The British Transplantation Society

Spring Meeting

Oxford

26th & 27th March 1996

Combined meeting with
The British Renal Association
&
The Dutch Renal Association 27th March

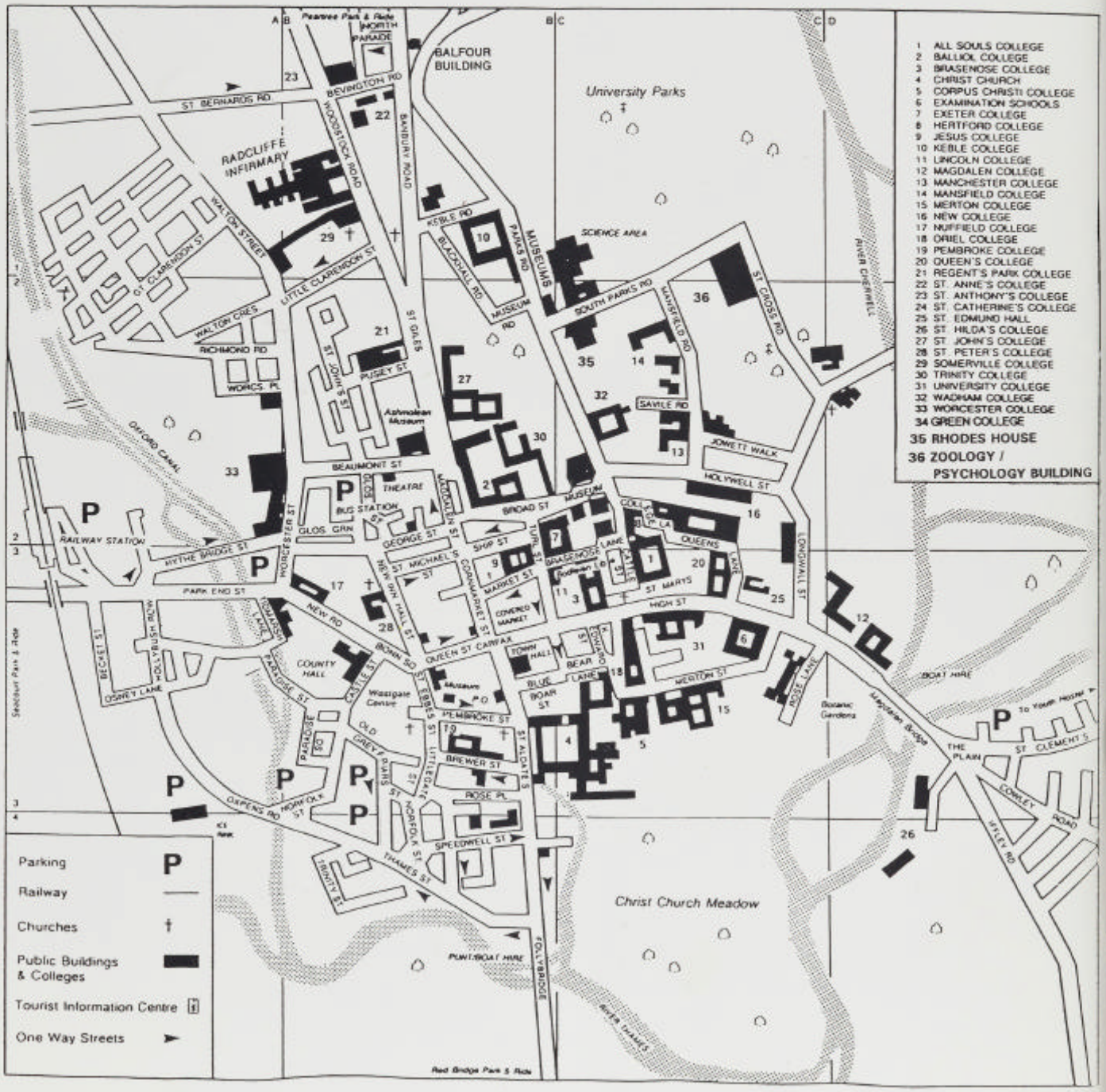
Venue: Department of Zoology, South Parks Road
Accommodation: Keble College

OXFORD MAP

Keble College, Parks Road = 10

Department of Zoology Building, South Parks Road = 36

University Museum = "Museums" Parks Road



ABSTRACTS SELECTED FOR PRESENTATION

ALLOREACTIVE HELPER T-LYMPHOCYTE PRECURSOR FREQUENCIES CORRELATE WITH HLA-DR ANTIGEN AMINO ACID RESIDUE MISMATCHES

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The generation of Interleukin-2 (IL-2) mediated helper activity is central to the cellular and humoral immune response induced by exposure to allogeneic histocompatibility antigens. We have analysed the influence of HLA matching on the *in vitro* response of primary alloreactive helper T-lymphocyte precursors (HTLp). Limiting dilution assays were employed to analyse frequencies of IL-2-producing HTLp in various HLA-mismatched responder:stimulator combinations.

