarial survival at 2 years was 39% after UD compared with 67% after ID-BMT ($p=<0.00001$). The following factors were independent predictors in multifactorial analyses of survival after UD-BMT: young recipient age, standard risk disease, diagnosis of ALL vs CML, HLA matched donor and use of serotherapy (monoclonal antibody or ATG) as pre-BMT immunosuppression. Choice of pre-BMT chemo-irradiation protocol and use of T-cell depletion (TCD) had no impact on survival after UD-BMT. Failure to engraft after UD-BMT was independently predicted in multifactorial analysis by increasing time from diagnosis to BMT and use of both ex-vivo and in-vivo TCD. HLA mismatching did not independently predict engraftment failure after UD-BMT. Reduced AGVHD after UD-BMT was predicted multifactorially by use of matched donors, pre-BMT serotherapy and ex-vivo TCD. In-vivo TCD was not an independent predictor of AGVHD. We conclude that donor histocompatibility, recipient age, stage of disease, diagnosis and choice of immunosuppressive protocol are independent predictors of survival after UD-BMT.

PAPER 9

ANTIEPITHELIAL CELL ANTIBODY IN PAEDIATRIC RENAL TRANSPLANTATION IN THE UK AND IRELAND 1987-1991

[1]Jane E Deal, [2]Andrea W Harmer, [3]Susan Martin, [2]Paul J Norman, [3]Helen Liggett, [1]Susan PA Rigden.

[1]Department of Paediatric Nephrology and [2]Tissue Typing Laboratory, Guy's Hospital London, [3]NW Regional Tissue Typing Laboratory, St Mary's Hospital, Manchester.

In order to ascertain the significance of the presence of an antiepithelial cell antibody (AECA) and renal graft loss in children transplanted in the UK and Ireland, retrospective data for renal transplants performed at 6 paediatric nephrology units between 1987-1991 were collected. Serum samples from the day of transplant or the cross match sample were obtained and tested independently for the presence of AECA using both a microcytotoxicity and a flow cytometry assay. Results were then compared to the clinical outcome.
Data were collected from 217 children undergoing 255 grafts. 27 grafts were excluded either because samples were not available for duplicate analysis (10) or because of a discrepancy between the results obtained by the 2 different assays (17/245 samples - 7%). AECA results were expressed as positive if both assays agreed.
151 grafts were first grafts and 65 were regrafts. In children receiving first grafts the prevalence of AECA was 9%; 48/151 (32%) were lost, 6 being AECA positive. No correlation was found between the presence of AECA and graft loss in first grafts. In children being regrafted the prevalence of AECA was 23%. 17/65 (26%) grafts were lost, 9 being AECA positive. A significant correlation was found between the presence of AECA and graft loss in these children (* $p < 0.001$);

All units	LOSS		SUCCESS	
	1st grafts	regrafts	1st grafts	regrafts
AECA+	6	9*	8	6*
AECA-	42	8*	107	42*

However when the data from Guy's was excluded no correlation was found. Further analysis of the Guy's data showed that AECA is not associated with graft loss in children undergoing a first renal graft but was associated with graft loss in children at Guy's hospital being regrafted (* *p < 0.02);

Guy's Hospital	LOSS		SUCCESS	
	1st grafts	regrafts	1st grafts	regrafts
AECA+	5	9**	2	5**
AECA-	20	4**	39	15**

In conclusion AECA does not appear to be important in paediatric renal transplantation in the UK and Ireland but may be important in a small subgroup of children being regrafted.

PAPER 10

A COMPARISON OF SEROLOGICAL AND DNA-BASED METHODS FOR CLASS I HLA-B TYPING IN RENAL TRANSPLANT MATCHING

SK Hemmatpour, S McQuilkin, PR Evans, *K Poulton, WM Howell

Wessex Histocompatibility Service, Southampton University Hospitals, Southampton SO16 6YD and *ARC Epidemiology Unit, Univ. of Manchester, Manchester M13 9PT.

The influence of patient-donor matching for the HLA-A, -B and -DR antigens on renal allograft outcome is well documented. Until recently, the majority of HLA class I typing was performed by serology. However, it is now possible to type individuals for the HLA class I loci using the polymerase chain reaction with sequence specific oligonucleotide probes (PCR-SSOP). In this study 170 individuals including 66 renal donor-recipient pairs, previously serologically typed, were PCR-SSOP typed for the HLA-B locus using an improved version of Yoshida's typing system (1) and results compared with those obtained by serology. A specific region of the HLA-B gene was amplified using HLA-B specific 5' primers (1) and two 3' primers in separate reactions. Initially, 23 digoxigenin labelled SSOPs were used specific for 4 hypervariable regions in exon 2 (1). A further 7 SSOPs were designed to detect sequences in 2 further hypervariable regions in exon 3. Using a combination of these primers and probes it was possible to assign HLA-B types to all 170 individuals with an improved resolution of previously reported ambiguous allele groups (1); for example, the 'B7' group was resolved into B7 (n=52), B42 (0) and B55/56 (n=7); the 'B14' group into B14 (n=8) and B39 (n=4); the 'B40' group into B40/41 (n=17) and B45/50 (n=2) and finally the 'B51' group into B51 (n=12) and B53 (0). One discrepancy was discovered in an individual that was serologically assigned as HLA-B35, B70 but PCR-SSOP typed as HLA-B35, B45/50. There were 9 HLA-B specificities (B41, 42, 45, 46, 54, 56, 63, 78, 79) that were not present in the samples studied and thus the efficacy of serology vs PCR-SSOP typing for these alleles could not be compared. These results indicate that clinical HLA-B typing by PCR-SSOP is suitable for renal transplant matching with a resolution at least equal to that obtained by serology.

1. Yoshida et al (1992) Hum Immunol, 34; 257-66.

PAPER 11

EPITOPE ANALYSIS IN HIGHLY SENSITIZED PATIENTS CAN REVEAL FINE ANTIBODY SPECIFICITY, PREDICT NEGATIVE CROSSMATCHES AND REDUCE WAITING TIME FOR TRANSPLANT

G J Laundy and B A Bradley

University of Bristol, Department of Transplantation Sciences, Southmead Health Services, Westbury-on-Trym, BRISTOL BS10 5NB

The accumulation of highly sensitized patients (HSP) with high frequency panel reactive antibodies (PRA) on renal transplant waiting lists is due mainly to the difficulty in identifying crossmatch negative HLA-DR compatible cadaver donors.

We have attempted to correlate antibody specificity in HSP sera with class I sequence variations. Our conclusions were then mapped as potential targets on the Björkman HLA model (Björkman *et al.*, 1987, Nature, **329**,506). The study group consisted of 20 random HSPs (10 female, 10 male, PRA 75%-100%) who had received between 0 and 3 allografts. Six were ungrafted. We were unable to establish PRA specificity in 9 HSPs (mean PRA, 99%) due to insufficient negative data. Five patients (mean PRA, 92%) had conflicting serological data where "negative windows" were identified but all 5 patients reacted positively with donor cells that would otherwise be considered acceptably mismatched. These data suggested the presence of confounding non-HLA antibody activity.

Significant correlation between panel reactivity and sequence variation was found in 6 HSPs and antibodies against a maximum of 3 public epitopes were defined in each patient (Table 1). 8/12 involved α-helical substitutions. Surprisingly, these 6 HSPs were predicted crossmatch negative with between 3 and 8 of the 10 most common British class I haplotypes. Four would be compatible with A1,3;B7,8 one of the most frequent HLA phenotypes. This analysis suggests that if organ donors with common HLA types were initially reserved for HSPs the steady accumulation on waiting lists could be reversed.

TABLE 1. Antibody specificity in 6 HSP sera

HSP	%PRA	Antibody†	HSP	%PRA	Antibody
GH	75	A-66K B-24T	JW	92	A-114H/116Y/127K B+C-163L
ML	90	A+B-82L/83R A-142T/145H	TC	83	B-24S A-142T/145H HLA-B55
AW	85	A-114R A-62E/65G A-149T	RD	80	A+B-82L/83R

† Predicted target residues. Thus, *'A+B-82L/83R'* indicates residue 82-leucine and 83-arginine on the HLA-A and B molecule respectively

PAPER 12

IS SPLINTAGE OF THE BILE DUCT ANASTOMOSIS IN ORTHOTOPIC LIVER TRANSPLANTATION (OLT) JUSTIFIED ?

DF Mirza, BH Ferraz Neto, S Rowley, D Candinas, A Dalgic, AD Mayer, JAC Buckels, P McMaster.

The Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham.

The biliary duct to duct(DD) anastomosis in OLT is usually splinted by a T-tube to monitor bile quality, facilitate cholangiography and allow decompression. It may however be a focus for sepsis, sludge deposition, and bile leaks following removal. 108 adult OLTs with DD anastomosis were studied prospectively, 54 with a T-tube(Group 1) and 54 without splintage(Group 2). Biliary imaging in Group 2 was by endoscopic/percutaneous cholangiography. Median followup in the two groups was 6 and 8 months respectively. The two groups were similar for indication, preservation solution, median cold(721 vs. 793 minutes) and warm(49 vs. 50) ischaemic times, and early graft function parameters. Biliary complications were as follows:

	Group 1	Group 2
Stricture	1	1
Anastomotic leak	2	4
Sphincter dysfunction	1	0
Sludge	1	1
Leak after T-tube removal	5	0

Two patients in group 1 and 3 in group 2 required biliary reconstruction. One patient in group 1 died due to bile leak after T-tube removal, and one graft in group 2 failed following biliary reconstruction.

The use of T-tubes is associated with an increased incidence of biliary morbidity. Endoscopic and percutaneous diagnostic cholangiography enable satisfactory imaging, and the routine use of T-tubes may not be justified.

PAPER 13

TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC STENT-SHUNT (TIPSS) INSERTION AS A PRELUDE TO ORTHOTOPIC LIVER TRANSPLANTATION (OLT) IN PATIENTS WITH SEVERE PORTAL HYPERTENSION

TG John, R Jalan*, DN Redhead†, PC Hayes*, HA Sanfey, OJ Garden

University Department of Surgery, Centre for Liver and Digestive Diseases and Department of Medicine* and Department of Radiology†, Royal Infirmary, Edinburgh EH3 9YW.

It has been postulated that TIPSS insertion in selected patients awaiting OLT may alleviate portal hypertension, optimise their preoperative clinical course and facilitate surgery. Between November 1992 and May 1994, 8 patients with end-stage cirrhosis, portal hypertension and severe gastroesophageal varices underwent TIPSS insertion prior to OLT (median interval 30 weeks; range 1-82 weeks). There were no procedure related complications and recurrent variceal haemorrhage was controlled in all patients. Comparison was made with ten cirrhotic patients with severe portal hypertension and varices in whom OLT was undertaken during the same period but without prior placement of TIPSS. The two groups were matched with respect to age, sex and Child's grade. There were two post-operative deaths in TIPSS patients, neither of which were TIPSS related. No significant differences (NS) were observed in operative times or perioperative transfusion requirements between the two groups (unpaired T-test) (Table).

(Median values)	TIPSS	No TIPSS	
Recipient hepatectomy (mins)	180	208	NS
Time to portal venous bypass (mins)	166	197	NS
'Warm ischaemia' time (mins)	60	59	NS
Total operation time (mins)	457	487	NS
Red Cell Concentrate (units)	8	7	NS
Fresh Frozen Plasma (units)	9.5	11	NS

Perivascular fibrosis at the porta hepatis and hepatic venous confluence was encountered in TIPSS patients, but did not prevent safe and satisfactory surgical dissection. Stent malposition with protrusion into the superior mesenteric vein and suprahepatic vena cava occured once and was associated with portal vein thrombus.

TIPSS placement in the preoperative management of patients with severe portal hypertension is a feasible option which does not preclude successful liver replacement, and 'buys time' for liver transplant candidates at risk of recurrent variceal haemorrhage. Accurate stent placement is important to avoid compromise of the recipient venous trunks.

PAPER 14

INCIDENCE AND OUTCOME OF DONOR ARTERIAL VARIATIONS IN LIVER GRAFTS- AN ANALYSIS OF 520 CONSECUTIVE CASES

AS Soin, PJ Friend, A Rasmussen, R Saxena, NV Jamieson, RY Calne

University of Cambridge Clinical School, Department of Surgery, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.

Recognition and preservation of anomalous donor arteries is essential to ensure adequate vascularity to the graft parenchyma and biliary tree. Even when preserved, the accessory vessels may be small and cause difficulties in reconstruction. The possibility of compromised biliary blood supply in reduced livers with abnormal vessels is another area of concern. To investigate the incidence and impact of variations in arterial anatomy, we analysed 520 consecutive allografts performed in the 6 year period from January 1988. Abnormal arteries were found in 160(31%) cases, including an accessory left hepatic (ALHA) in 74, accessory right (ARHA) in 48, both, ALHA and ARHA in 9, main hepatic from superior mesenteric artery in 12, and miscellaneous others in 17. The presence of abnormal anatomy was not associated with subarachnoid haemorrhage from cerebral aneurysm as the cause of donor death. There was no significant difference in the incidence of biliary complications between grafts with normal (19%) and anomalous (18%) arterial supply. However, both patients who had a left lateral segmental graft (LLSG) with the inevitable sacrifice of an ARHA developed biliary stricture. Similarly, all 4 patients with LLSG based on ALHA alone either lost their grafts very early (nonthrombotic infarction 1, primary non-function 1) or developed biliary complications (n=2). The major contribution of the right main or when present, ARHA to the supply of the main bile duct has been previously noted by us in an anatomical study of hepatobiliary vascular supply in cadaveric liver resin casts. The incidence of arterial complications was 4% with no significant difference between those with normal(3%) and abnormal(6%) anatomy. The incidence of hepatic infarction, chronic rejection and overall graft loss was also similar in the two groups. The findings were similar when individual anatomical variations were analysed separately. In conclusion, anomalies of hepatic arterial anatomy occur in a third of all livers. In general, appropriately noted and reconstructed arterial variations do not compromise graft outcome. However, reduction of livers to left moieties leaving the complete bile duct with sacrifice of the main or right accessory hepatic artery is likely to render the duct ischaemic and should be avoided.