Mean HTLp frequencies were significantly ($p<0.02$) higher in HLA-DR antigen mismatched ($n=24$) than HLA-DR matched ($n=6$) assays. Significant differences in the effect of one ($p<0.02$) or two ($p<0.05$) mismatched HLA-DR antigens were also detected. Mean HLA-A,-B,-Cw mismatches were not significantly different in each group (3.5 in DR-matched, 4.6 in DR-mismatched) and did not appear to be involved in alloantigen-induced IL-2 release. Linear regression analysis of HLA antigen protein sequence differences revealed a positive correlation ($r=0.545$, $p<0.002$) between HTLp frequency and the number of mismatched amino acid residues at the HLA-DRB loci. A predominant influence of bound peptide in the stimulation of primary HTLp was suggested by the greater significance of mismatched residues in the beta sheet ($r=0.535$, $p<0.005$) than the alpha helical ($r=0.467$, $p<0.01$) regions of the HLA-DRB molecule.

Our results demonstrate the major influence of HLA-DR sequence mismatching on alloreactive HTLp frequencies but indicate that additional genetic or environmental factors affect the alloreactive helper T-cell repertoire.

REMOVAL OF IL-4 PREVENTS THE INDUCTION OF TRANSPLANTATION TOLERANCE:- CONDITIONAL SUPPORT FOR THE TH1/TH2 PARADIGM

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Background and aims Mouse CD4+ T cells can be divided into Th1 and Th2 subtypes depending on the signature cytokines which they secrete on exposure to antigen. In vitro, products of each subtype can mediate powerful reciprocal regulation of the other subset. Because cellular responses frequently associated with graft rejection tend to be driven by Th1-type cytokines it has been tempting to speculate that tolerance is dependent on Th2 cytokines. We have used a tolerance model known to involve CD4+ T cell regulation to determine whether tolerance induction is dependent on the availability of IL-4 during primary exposure to alloantigen.

Methods C3H.He mice (H-2^k) pretreated with anti-CD4 antibody and donor specific transfusion (DST) accept fully allogeneic B.10 (H-2^b) hearts transplanted 28 days later (MST >100 days). In order to determine whether IL-4 is necessary during the initial contact between recipient T cells and donor antigen in the DST, C3H mice were pretreated with the anti-CD4/DST protocol with or without the neutralizing anti-IL4 antibody 11B11.

Results Pretreatment with the anti-CD4/DST protocol led to indefinite graft survival (MST >100 days, n=8) consistent with our previous data. However, in two independent experiments mice pretreated with the same protocol plus the anti-IL4 antibody had median graft survival times of 33 days (n=5) and 43 days (n=9). To our knowledge this is the first demonstration that the induction of tolerance to alloantigen in immunologically mature animals is dependent on the presence of IL-4.

Conclusion These data indicate that the Th1/Th2 paradigm may provide a useful framework for the understanding of transplantation tolerance particularly where continued graft survival is dependent on CD4+ T cell regulation. The results demonstrate that subtle modifications of the local cytokine environment at defined stages in the immune response can alter graft outcome and give grounds for cautious optimism that attempts to encourage the expansion of antigen-specific Th2 type cells, perhaps using anti-cytokine therapy, may provide a practical route to clinical transplantation tolerance.

DONOR FACTOR V Q506 MUTATION PREDISPOSES TO HEPATIC VASCULAR THROMBOSIS FOLLOWING LIVER TRANSPLANTATION

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The most frequent laboratory abnormality found in patients with venous thrombosis is an abnormal factor V coagulant synthesised in the liver in which arginine at position 506 is replaced by glutamine. Hepatic vascular thrombosis may complicate up to 15% of liver transplant procedures. The influence of factor V Q506 allele on the risk of hepatic vascular thrombosis following liver transplantation was therefore sought using paired recipient and donor DNA obtained from 124 consecutive procedures.

Using PCR and Mn II digestion, a rapid and reproducible assay, 25 heterozygous mutations were identified; 14 in recipients alone, 8 in donor alone and 3 in both. The heterozygous frequencies were 8.8% (95% C.I. 4.5-15.3) in donors and 13.7% (8.2-21.0) in recipients. Following transplantation there were 25 thrombotic events; 12 hepatic artery, 7 portal vein and 6 non-hepatic.

The Q506 allele was present in the donor in 4/19 cases complicated by hepatic vessel thrombosis and 4/105 without. The relative risk for hepatic vessel thrombosis in the presence of the Q506 allele was therefore 5.53 (95% C.I. 1.5-20.2). The presence of this allele in the recipient was not associated with hepatic vessel thrombosis.

Early identification of the Q506 allele in the donor would allow prophylactic anticoagulation following transplantation and may reduce the risk of hepatic vascular complications.

THE INFLUENCE OF DONOR/RECIPIENT HLA PHENOTYPE AND DEGREE OF HLA MISMATCH ON THE DEVELOPMENT OF TRANSPLANT ASSOCIATED CORONARY ARTERY DISEASE IN HEART TRANSPLANT PATIENTS.

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The development of transplant associated coronary artery disease (TxCAD) is the manifestation of chronic heart rejection. This condition is likely to be under the influence of immunological and non-immunological risk factors.

We examined 3 groups of patients: 'early' TxCAD (coronary artery disease which developed within 2 years of transplantation), 'late' TxCAD (3-14 years following transplantation) and 'No' TxCAD (no evidence at any angiogram). Between 1980 and 1994 534 patients met inclusion criteria for this study, namely yearly angiographic studies and HLA typing results. We undertook 2 separate analyses: (1) where there was a minimum of a 50% stenosis, and (2) where there was a minimum of 25% stenosis in at least a single vessel.