PAPER 15

DONOR-RECIPIENT MICROCHIMERISM IS NOT REQUIRED FOR ADULT INDUCED TRANSPLANTATION TOLERANCE

Andrew Bushell, Peter J. Morris and Kathryn J. Wood

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

Aim It has been suggested that chimerism between donor and recipient is an absolute requirement for adult-induced transplantation tolerance. We have sought to answer this question in a well defined mouse model in which tolerance is induced by pre-treatment with donor antigen and anti-CD4 monoclonal antibody.

Methods C3H.He mice (H-2^k) pre-treated 28 days before transplant with the depleting anti-CD4 antibody YTA 3.1.2 and a single iv. donor specific transfusion (DST) accept B.10 (H-2^b) hearts indefinitely (MST >100 days). Tolerance is donor-specific and is not induced by either antibody alone (MST 26 days) or DST alone (MST 21 days). We reasoned that if stable engraftment of stem cells from the transfusion is essential for this effect, then mice pre-treated with irradiated DST under anti-CD4 cover should not become tolerant.

Results An equivalent anti-CD4/DST protocol using irradiated DST (2000 rads) resulted in poor graft survival (MST 12 days, n=8 cf. untreated controls MST 8 days, n=5) initially supporting the view that micro-chimerism may indeed be essential for the success of the anti-CD4/DST protocol. However, additional data indicate that for tolerance to be achieved in this model, the small number of $CD4^+$ T cells which escsape depletion (~30%), must encounter donor antigen during a relatively short period when they are coated with anti-CD4 antibody. Irradiated cells (which are less than 5% viable after 24 hours) may simply fail to persist for long enough to make this encounter. In order to ensure the persistence of donor cells during the period of antibody coating, recipients were pre-treated with the anti-CD4/irradiated DST protocol, then given three additional irradiated DST's at daily intervals. This resulted in striking graft prolongation (MST >100 days, n=7) and tolerance to alloantigen essentially identical to that achieved using anti-CD4 antibody and unmodified blood.

Conclusion We have shown unequivocally that transplantation tolerance in this model does not depend upon the establishment of donor-recipient micro-chimerism following antigen pre-treatment. This observation is particularly relevant to the current debate concerning the role of chimerism in clinical transplantation and may also shed new light on the mechanisms involved in the well known but poorly understood blood transfusion effect.

PAPER 16

THE MECHANISM OF ANTI-CD4 INDUCED T CELL INHIBITION AND EARLY INTRACELLULAR SIGNALLING EVENTS

Jaques B, Neison P, Bolton EM, Bradley JA

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

The cellular and molecular mechanisms whereby non-depleting anti-CD4 monoclonal antibodies (mAb) may induce transplant tolerance are unclear. MRC OX-38 is a mouse IgG2a mAb which binds to the membrane distal domain of rat CD4. We and others have shown that this antibody strongly inhibits allogeneic T cell responses *in vitro* and may induce transplant tolerance *in vivo*. During initial T cell encounter with alloantigen, OX-38 may either block the functional interaction of CD4 with MHC class II or may interrupt normal intracellular signalling events necessary for T cell activation.

In this study evidence that anti-CD4 perturbs intracellular signalling was obtained by demonstrating that OX-38 inhibits the proliferative response of purified CD4 T cells in response to TCR cross-linking by immobilised TCR mAb (R73) i.e. a system independent of class II MHC-CD4 interaction. In this system analysis of early intracellular signalling events showed that OX-38 led to subtle differences in the kinetics of tyrosine kinase phosphorylation and to striking differences in the overall pattern of phosphorylation in CD4 T cells. The most notable observation was the loss of association of a 56 kd phosphorylated protein (shown by immunoblotting to be lck) and the dephosphorylation of a smaller (as yet unidentified) protein after ligation of CD4 by OX-38. Both the CD4 T cell inhibition and the altered phosphorylation events were enhanced when anti-CD4 was further cross-linked on the cell surface.

The observations that the anti-CD4 mAb OX-38 inhibits T cell activation through the induction of negative intracellular signalling events which are amplified when CD4 is cross-linked may be of relevance in the design of improved protocols for anti-CD4 therapy.

PAPER 17

DONOR-TYPE SINGLE MHC LOCUS PRODUCTS COMBINED WITH ANTI-CD4 mAb CAN INDUCE TOLERANCE TO FULLY ALLOGENEIC CARDIAC ALLOGRAFTS

SAITOVITCH David , MORRIS Peter J. , WOOD Kathryn J.

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

For success, clinical transplantation still relies on the administration of non-specific immunosuppression for the prevention and treatment of allograft rejection. As a consequence, infections are a major threat to transplant patients. Donor-specific transfusions (DST) can prolong allograft survival, but may also cause allosensitization and the transmission of infectious diseases. Because $CD4^+$T cells are pivotal in the rejection process we have searched for more specific forms of immunosuppressive therapies by combining alloantigen pretreatment with anti-CD4 mAb therapy.
To avoid the potential adverse consequences of blood transfusion, recipient cells (L cells, H-2^k) transfected with the MHC genes encoding the donor antigens H-$2K^b$, H-$2D^b$, H-$2IA^b$ were used as a substitute for DST. Transfectants expressing all three donor MHC products in combination with a depleting anti-CD4 mAb (YTA 3.1) induced tolerance in almost all recipients independently of the cell dose given: 1 x 10^6 cells, MST > 100d (n=9) and 5 x 10^6 cells, MST > 100d (n=7).
The tissue type of the organ donor is not usually known before transplantation. Therefore, the next step of this investigation was to determine if syngeneic cells expressing only one donor MHC gene product were also effective. C3H/He (H-2^k) mice were pretreated with syngeneic L cells transfected with single donor class I or class II MHC genes (K^b, D^b or IA^b) combined with YTA 3.1, 28 days before transplantation of a C57BL/10 (H-2^b) heart. Indefinite allograft survival (> 100 days) was achieved in 85% of the recipients treated with the cells expressing donor class II, IA^b, (1 x 10^6 cells, n=7, MST >100d) and 70% with donor class I ,K^b, (5 x 10^6 cells, n=8, MST > 100d) molecules. In contrast, cells expressing class I D^b locus products were less effective at all cell doses investigated. The control groups were as follows: untreated recipients, MST = 10 days (n=5); YTA 3.1 only, MST = 21 days (n=8); L-K^b cells only, MST = 9 days (n=5).
Conclusions: 1) Syngeneic cells expressing single donor MHC locus products are able to induce tolerance to alloantigens in vivo when combined with anti-CD4 mAb before transplantation; 2) there is a tolerogenic hierarchy amongst the MHC locus products.