For both analyses we were unable to find any significant association between the number of HLA mismatches for Class I (HLA-A, -B) or class II (HLA-DR) and any of the 3 patient groups. we were also unable to idnetify any particular class I or II phenotype for recipient ao donor that exerted a protective or deleterious effect.

	AMM	BMM	DRMM	ABMM	ABDRMM
Early	1.37+/-0.08	1.55+/-0.06	1.43+/-0.07	2.93+/-0.11	4.31+/-0.15
Late	1.39+/-0.06	1.67+/-0.05	1.33+/-0.07	3.07+/-0.08	4.50+/-0.13
No CAD	1.31+/-0.67	1.55+/-0.04	1.34+/-0.05	2.86+/-0.07	4.21+/-0.09
p value	0.56	0.19	0.55	0.19	0.23

Mean mismatch +/- S.E

From these data it appears that the time of development of TxCAD is not significantly affected by the degree of HLA mismatch or by donor/recipient HLA phenotype. Possible explanations for this lack of association be discussed.

EFFICIENCY OF HEART AND LIVER RETRIEVAL

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The availability of transplantable organs is dependent both on the rate of referral of 'beating heart donors' and on the efficiency of organ retrieval. Statistics collected for the Human Organ Transplant Registry and published by UKTSSA for 1992 to 1994 were used to calculate the proportion of donors from whom these organs were retrieved and to estimate the potential number of additional organs that could have been retrieved. We observed that organ donor referral rates varied widely between regions (12.31 to 19.72 pmp pa). The overall efficiency of retrieval of kidneys was 97% with little variation between regions, but retrieval of livers ranged from 43 to 83% and hearts from 29 to 42%. The number of 'not-retrieved' livers and hearts was estimated using the average to calculate the minimum value (63.6% and 36.1% respectively) and the highest retrieving region as the maximum value (Anglia and Oxford for hearts and W.Midlands for livers).

Health Region [Catchment population]	Organ donors 1992-1994 [pmp pa]	% donors use for: Livers	Hearts	Estim not retrieved: [min-max] Livers	[min-max] Hearts
Northern & Yorks [6.38]	333 [17.40]	70.6	39.3	0 - 42	0 - 8
Trent [4.69]	214 [15.21]	60.7	36.4	6 - 48	0 - 11
Anglia & Oxford [5.63]	208 [12.31]	67.3	***41.8***	0 - 33	0
N. Thames [7.31]	302 [13.77]	69.5	32.1	0 - 41	12 - 29
S. Thames [6.31]	260 [13.73]	57.7	29.2	15 - 66	18 - 33
S. West & Wessex [5.80]	275 [15.80]	60.4	35.6	9 - 62	1 - 17
W Midlands [5.23]	225 [14.34]	***83.1***	36.9	0	0 - 11
N Western [7.30]	296 [13.52]	42.6	35.8	62 - 120	1 - 18
Wales [2.22]	122 [18.32]	68.8	36.1	0 - 17	0 - 7
Rep. Ireland [3.60]	213 [***19.72***]	57.3	34.7	14 - 55	3 - 15
N. Ireland [1.59]	81 [16.98]	65.4	40.7	0 - 14	0 - 1
Scotland [5.07]	274 [18.01]	66.1	38	0 - 47	0 - 10
Totals [61.13]	2803 [15.26]	***63.6***	***36.1***	106 - 545	35 - 160

Assuming there were no regional variations in donor eligibility criteria, these data suggested that an additional 106-545 livers and 35-160 hearts could have been retrieved during this period. Furthermore if all regions had achieved an organ donor referral rate equivalent to the highest (Rep. Ireland) an additional 813 donors could have yielded an extra 1577 kidneys assuming 97% efficiency, 517-676 livers and 293-340 hearts. We conclude that the shortfall in the supply of organs is due both to the non-referral of organ donors, and to the suboptimal retrieval of livers and hearts.

THE EFFECT OF THE EUROPEAN DONOR HOSPITAL EDUCATION PROGRAMME (EDHEP) ON ORGAN DONATION RATES: A PROSPECTIVE RANDOMISED STUDY IN 20 INTENSIVE CARE UNITS (ICUs) IN NORTH WEST ENGLAND

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The EDHEP workshops for ICU staff are designed to promote positive co-operative attitudes to cadaver organ donation and to improve intensivists' skills in requesting permission to donate from bereaved relatives. We present here the first prospective controlled trial of the effect of EDHEP day release courses on organ donation rates.

Twenty North West Regional hospitals ICUs were randomised to attend workshops (Group I) or not (Group II) between 1.2.95 and 31.12.95. Simultaneously staff in both Groups underwent repeated psychological evaluations of their attitudes and skills in organ donor situations. Donation rates were measured in Groups I and II, in other (non-involved) Regional Hospitals (Group III) and in UK hospitals outside the North West Region (Group IV). We compared organ donation rates during the study period with a similar 11 month period in 1994.