PAPER 18

TRANSPLANT TOLERANCE MAY BE ASSOCIATED WITH A DIMINISHED TH2 CYTOKINE RESPONSE

JR Tweedle, SE Middleton, HE Marshall, JA Bradley, EM Bolton.

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

The contemporary paradigm that transplantation tolerance corresponds to a diminished Th1 and, in many cases, a heightened Th2 T cell response fails to accomodate within it the possibility that alloantibody (usually ascribed to a Th2 response) may be responsible for acute rejection. We have addressed this paradox by developing a protocol for inducing tolerance to class I disparate kidney allografts in a rat model in which alloantibody is known to mediate acute graft rejection.
PVG ($RT1^u$) recipients were rendered tolerant to class I (A^a) disparate kidney allografts by administration of 4 weekly donor specific pre-transplant blood transfusions combined with a short course of cyclosporin A given with the first transfusion. This protocol was highly effective at inducing tolerance and tolerant animals readily accepted a further donor kidney without additional immunosuppression. Tolerance induction correlated with abrogation of a cytotoxic alloantibody response whereas, in contrast, omission of cyclosporin A from the protocol led to a heightened antibody response and rapid graft destruction. The intragraft cytokine mRNA expression in tolerant and rejecting grafts was assessed by semiquantitative RT PCR. The Th1 cytokines (IL-2 and IFNγ) were detectable at similar levels in tolerant and rejecting grafts but, interestingly, message for the Th2 cytokines (IL-4 and IL-10) was detectable in rejecting but not in tolerant kidney allografts.
These results in a clinically relevant model for tolerance induction suggest that, where antibody is responsible for graft rejection, induction of tolerance is associated with a decreased Th2 response, thus challenging the existing paradigm.

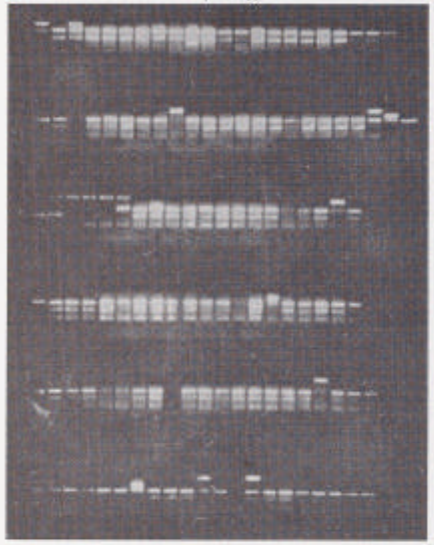
PAPER 19

HLA "PHOTOTYPING" BY SEQUENCE-SPECIFIC PRIMERS.

M. Bunce, C.M. O'Neill, G.C. Fanning, M.C.N.M. Barnardo, P.J. Morris & K.I. Welsh.

Transplantation Immunology, Nuffield Dept. of Surgery, Oxford Transplant Centre, Churchill Hospital, Oxford OX3 7LJ. FAX 0865 226162.

HLA typing by sequence-specific primers (SSP) has proved to be both rapid and accurate for HLA-DR and HLA-DQB and recently SSP systems have been described for HLA-A and HLA-C. We have now improved on the existing systems and developed medium resolution HLA-B SSP typing which has enabled us to produce for the first time an HLA "phototype" where all the relevant loci of an individual can be displayed on a single easily stored and transmitted image. The photograph below illustrates such a photoype.



Gel Order: Row 1 lanes 1-22 HLA-A. **Row 2** lanes 1,2 HLA-A, lanes 4-23 HLA-C. **Row 3** lanes 1-20 HLA-B. **Row 4** lanes 1-20 HLA-B. **Row 5** lanes 1-8 HLA-B, lanes 10-22 HLA-DR. **Row 6** lanes 1-12 HLA-DR, 14-22 HLA-DQB

All PCR reactions except the first 6 HLA-B lanes contain a 796bp amplification control. The first 6 HLA-B reactions contain a 256bp control.

Positive reactions in lanes 1 & 3 of row 1 correspond to HLA-A*01,*02. Positive reactions in lanes 9, 21 & 22 of row 2 correspond to HLA-Cw*06. Positive reactions in lanes 1,2 ,6,8,19 of row 3, lanes 15 of row 4 correspond to HLA-B*5001, B* 1701, Bw4 and Bw6. Positive reactions in lane 18 of row 5 and lane 11 of row 6 correspond to DRB1*0701 and DRB4*01. Positive reaction in lane 14 of row 6 corresponds to DQB*0201.

Our SSP system offers DNA typing of cadavers in under 2.5 hours with the advantage of higher resolution and increased definition compared to serology. We estimate the cost of phototyping in reagent terms at £15. Although far in excess of the present UKTSSA requirements for solid organ exchange the system is cheap and accurate enough to allow new single or multi-centre strategies to be considered (eg those based on sequence difference or peptide repertoire) rather than somewhat crude serological differences.

We will present our HLA-B SSP in detail along with the resolution updates on the SSP typing for the other loci and our general methodology for SSP.

PAPER 20

HUMAN ENDOTHELIAL STIMULATION OF ALLOGENEIC T CELLS VIA A CTLA-4 INDEPENDENT PATHWAY

Christopher Page, Craig Thompson, Magdi Yacoub and Marlene Rose

Department of Cardiothoracic Surgery, National Heart and Lung Institute, Heart Science Centre, Harefield Hospital, Harefield, Middlesex, UB9 6JH

It is accepted that T cells require at least two signals to undergo proliferation and cytokine release; an antigen dependent signal mediated via the TCR and an antigen independent signal mediated via one or more accessory or adhesion molecules. Interaction between CD28 or CTLA-4 and the B7 coreceptors found on many Antigen Presenting Cells is known to be essential for antigen specific (including alloantigen) expansion of T cells *in vitro* and *in vivo*. CTLA-4-Ig is a fusion protein with very high affinity for B7. It has been used *in vivo* to block both allograft and xenograft rejection.

Most of the work investigating second signal requirement have used "professional" Antigen Presenting cells. In view of the observations that Class II positive human endothelial cells can cause direct allostimulation of resting CD4+ and CD8+ T cells, we have investigated the requirement of CTLA-4 in this response. The current studies show that the proliferative response of allogeneic CD4+ and CD8+ T cells to γ-IFN treated HUVEC is inhibited by MoAbs against MHC Class II and Class I antigens respectively, but not by CTLA-4-Ig. In contrast lymphocytes proliferating in response to allogeneic splenocytes are inhibited by CTLA-4-Ig. Cell surface binding studies using Flow Cytometry demonstrated failure of endothelial cells to bind either CTLA-4-Ig or MoAbs against B7 receptors. In conclusion, different Antigen Presenting Cells use different costimulatory signals. The possibility that this leads to a different cytokine profiles needs to be investigated to further understand the role of endothelial cells in transplant rejection.

PAPER 21

THE PRESENCE OF ACTIVATED DONOR HLA CLASS I REACTIVE T LYMPHOCYTES (CTLs) IS ASSOCIATED WITH REJECTION OF CORNEAL GRAFTS

D L Roelen, E van Beelen, S P M J van Bree, J J van Rood, H J Völker-Dieben and F H J Claas

Department of Immunohaematology and Blood Bank, University Hospital Leiden, POB 9600 2300RC Leiden, The Netherlands

Although the cornea is considered to be an "immunological privileged site", corneal transplantation can result in immunological rejection followed by graft failure, especially in patients with vascularized corneas. Several studies suggest a beneficial effect of matching for the HLA class I antigens on corneal graft survival, although a large study (CCTS) failed to confirm this. To circumvent an endless discussion on studies either confirming or denying the relevance of HLA matching, we decided to approach this problem in another way. A more direct way to assess the importance of HLA class I antigens in corneal transplantation is to measure whether rejection of an allograft is associated with priming of cytotoxic T lymphocytes (CTLs) recognizing the mismatched HLA antigens of the donor.
In the present study 13 patients with good graft function and 10 with ongoing rejection of their corneal allografts were analyzed for the presence of CTL directed against mismatched donor HLA class I antigens, by limiting dilution assays. CTLs were divided into naive and primed CTLs based on the measurement of their *in vitro* sensitivity or resistance to anti-CD8 or cyclosporine A.
Cytotoxic T cell precursors (CTLp) frequencies directed against the mismatched donor HLA class I antigens were similar in non-rejectors and rejectors. However, rejection was strongly associated with the presence of primed donor-specific CTL whereas these primed cells were absent in case of good graft function.
These data show that HLA antigens of a transplanted cornea are immunogenic and targets for rejection by cytotoxic T cells. Therefore, this study strongly supports the need for HLA-A and -B matching in corneal transplantation in patients with a high probability of rejection.

PAPER 22

INDUCTION OF TRANSPLANT TOLERANCE BY INTRATHYMIC INJECTION OF DONOR BONE-MARROW CELLS IS CRITICALLY DEPENDENT ON DONOR-RECIPIENT HAPLOTYPE

Walker KG, Bolton EM, Bradley JA

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

Intrathymic injection (ITI) of donor cells or alloantigen together with transient immunosuppression (e.g. by ALS) has recently emerged as a novel strategy for inducing transplant tolerance in a variety of experimental models.
We have confirmed the validity of this approach in a rat model of heterotopic heart transplantation in which recipients received an ITI of an optimal number (2.5×10^7) of donor bone-marrow (BM) cells together with 1 ml ALS i.p. 14 days before transplantation. The efficacy of this protocol was critically dependent on donor-recipient haplotype and strongly influenced not only by donor-recipient MHC disparity but also by non-MHC background genes. Thus ITI of donor BM effectively rendered Lou but not PVG (both $RT1^u$) recipients tolerant to Lewis ($RT1^l$) heart grafts (MST > 70 days and 7 days respectively). In several fully allogeneic rat strain combinations tested, ITI produced no increase in graft survival (e.g. DA and BN into Lewis) and in others survival was only slightly prolonged (e.g. DA and Lewis into PVG). Interestingly ITI of parental BM induced tolerance to semi-allogeneic DA x PVG and Lewis x PVG F_1 heart grafts in PVG recipients (MST > 100 days) indicating the importance of the antigenic strength of the graft in this model. Failure to induce permanent tolerance to fully allogeneic hearts in PVG recipients was attributable to emergence of new donor reactive T cells from the thymus rather than failure of ALS to disable the peripheral T cell pool since thymectomised recipients given ALS permanently accepted DA and Lewis heart grafts (MST > 100 days). Overall these results confirm that ITI is a promising approach for inducing transplant tolerance but its efficacy appears critically dependent on both donor-recipient haplotype and antigenic strength.

PAPER 23

POLYMORPHISM AT THE TNF-Nco1 LOCUS PREDICTS EARLY REJECTION IN CARDIAC TRANSPLANT RECIPIENTS.

G. Gallagher, F. Hornero, D.A. Campbell, & D. Richens.

University Departments of Surgery and Cardiac Surgery, Glasgow Royal Infirmary.

Recent research in animal models has demonstrated an apparent relationship between graft rejection and TNF production. Studies have shown that administration of TNF can promote rejection in heart transplantation models and conversely, that a single administration of anti-TNF antibody can inhibit rejection and promote graft survival. However, the role of TNF in the rejection process in man remains largely undefined. Several independent studies in autoimmune diseases and using cells from normal individuals have demonstrated that a polymorphic marker within the TNF locus on chromosome 6 has been associated with high or low TNF production. This polymorphism is defined as the presence or absence of a recognition site for the Nco-1 restriction enzyme, such that the allele B*1 contains the Nco-1 site and B*2 does not; the B*2 phenotype has been associated with high TNF production.We examined the presence of these two alleles in a population of 30 cardiac transplant recipients in relation to the number of rejection episodes; the median follow-up period was 420 days. We then determined whether the individual allelotypes possible at this locus further influenced the course of graft survival.

Results.

	rejection events (n ± SD) for total follow-up period	rejection events (n ± SD) for initial 90 days post-grafting
B*1(n=19)	4.3 ± 2.4 ns	2.3 ± 1.6 ns
B*2(n=22)	4.1 ± 2.0 ns	2.6 ± 1.3 p = 0.008
B*1/B*1 (n=8)	2.7 ± 2.4 ns	0.7 ± 0.7 p = 0.008*
B*1/B*2 (n=11)	5.3 ± 1.8 p = 0.014	3.0 ± 1.2 p = 0.012
B*2/B*2 (n=11)	2.7 ± 1.6 ns	1.9 ± 1.0 ns

* lower rejection

Conclusion.
The data demonstrate that the B*2 phenotype is strongly associated with increased rates of rejection within the first 90 days post-transplant, implicating TNF in the rejection process. Interestingly, our data also show that graft recipients homozygous for the B*1 allele appear to be protected from early rejection.