Results:

(Number of donors per study period)	**Group (n)**	**I (10)**	**II (10)**	**III (42)**	**IV (433)**
	1994	22	28	43	703
	1995	33	42	47	678
	Change	**+50%**	**+60%**	**+9.3%**	**-3.6%**

Conclusions: We report a substantial increase in organ donation in those ICUs whose staff attended EDHEP workshops, and also in "control, evaluated only" ICUs compared with those ICUs in the UK not involved in EDHEP. The rise in donation in "control" ICUs may be explained by increased awareness of need for donation induced by the evaluation process. We conclude that the EDHEP course is an effective way of increasing donation. Staff evaluation sessions alone raise awareness and are also productive.

SIMULTANEOUS BLOCKADE OF THE CD28 AND CD40 PATHWAYS SYNERGIZES TO PROMOTE LONG TERM SURVIVAL OF MURINE CARDIAC ALLOGRAFTS.

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We have previously shown that although blockade of either the B7/CD28 or the gp39/CD40 pathway can prolong murine cardiac allograft survival, neither uniformly produces complete immunologic unresponsiveness. The purpose of the current study was to investigate the effect of simultaneous blockade of both pathways on cardiac allograft survival.

C3H/HeJ recipients of BALB/c vascularized heterotopic cardiac allografts (n=7) were treated perioperatively with 4 doses of CTLA4-Ig (200 µg each) and 3 doses of MR1 (250 µg each), a hamster mAb specific for mouse gp39. Control recipients received 4 doses of CTLA4-Ig alone (n=12), 3 doses of MR1 alone (n=12), or no treatment (n=7). Although all of the treatment groups had prolonged survival of the cardiac allografts compared with untreated controls (CTLA4-Ig: median survival time [MST]=50 days; MR1: MST=70 days; CTLA4-Ig+MR1: MST >70 days; untreated controls: MST=12 days), histologic analysis of the hearts 50-75 days following transplantation demonstrated severe coronary arterial intimal thickening and fibrosis consistent with chronic rejection in both the CTLA4-Ig and the MR1 treated recipients. In distinct contrast, cardiac allografts from recipients treated with the combination therapy were essentially indistinguishable from normal heart, suggesting that blockade of both pathways synergizes to inhibit chronic allograft rejection.

Analysis of the allografts using RT-PCR at 8 days following transplantation showed that both Th1 and Th2 T-cell cytokine transcripts, T cell effector transcripts and macrophage effector transcripts are significantly diminished in the combination group as compared to recipients treated with CTLA4-Ig or MR1 alone. These results suggest that CTLA4-Ig and MR1 may synergize to prolong allograft survival by blocking both T cell activation and cognate T cell help for effector functions.

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ANGIOTENSINOGEN, ACE AND AT_1 RECEPTOR GENE POLYMORPHISMS AND THE DEVELOPMENT OF TRANSPLANTATION ASSOCIATED CORONARY ARTERY DISEASE

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We investigated the influence of genetic factors on the development of transplantation associated coronary artery disease (TxCAD). Polymorphisms in 3 genes of the renin-angiotensin system (RAS) (ACE, angiotensinogen and the ACE receptor AT_1) have previously been shown to be associated with an increased risk of ischaemic heart disease. We have used PCR amplification to genotype 80 heart transplant recipients and donors, 44 of whom developed TxCAD within 2 years of transplantation and 36 of whom remained disease free at two years.

No association was found with either the AT_1R C variant or the angiotensinogen M235T polymorphism with the development of TxCAD. Furthermore, no association was shown between the recipient ACE genotype and TxCAD. However, the donor population of the non-TxCAD group had a significantly ($p<0.05$) lower frequency of the D allele (0.35) than either the TxCAD group (0.54) or the general population (0.57). Thus, there was a negative association between the frequency of the I allele of the donor and the development of TxCAD. Other risk factors analysed included original heart disease of the recipient, cholesterol, LDL and HDL levels, donor and recipient age, recipient body mass index and number of acute rejection episodes. Of these factors, only the number of acute rejection episodes experienced within the first post-transplant year was found to be significantly associated with an increased risk of developing TxCAD ($p<0.05$).

We conclude that the local (tissue), rather than the circulating, RAS may be implicated in the pathogenesis of coronary artery disease.

THE EFFECT OF ANTI-CD2 MONOCLONAL ANTIBODY ON *IN VITRO* AND *IN VIVO* ALLOREACTIVITY

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CD2 is a cell-surface receptor involved in signal transduction and adhesion and plays an important co-stimulatory role in T cell activation. Monoclonal antibodies (mAbs) directed against CD2 may be of value, therefore, in preventing graft rejection and inducing transplant tolerance.

In this study, the ability of a mouse anti-rat CD2 mAb (OX-34) to inhibit *in vitro* and *in vivo* T cell alloreactivity was studied. Addition of OX-34 to cultures of purified rat CD4 T cells inhibited their ability to proliferate and produce IL-2 in a primary MLC. Supplementing the cultures with exogenous IL-2 restored T cell proliferation. *In vivo* treatment of DA ($RT1^a$) rats with OX-34 produced a dose dependent prolongation in the survival of fully allogeneic Lewis ($RT1^l$) heart grafts. Administration of OX-34 at the time of transplantation (5mg/kg days -1 and 0) led to long-term heart graft survival (MST >60 days versus MST 9 days in control animals). Lower doses of OX-34 (2.5mg/kg or 5mg/kg on day 0) increased graft survival only marginally (MST 11 and 13 days respectively). Interestingly all three doses of OX-34 tested gave similar levels of T cell depletion and modulation of CD2 on residual T cells. However, complete saturation of CD2 binding sites on residual T cells was only found with the highest dose of OX-34 suggesting that saturation is important for ensuring graft survival.