PAPER 24

CHRONIC REJECTION IS INFLUENCED BY A GENE POLYMORPHISM IN THE ANGIOTENSIN-CONVERTING ENZYME

DA Cunningham, SJ Crisp, MJ Dunn, ML Rose and MH Yacoub

Department of Cardiothoracic Surgery, National Heart and Lung Institute, Heart Science Centre, Harefield Hospital, Harefield, Middlesex, UB9 6JH.

It has recently been shown that a polymorphism in the gene encoding angiotensin-converting enzyme may be a risk factor for myocardial infarction and other cardiovascular diseases. Individuals who are homozygous for the deletion allele (DD) are more susceptible to cardiovascular diseases and have higher levels of plasma ACE than individuals with the insertion (II). Accelerated coronary artery disease is one of the most serious complications following heart transplantation. Here we have used polymerase chain reaction (PCR) amplification to determine the ACE genotype of both recipients and donors of 48 patients who developed accelerated coronary artery disease (CAD) within 2 years of cardiac transplantation and in a group of 38 disease free transplant patients.

No significant differences were seen in the frequencies of the recipient genotypes between those patients who developed CAD and those who did not develop CAD. However, when donor genotypes were investigated, there was a significantly higher frequency of the I allele in donors whose recipients did not develop CAD ($P = < 0.05$)than in donors whose recipients did develop CAD. Moreover, the frequency of the I allele in the group whose recipients remained free of CAD was significantly higher than in the normal Northern European population ($P < 0.01$).

It must be assumed that levels of local ACE (ie within the coronary arteries) will be determined by the donor genotype whereas circulating ACE levels will be determined by the recipient genotype. The present results suggest that the ACE genotype of the donor plays a part in development of CAD, and that local levels of ACE are more important than circulating levels.

PAPER 25

INFLUENCE OF AGE IN RENAL TRANSPLANTATION: A SINGLE CENTRE, TEN YEAR EXPERIENCE

P.A.Andrews, F.Compton, C.G.Koffman, J.S.Cameron

Renal Unit, Guy's Hospital, London SEl 9RT

The availability of donor organs remains the rate limiting factor in renal transplantation, yet age limits for transplantation and the age profile of patients accepted onto renal replacement therapy is steadily increasing. Given this, should we be transplanting elderly patients? Several studies have examined the outcome of patients transplanted aged 55 or over, but most have been small, multi-centre, or with limited follow up. We present the results of transplantation in a single centre with an aggressive surgical policy and actuarial ten year (mean 6 year) follow up.

From 1984-93, 611 transplants were performed, of which 121 were in recipients aged >60. Induction immunosuppression was not age-dependent, and the proportion of elderly recipients did not change over this period. The following actuarial trends were noted with increasing age: lower patient survival; lower graft survival; increased incidence of vascular disease and malignancy; increased length of hospital admission. When death with a functioning graft was excluded, graft survival age >60 was not impaired, indicating that the decline in graft survival reflected increased mortality rather than graft dysfunction. Steady improvement in 1 and 3 year patient survival was noted over the 10 year period in recipients aged >60, probably reflecting improved patient screening and selection. Transplantation in elderly recipients improved quality of life, but not patient survival, compared to an age-matched dialysis population. However, we estimate that 23 grafts were lost due to excess elderly patient deaths, grafts that could have been better used in a younger population.

Conclusion: the optimal use of donor organs and of patient selection remains to be defined: ethical and pragmatic approaches may conflict.

PAPER 26

USE OF THE NEW FORMULATION OF CYCLOSPORIN (NEORAL) IN LIVER TRANSPLANT RECIPIENTS WITH BILIARY DIVERSION AND PROLONGED CHOLESTASIS.

DF Mirza, D Candinas, BH Ferraz Neto,
D Mayer, JAC Buckels, P McMaster

The Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham.

The conventional oral formulation of cyclosporin (Sandimmun) is highly lipophilic with unpredictable absorption. In liver transplant recipients with biliary diversion or prolonged cholestasis, increases in dosage usually fail to achieve desired trough levels. The microemulsion formulation of cyclosporin (Neoral) has the advantages of predictable pharmacokinetics and better absorption, and is less dependant on bile production. From December 1993 to May 1994, 5/74 liver transplant recipients were converted on a compassionate basis from conventional cyclosporin to Neoral for the following reasons: prolonged cholestasis (n=2), biliary diversion (n=2), allergy to intravenous cyclosporin (n=1). Stable trough cyclosporin levels >200 ng/ml were achieved in all patients within a median period of 3 days (range 1-5). A reduction in the dose was achieved following conversion, as follows:

No	Reason	oral cyclosporin dose (mg/kg/day) preconversion	conversion to Neoral	1 month after conversion
1)	cholestasis	33	13	5
2)	cholestasis	22	8	4
3)	bile diversion	30	10	7
4)	bile diversion	10	7	4

In conclusion, conversion to Neoral in liver transplant recipients with biliary diversion or cholestasis achieves stable drug levels at reduced dosage requirements, with further reduction in dosage once liver function improves.

PAPER 27

EARLY GRAFT LOSS

Campbell B A, Abraham J S, Scott P D, Parrott N R, Pearson R C, Johnson R W G

Renal Transplant Unit, Manchester Royal Infirmary, Manchester

In a series of 688 consecutive adult cadaveric renal transplants, between April 1988 and July 1994, 33 (4.8%) renal allografts failed in the first thirty days.

In 30 (90.0%) failure was a consequence of vascular thrombosis. Renal artery thrombosis was responsible for the failure of 14 allografts and renal vein thrombosis for the other 16. In 20 (60.6%) allografts failing as a result of vascular thrombosis the donor kidney was from the right side, when renal vein thrombosis only was considered this increased to 80.1% (P<0.05). Only 4 allografts failed because of acute rejection which is now an uncommon cause of early allograft failure. Right sided donor kidneys, with a short renal vein and a long renal artery appear particularly at risk and require careful attention to surgical technique during revascularisation. Techniques which may contribute to the avoidance of vascular thrombosis include :- full mobilisation of the recipient's iliac vein which requires division of the internal iliac artery; the fashioning of an extension to the vein from the attached inferior vena cava. Prophylactic anticoagulation would be an alternative approach.

PAPER 28

INTERSTITIAL FIBROSIS AND OUTCOME IN RENAL TRANSPLANTATION

M L Nicholson, S J Harper, C M Edwards, T A McCullogh, M J R Feehally, P N Furness

University Department of Surgery, Leicester General Hospital, Leicester

Chronic cyclosporin (CsA) therapy is often attended by a progressive and irreversible decline in renal function. The principle histological finding in such cases in marked interstitial fibrosis. In this study allograft interstitial fibrosis has been assessed in sequential biopsies and correlated with renal function and graft survival.

107 consecutive renal transplants immunosuppressed with cyclosporin were studied. Three different drug regimens were used, (a) CsA starting at 17 mg/kg/day + prednisolone (b) same as (a) + nifedipine (c) CsA starting at 10 mg/kg/day + azathioprine 1 mg/kg/day + prednisolone. Needle core transplant biopsies were performed pre operatively and at 1, 6 and 12 months post transplant. Renal function was assessed by isotope glomerular filtration rate (GFR) measurements at 1, 6 and 12 months. A single observer, who was unaware of patient details, assessed allograft fibrosis by histomorphometric analysis of the interstitial volume fraction (IVF).

Renal allograft IVF percentage scores (95% confidence intervals)

Pre op	17.2 - 22.0	
1 month	23.1 - 27.2*	
6 months	26.3 - 32.7*+	*p<0.05 v pre-op
12 months	26.7 - 32.2*+	+p<0.05 v 1 month

IVF increased with time but stabilised at 6 months (table). GFR was negatively correlated with IVF at all time intervals; this relationship reached statistical significance at 6 months (p=0.05) but not at 1 month (p=0.157) or 12 months (p=0.069). Six month IVF predicted subsequent allograft survival and there were no failures in transplants with an IVF <25%.

Renal allograft IVF correlates well with renal function and long term transplant survival. It provides an objective measure of chronic allograft damage in transplants immunosuppressed with cyclosporin.

PAPER 29

SODIUM BASED LACTOBIONATE/RAFFINOSE LIVER PRESERVATION SOLUTION PREVENTS EARLY HYPOMAGNESAEMIA AND THROMBOCYTOPENIA AFTER ORTHOTOPIC LIVER TRANSPLANTATION

TR.Kurzawinski, B.Fuller, K.Cheetham, JN.Appleby, R.Whitta, L.Selves, A.Burroughs, B.Davidson, K.Rolles.

Liver Transplantation Unit, Royal Free Hospital and School of Medicine, London UK.

Organ preservation solutions generally contain a high potassium concentration to minimize loss of intracellular cations during storage. We have compared Na (NaLR) and standard K (KLR) based lactobionate/raffinose solution for liver preservation.

72 patients were randomized to liver preservation with a NaLR (20M, 16F, median age 48.5 (22-63) years) or a KLR (20M, 16F, median age 48.5 (5-68) years). The quality of the liver preservation was assessed by highest serum concentration of bilirubin, AST, ALT and lowest platelet count within 48h of transplantation and peroperative potassium, blood, albumin and plasma requirements. Concentrations of endothelin, bilirubin, LDH and lactate were measured in albumin liver flush performed before revascularization. Serum total magnesium concentration before and 12h after operation, acute rejection episodes and reperfusions syndromes were recorded. Actuarial graft survival was calculated. Mann Whitney U test was used for statistical analysis.

Indication for orthotopic liver transplant (OLT), cold and warm ischaemic time were similar in both groups. There were no differences in maximum levels of bilirubin, AST, ALT or intraoperative plasma, albumins and blood requirements. Levels of endothelin, bilirubin, LDH and lactate in wash out fluid were similar in both groups. Peroperative potassium requirements, number of acute rejection episodes and reperfusion syndromes were not different. The preop serum Mg concentration were similar in both groups (0.77mmol/l vs 0.78mmol/l). There was a significant fall in serum magnesium post op in those preserved with KLR (0.61) but not with NaLR (0.68),($p=0.017$). Post op platelet count was lower in patients transplanted with liver preserved with KLR (64 vs 50.5, $p<0.05$). Actuarial 1 and 6 months graft survival was alike in both groups.

Na and K based lactobionate/raffinose solutions are equally effective for liver graft preservation and outcome of OLT, and this may assist in the development of single preservation solution for multiorgan retrievals. The NaLR solution reduces post operative hypomagnesaemia and thrombocytopenia.

PAPER 30

THE F344 - LEW MODEL OF CHRONIC REJECTION IN RENAL ALLOGRAFTS RE-EXAMINED WITH MORPHOMETRY AND IMMUNOHISTOCHEMISTRY.

S.R.Stubington, J.S.O'Rourke, M.C.Bishop, K.M.Rigg, I.J.Beckingham

Renal Transplant Unit, Nottingham City Hospital, Nottingham, NG5 1PB

Chronic rejection is now the major cause of renal graft loss after the first six months, but we remain largely ignorant of its pathogenesis. For a logical approach to the prevention and treatment of chronic rejection we must look to animal models to unravel the sequence of events and subsequently provide a 'test-bed' for future research.

We performed unilateral renal transplants from F344 donor rats to immunologically similar LEW recipients without immunosuppression, leaving the LEW right kidney in-situ and so departing from the original model described by White in 1969.

The histological features of chronic rejection (arterial intimal thickening, glomerulopathy and tubulo-interstitial fibrosis) developed in the allograft kidneys but were absent in isograft controls. Computerised morphometric analysis revealed a significant increase in the arterial intimal thickness in allografts from 2.66 ($\pm$0.32) um^2/um to 5.2 ($\pm$0.9) um^2/um (Kruskal-Wallis $H=17.4$, $p<0.001$) by three months. A three stage streptavidin-biotin alkaline phosphatase complex method showed that this increase in thickness was mainly due to infiltration by, and proliferation of smooth muscle actin positive cells. A similar technique demonstrated the deposition of IgG1 on the vascular endothelium which persisted throughout the development of chronic rejection. There were increased numbers of ED1 positive macrophages and dendritic cells one month following transplantation, but no change in the ED2 positive macrophage subset. There was an increase in the expression of receptors for PDGF-B, FGF and TGF-B, but not IGF or EGF in the arterial media and intima preceding and during the development of chronic rejection.

A unified hypothesis for the development of chronic rejection is presented.

Abbreviations:

PDGF-B	=	Platelet-derived growth factor beta
FGF	=	Fibroblast growth factor
TGF-B	=	Transforming growth factor beta
IGF	=	Insulin-like growth factor
EGF	=	Epidermal growth factor

Reference: White.E., Hildemann.W.H., Mullen.Y (1969); Transplantation 8(5): 602-617.

PAPER 31

HUMAN IgG XENOREACTIVE ANTIBODIES BIND TO PORCINE AORTIC ENDOTHELIAL CELLS AND ARE FUNCTIONALLY ACTIVE.