These results suggest that the CD2 molecule is an effective target for therapeutic antibodies designed to promote long-term allograft survival and we are currently exploring in more detail the mechanisms responsible.

T-CELL VACCINATION (TCV) PROLONGS HEART BUT NOT ISLET OR SKIN ALLOGRAFT SURVIVAL IN RATS

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Purpose: To prolong heart, islet and skin allograft survival by autologous TCV without immunosuppression. **Methods:** W/F rats were recipients and BN rats were donors. W/F vaccine donors were sensitized with BN skin grafts. Lymphocytes from draining lymph nodes were stimulated in culture for 48 hours, attenuated and injected into naive W/F rats on days -14, -7, +5, +12 [day 0: transplantation (tx)]. On day 0 the vaccinated rats received either a heterotopic heart, 2000 islets (diabetic recipients) or a skin graft from BN donors. **Results:** Graft Survival (GS): Table: Heart (beating), Islets (blood glucose< 200 mg/dl), Skin (intact)

Treatment	N	Graft Survival (days)	Median	p-values
Heart /None	6	7, 9, 9, 10, 11, 16	9.5	
Heart /TCV	13	2, 3, 3, 3, 9, 20, 29, 32, 35, 52, 64, 67, >183	29	p=0.3
Islets/None	5	1, 1, 4, 5, 5	4	
Islets/TCV	9	1, 1, 1, 1, 2, 2, 2, 2, 4	2	p=0.26
Skin/None	6	15, 17, 18, 19, 19, 21	18.5	
Skin/ TCV	5	18, 18, 18, 20, 20	18	p=0.65

Four TCV treated heart tx recipients had shorter GS than the shortest GS in controls (30% TCV treated, 0% controls, p=0.25). However, eight TCV treated heart tx recipients had longer GS than the longest GS in controls (61% TCV treated, 0% controls, p=0.02). **Conclusions:** This TCV protocol can either prolong or shorten (possible vaccine contamination with donor leukocytes) heart allo-GS but it did not prolong islet or skin allo-GS indicating that more potent protocols must be devised for the not immediately vascularized grafts.

DISSECTION OF CYCLOSPORIN-A SENSITIVE AND RESISTANCE T CELL ALLO-IMMUNITY

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Clinically, alloreactivity can be divided into Cyclosporin-A (CyA) sensitive and CyA resistant. This can be simulated *in vitro* by cell mediated lympholysis (CML) assay in the presence of excess IL-2 with/without various doses of CyA. We tested this in 8 individuals: haemodialysis (HD, n=3); continuous ambulatory peritoneal dialysis (CAPD, n=3) and normal healthy adults (n=2). Of these 6 were male and 2 female. An allogeneic one way mixed lymphocyte assay was set-up by incubating responder cells suspended in different concentrations of CyA (0 to 1500 ng/ml) with irradiated (3000 rad) stimulator cells in culture media with rIL-2 (25 units/ml). Targets were grown by 2μg/ml Phytohaemagglutinin (PHA) and rIL-2(20 units/ml). A non-radioactive Europium release assay was optimised and modified in our laboratory to perform the *in vitro* CML assay. T cell alloimmunity and autoimmunity was measured as % lysis. This was calculated for each target on a scale from 0% (spontaneous release) to 100% (maximum release). Specific radioimmuno-assay was used for confirming concentration of CyA. Each responder was cultured with irradiated stimulator cells from a randomly selected normal healthy HLA mismatched adult. At 7 days the responder cells were tested against three targets: the original stimulator; a third party, and the autologous target. Specific alloreactivity was negative to weak in 3 HD patients and positive in 5 others (CAPD and normal). In 2 cases cytotoxic T cell alloimmunity (TCA) was suppressed by low concentration of CyA (50-100) ng/ml. In 3 cases TCA was incompletely suppressed by low doses CyA and partially resistant to doses up to 1000 ng/ml. In one case, where TCA was suppressed by CyA, weak CyA resistant CTL activity appeared to be directed towards autologous blasts. In 5 of 8 cases evidence was obtained for CyA sensitive TCA. In 2 of 5 cases clear evidence of CyA resistant TCA and in 1 of 5 cases evidence for CyA resistance autoimmune CTL was obtained. This preliminary study allowed us to identify and quantitate CyA resistant and sensitive TCA in HD and CAPD patients prior to transplantation. Further studies (in progress) will allow us to identify the molecular targets associated with these different subsets of CTLs and their relevance to kidney transplant rejection.

TACROLIMUS (FK506) AS RESCUE THERAPY FOLLOWING CARDIAC TRANSPLANTATION

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BACKGROUND

Allograft dysfunction develops in a proportion of heart transplant recipients without significant cellular infiltrate in endomyocardial biopsies and with normal coronary arteries at angiography. The mechanisms responsible for this presentation are unclear and the prognosis is poor. We report encouraging experience with FK506 given with cyclophosphamide and prednisolone therapy in five heart transplant recipients with poor graft function with normal endomyocardial biopsies and coronary angiography.