S.P. Cooke, R.A. Hederer and C.O.S. Savage

Vascular Biology Research Centre, King's College, London

Xenoreactive antibodies (XAb), particularly of the IgM class, play a major role in the hyperacute rejection of xenografts. We investigated the identity of antigens expressed by Porcine Aortic Endothelial Cells (PAEC) that bind human IgG XAb. We identified high titres of IgG XAb in both individual and pooled normal human sera by ELISA using fixed and unfixed endothelial cell monolayers. This binding was $F(ab')_2$ mediated and the bound XAb were predominantly of the IgG_2 subclass. Only a minor component of this IgG XAb binding was directed at residues in an α1-3 linkage with galactose on PAEC surfaces. There was no binding of these XAb to antigens expressed by porcine red blood cells and/or lymphocytes. Labelling PAEC with ^{125}I followed by immunoprecipitation showed reproducible binding of IgG XAb to 75Kd, 110Kd, 180Kd and 210Kd components of PAEC cell surfaces. The 110Kd and 180Kd components were sensitive to digestion by Endoglycosidase F suggesting the presence of N-linked glycosylation sites. These IgG XAb were unable to activate complement but were able to induce increased adhesion of neutrophils to the endothelium and to mediate a cytotoxic response to PAEC by peripheral blood mononuclear cells via an antibody dependent cellular cytotoxicity mechanism. In conclusion, human IgG XAb recognise antigens on the surface of porcine vascular endothelium. Thus IgG XAb , in addition to previously described IgM class XAb, have to be considered as potential mediators of hyperacute rejection of porcine xenografts.

PAPER 32

RELATIVE INFLUENCE OF DELAYED GRAFT FUNCTION AND ACUTE REJECTION ON RENAL TRANSPLANT SURVIVAL

Nicholson ML, Horsburgh T, Edwards CM, Morgan JDT, Veitch PS, Bell PRF

University Department of Surgery, Leicester General Hospital, Leicester

The effect of delayed graft function (DGF) on long term outcome of renal transplantation remains controversial. Most studies have only considered the influence of delayed graft function using univariate analysis and do not stratify for rejection (REJ). In this study Cox stepwise logistic regression analysis was used to define factors exerting an independent and significant effect on graft survival.

Data was collected from 308 consecutive cadaveric renal transplants immunosuppressed with cyclosporin. The parameters entered into the multivariate model were: age, sex, race, diabetes, transplant number, donor source, blood transfusion, donor age, antibody status, HLA matching, ischaemic times, delayed graft function and rejection. Delayed graft function was defined as the requirement for dialysis in the first seven days and all rejection episodes were diagnosed using needle core allograft biopsy.

Delayed graft function occurred in 87 patients (28%) and a rejection episode within 3 months occurred in 147 patients (48%). Delayed graft function ($p=0.004$) and rejection ($p=.03$) both had an independent and signficant adverse influence on graft survival but the effect of delayed graft function was more marked (Table).

Group	Actuarial Graft Survival (%) 1 year	2 year	4 year
No DGF + No REJ	97	92	89
No DGF + REJ	83	74	69
DGF + No REJ	73	63	60
DGF + REJ	55	42	40

Efforts to improve graft survival in cadaveric renal transplantation should concentrate on reducing the incidence of delayed graft function.

PAPER 33

AN INVESTIGATION OF THE ROLE OF URETERIC ANASTOMOSES AND URETERIC STENTS IN RENAL TRANSPLANT RECIPIENTS

HCC Pleass, KR Clark, KM Rigg, KS Reddy, JLR Forsythe, G Proud, RMR Taylor

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

Urological complications following renal transplantation produce significant patient morbidity and mortality. A prospective randomised trial was established to compare two techniques of ureteric anastomosis, as well as the use of ureteric stents, in the prevention of urological complications. In total 300 patients have been recruited after informed consent, and randomised to one of four different groups. The anastomotic techniques used were either direct spatulated (modified Leadbetter-Politano), or external ureteroneocystostomy (UNC). These anastomoses were performed either with, or without a ureteric stent according to the randomisation. Complications were recorded on a standard proforma and patients were assessed whilst in hospital and again at three months post transplantation.

Of the patients who were randomised to receive a ureteric stent, whichever anastomosis was performed, none has experienced a major urological complication. It was clear within this trial, that ureteric stents significantly reduce the incidence of ureteric obstruction ($p<0.05$) and urinary leak ($p<0.05$) within the follow up period. Although the incidence of major urological complication was not statistically different between unstented direct spatulated and unstented external UNC anastomoses, the direct spatulated anastomosis is associated with a significantly higher incidence of clot retention ($p<0.05$).

We therefore advocate the use of an external UNC anastomosis with a prophylactic ureteric stent to reduce the morbidity of the renal transplant procedure.

PAPER 34

THE EFFECT OF HLA MIS-MATCHING ON RENAL ALLOGRAFT SURVIVAL: A REPORT FROM THE UKTSSA KIDNEY AUDIT GROUP

C A Rogers, M A Belger, R J Bawden, S Martin, J D Briggs

For The Kidney Audit Group, UK Transplant Support Service Authority, Bristol

In the UK, many renal transplants involve organ exchange and since 1989 beneficial matching has provided the basis for this exchange. This paper reviews the influence of HLA mis-matching on transplant survival and the beneficial matching concept.

Sufficient data for analysis was available for 11,436 transplants (68% of transplants reported) performed from January 1983 to June 1993 from 37 centres in the UK and Republic of Ireland.

Multifactorial analysis was used to evaluate the influence of HLA mis-matching and donor factors for both overall survival and distinct transplant epochs (0-3 mths, 3-12 mths, 1-3 yrs, 3+ yrs). Significant recipient characteristics were modelled through the inclusion of a covariate.

For overall survival no differences in risk were found within the beneficially matched group but for other transplants the risk of failure increased significantly with DR mis-matches (Relative Risks = 1.35, 1.49, 1.95, $p < 0.01$ for 0, 1 and 2 DR mis-matches respectively). The epoch analysis supported this trend, but in the 1-3 yrs epoch only 2 DR mis-matched transplant reached statistical significance (RR = 1.60, $p < 0.05$), suggesting a possible lessening of the DR effect. However, for transplants surviving beyond 3 years both 1 and 2 DR mis-matched transplant carried an increased risk (RR + 1.67, 2.09, $p < 0.01$ and 2 DR mis-matches respectively).

Kidneys transplanted locally carried a significantly reduced risk of failure in the first 3 months (RR = 0.75, $p < 0.0001$) after which no differences were found. However transplants from older donors (> 55 yrs) carried a significant and consistent high risk throughout (RR = 1.55, $p < 0.0001$ for overall survival).

This analysis suggests that while HLA mis-matching influences outcome and therefore should continue to be considered in the allocation procedure, donor age also has an important influence and should therefore be considered as well.