METHODS

Five patients (4 males and 1 female; aged 46, 51, 52, 53 and 63 years) developed severe biventricular failure 1, 14, 15, 21, 36 months after orthotopic cardiac transplantation despite immunosuppression with cyclosporin A, Azathiprine, oral prednisolone, cyclophosphamide and intravenous methyl prednisolone therapy. Endomyocardial biopsies and coronary angiography was normal in each case. One patient required mechanical ventilation, inotropic support and intra aortic balloon counterpulsation. Tacrolimus was substituted for cyclosporin A and the dose adjusted to achieve trough serum levels of 5-15 ng/ml. Patients continued on cyclophosphamide 2 mg/kg/d and prednisolone 0.2 mg/kg/d.

RESULTS

Cardiac function and exercise capacity improved significantly in each case. Two patients developed cytomegalovirus infection (1 pneumonitis, 1 encephalitis) successfully treated with ganciclovir. No other adverse effects attributable to tacrolimus were observed. The patients remain well with no evidence of heart failure and with shortening fractions of 35% at 3, 8, 8, 8, and 9 months after commencing tacrolimus.

CONCLUSION

Tacrolimus should be considered as adjunctive therapy to conventional immunosuppression for heart transplant recipients with poor graft function in the absence of cellular rejection or coronary artery disease.

INCIDENCE OF REJECTION IN COMBINED LIVER-TOTAL BOWEL TRANSPLANTATION VS. TOTAL BOWEL TRANSPLANTATION ALONE: A PROSPECTIVE STUDY IN A PORCINE MODEL.

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It has been suggested from small animal studies that the liver graft may protect the simultaneously transplanted bowel. We studied in a preclinical pig model the addition of a liver graft to the transplanted bowel vs. solitary bowel Txs with regard to survival, rejection (Rej) and GVHD. **MATERIALS AND METHODS:** Yorkshire Landrace pigs underwent either combined liver-total bowel transplantation (LTBTX; Group 1) or solitary total bowel transplantation (TBTX; Group 2). In Group 1 (n=9), liver, small and large bowel were transplanted en bloc. In Group 2 (n=5), pigs underwent portally drained TBTX. In both groups iv FK506 (0.2 mg/kg/day) and iv prednisolone (2 mg/kg/day) were given for induction and maintenance therapy. Daily laboratory tests included liver function tests, amylase, IL2, IL6, IL7 and FK506 levels. Biopsies were obtained daily from the ileostomy to study the incidence of graft Rej. **RESULTS:** Pig survival (Kaplan-Meier) on postTX days 7, 10, 14, 21 and 28 was 100%, 78%, 22%, 11% and 0% in Group 1 vs. 100%, 100%, 100%, 100% and 60% in Group 2. There were no Rej-related deaths. Daily biopsies showed that Rej grades were significantly better in Group 2 on individual days; the difference did not reach significant over time ($p=0.2$). In both groups, vascular Rej was encountered in <10% of daily biopsies. Skin graft-vs-host-reaction (GVHR) was identified in 33% in Group 1 and 80% in Group 2. **CONCLUSIONS:** 1. FK506 was effective in preventing graft loss from Rej in both groups. 2. Pigs with LTBTX had a higher infection and surgical complication rate than TBTX pigs. 3. LTBTX has no immunologic advantage over TBTX.

SEMI-QUANTITATIVE POLYMERASE CHAIN REACTION (PCR) OF CYTOMEGALOVIRUS (CMV) DNA IN SERUM IDENTIFIES LIVER AND BONE MARROW TRANSPLANT RECIPIENTS AT RISK OF CMV-RELATED DISEASE

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CMV replication is common after liver or bone marrow transplantation and can be identified readily by PCR amplification. However, many infectious episodes are clinically silent and do not require treatment with ganciclovir which is marrow toxic. We have assessed a semi-quantitative PCR to determine whether patients at risk of symptomatic disease can be identified.

Serial samples (from time zero to 6 months) were screened from 32 liver graft and 17 bone marrow transplant recipients. Nine recipient/donor pairs were seronegative for CMV, in whom 48 urine and 79 serum samples were PCR negative. Forty patients were therefore at risk of CMV infection; 17/40 hosted active CMV infection as determined by PCR. Serum was PCR positive in 15/17, urine in 14/17; notably, 5/17 patients were PCR positive but negative for CMV by conventional techniques.

Semiquantitative PCR was achieved by amplifying 'wild type' sequences alongside a deleted, exogenous control. PCR products were quantified by an ABI 373A automatic DNA sequencer (Genescan 1.1 software).

10/15 patients seropositive by PCR had CMV DNA > 2.55×10^5 genome equivalents/ml; all 10 were symptomatic with pyrexia, hepatitis, retinitis or pneumonia. All 5 patients with CMV DNA below this threshold remained healthy ($p < 10^{-8}$). In contrast, quantitation of CMV DNA in urine was not discriminatory.

Semi-quantitative PCR of CMV DNA in serum is more sensitive than conventional tests and allows identification of patients at risk of symptomatic disease and hence targetting for anti-viral therapy.

THE MANAGEMENT OF CYTOMEGALOVIRUS INFECTION FOLLOWING CARDIAC TRANSPLANTATION

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Cytomegalovirus (CMV) is an important cause of morbidity and mortality after cardiac transplantation and may be associated with graft coronary disease and rejection. Our protocol includes CMV serology (IgG, IgM) of recipient and donor (D). In recipients of a CMV antibody mismatch (R- D+) hyper-immune immunoglobulin is prescribed as prophylaxis for four months. In the postoperative follow-up weekly CMV rapid antigenaemia test (RAT) is assessed to six weeks, fortnightly to three months and then six weekly until the end of the first year. At further follow-up, CMV RAT is assessed when symptoms indicate. We wish to determine whether routine screening dictates a change in the clinical management of CMV infection.

Since February 1992 120 patients have survived more than 30 days. Clinical follow-up ranged from 1 to 48 months with a median of 23 months. They are divided amongst four groups, - R+ D+ (29/120), R+ D- (39/120), R- D+ (23/120), R- D- (29/120). Episodes of CMV antigenaemia were registered in 55 patients (55/120, 42%), none in the R- D- group. CMV antigenaemia occurred in 58.6% R+ D+ (17/29), 59% R+ D- (23/39) and 65.2% of the mismatch R- D+ (15/23). 24/55 (43.6%) required Gancyclovir treatment for symptomatic CMV infection, 6/29 (21%) R+ D+, 8/39 (20%) R+ D- and 10/23 (43%) R- D+. Patients who were CMV antigen positive without symptoms 31/55 (56.4%) were not treated with Gancyclovir and remained well. No patients died of CMV disease.

Regular screening for CMV is unnecessary in the R- D- group. The group most at risk is the mis-matched R- D+ who received prophylaxis. However, of those who became CMV RAT positive 66% (10/15) developed symptoms which responded to treatment. Overall, only the patients who were symptomatic (CMV disease) were actively treated. We suggest that the important factor in the successful management of CMV disease is a high index of clinical suspicion and is not helped by routine monitoring of patient CMV status.

EFFECT OF DONOR-SPECIFIC BONE MARROW TRANSFUSION (BMT): A COMPARATIVE STUDY BETWEEN KIDNEY TRANSPLANTATION (KTX) AND INTESTINAL TRANSPLANTATION (ITX)

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Immunomodulatory strategies may help to reduce the need for nonspecific immunosuppression after organ transplantation (Tx). This is particularly important in intestinal Tx (ITx) where rejection and sepsis remain major difficulties. Donor-specific bone marrow transfusion (BMT) induces tolerance in rodent models of kidney Tx (KTx). Using a preclinical model, we prospectively studied the effect of BMT -10^7 or 10^8 unpurified BM cells/kg-on rejection, GVHD, infection, and survival after KTx (n=42) and ITx (n=44) in FK506-treated or untreated Landrace pigs. Reactive donor/recipient pairs (CPM>10.000) were used. After KTx, there was a tendency toward delayed rejection in BMT vs controls and toward less rejection in BMT+FK506 vs FK506 controls (-33%;p=.2). Although not significant, survival was prolonged in BMT pigs (9.5 vs 8 days in controls) and in BMT+FK506 pigs (20.5 vs 18.5 days in FK506 controls). In FK506 pigs, BMT caused 33% GVHD and increased infection rate (+30%;p=.07). After ITx, BMT caused a marked tendency toward more rejection, particularly pronounced in BMT+FK506 vs FK506 controls (+38%;p=.09), and this was associated with reduced survival (21 days vs 37 days, respectively, p=.1). Finally, groupwise comparison showed an order of susceptibility to GVHD and infection as follows: BMT+FK506 > FK506 > BMT > controls. No difference was seen between 2 BM dosages -10^7 or 10^8 cells/kg-after KTx or ITx.

CONCLUSIONS:

The effect of BMT is organ-specific: After KTx, BMT tended to reduce rejection and prolong survival whereas the opposite - accelerated rejection and reduced survival - was seen after ITx. BMT increased susceptibility to GVHD and infection after both KTx and ITx. Immune strategies that are potentially beneficial in KTx may not necessarily apply to highly immunogenic ITx. Before being used clinically, BMT needs to be refined in order to increase its tolerogenic potential without causing GVHD.

ALLOGRAFT REJECTION BY MICE LACKING INDUCIBLE NITRIC OXIDE SYNTHASE

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Nitric oxide (NO) is an important immunoregulatory and inflammatory mediator. It is both cytostatic and cytotoxic at high concentrations and generated in large amounts by activated macrophages/monocytes which express inducible NO synthase (iNOS). To evaluate the role of NO as an effector molecule during allograft rejection, mutant mice (129 x MF1) which lack iNOS were used.

Homozygous iNOS deficient mice show, when compared with their heterozygous counterparts, a reduced non-specific inflammatory response when challenged with carrageenan. Moreover, *in vitro* analysis confirmed that peritoneal macrophages from iNOS deficient mice did not contain detectable iNOS protein and, in contrast to heterozygotes, were unable to produce increased levels of NO following activation. Nevertheless, iNOS deficient mice showed no impairment in their ability to reject Balb/C skin allografts when compared with heterozygote control animals (MST 10, range 8-15 days versus MST 8, range 7-13 days respectively). To determine whether NO is a critical effector molecule during DTH in the absence of cytotoxic effector cells, graft recipients were treated with a depleting anti-CD8 mAb on days 0, 3, 7 and 10 following skin transplantation. Again the kinetics of graft rejection were similar in homozygous iNOS deficient and control heterozygote recipients (MST 10, range 9-15 days versus MST 10, range 9-13 days respectively).

These results suggest that NO does not play a critical role in the effector mechanisms responsible for rejection of skin allografts. Because of considerable redundancy in the inflammatory molecules responsible for tissue destruction during DTH responses, inhibition of iNOS is, by itself, unlikely to be of therapeutic importance in preventing graft rejection.

COMPARISON OF THE IMMUNOMODULATORY PROPERTIES OF HEPARIN AND A NON-ANTICOAGULANT HEPARIN DERIVATIVE.

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Heparin has been shown to have immunosuppressive activity in animal models of allograft rejection. The mechanism by which heparin produces this effect is unclear. This group has recently described the observation that heparin antagonises the proinflammatory cytokine Interferon-γ (IFN-γ) and prevents upregulated expression of ICAM-1 and the induction of class II MHC antigens by IFN-γ stimulated endothelial cells. In this study we compared the immunomodulatory activity of heparin with that of a non-anticoagulant derivative termed 'Natrium' (Leo Pharmaceutical Products), produced from heparin by chemical oxidation and reduction.

Radio-ligand binding assays were used to quantify the total binding of IFN-γ to cultured human umbilical vein endothelial cells (HUVEC). Addition of either heparin or Natrium at a concentration of 100μg/ml was found to significantly inhibit the binding of IFN-γ (in both cases $p<0.02$).

It was found that both heparin and Natrium were equally effective at inhibiting the induction of class II MHC antigens by IFN-γ stimulated HUVEC. The immunogenicity of endothelial cells was assessed by co-culture of IFN-γ stimulated HUVEC with allogeneic CD4+ T lymphocytes. It was found that the lymphoproliferative response produced by endothelial cells which had been stimulated with IFN-γ in the presence of heparin or Natrium was significantly smaller than that produced by cells which had been stimulated in a drug-free system ($p<0.05$).

Results from this study indicate that the immunosuppressive activity of heparin may be related to its ability to prevent IFN-γ from binding to target cells within an allograft and enhancing their immunogenicity. Anticoagulant activity appears to be irrelevant to this effect.

THE USE OF ELISA AND CYTOTOXICITY SCREENING FOR THE DETECTION OF HLA SPECIFIC ANTIBODIES RELEVANT TO TRANSPLANT OUTCOME.

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The aim of this retrospective study was to correlate the presence of HLA specific antibodies detected using an ELISA technique, as compared with complement dependent cytotoxicity (CDC), with transplant outcome. The ELISA kit PRA-STAT (NEXtran) was used for the detection and definition of HLA Class I specific IgG antibodies in 295 sera, taken pre- and post-transplant, from 95 patients who received 98 renal transplants between 1987 and 1994. The patients were divided into two groups matched for age and HLA mismatching: Group I - transplant failed within first month, 42 primary, 7 retransplants; Group II - successful transplant, 40 primary, 10 retransplants.

The concordance between PRA-STAT and CDC for the detection of HLA specific antibodies was 87.1% (257/295). For 4 patients (7 sera) donor HLA specific IgG antibodies were detected only by PRA-STAT (2 pre-, 2 post-transplant) and all 4 transplants failed. For 6 patients (8 sera) donor HLA specific antibodies were detected only by CDC (2 pre- 4 post-transplant) and all 6 Tpx failed. In the 2 patients for whom the antibodies were detected by CDC pre-transplant they were shown to be IgM alloantibodies.

In conclusion, PRA-STAT can detect HLA specific IgG antibodies relevant to transplant outcome that are not detected by CDC. However it cannot detect IgM alloantibodies that we have also shown to be important.

SEMI-ALLOGENEIC (F1) VERSUS FULLY ALLOGENEIC BLOOD TRANSFUSIONS: DIFFERENCES IN THEIR ABILITY TO INDUCE SPECIFIC IMMUNOLOGICAL UNRESPONSIVENESS

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The beneficial effect on graft survival achieved by pretransplant blood transfusions is well established. However the type of MHC mismatch between transfusion donor and recipient seems to play a role in determining the outcome.

The hypothesis that this sharing of MHC antigens is correlated with the level of sensitization or tolerization was studied in mice (C3H/He) by pretreatment with semi-allogeneic ((C3HxC57BL/10)F1) or with fully allogeneic (C57BL/10) whole blood transfusions.

In vitro limiting dilution analysis (LDA) for donor-specific T helper (HTLp) and cytotoxic T lymphocyte precursors (CTLp) performed on splenocytes isolated from transfused recipients 2 or 4 weeks after transfusion, showed that both the duration and magnitude of the response was reduced after a semi-allogeneic compared to a fully allogeneic transfusion. Interestingly, after a semi-allogeneic transfusion both HTLp and CTLp frequencies had returned to naive levels 4 weeks after transfusion whereas after infusion of fully allogeneic blood they still remained significantly elevated at this time point. When a fully allogeneic heart (C57BL/10) was transplanted 4 weeks after transfusion a (small but) significant improvement in graft prolongation ($p<0.01$) was observed following pretreatment with a semi-allogeneic transfusion compared to that obtained after fully allogeneic transfusion (median survival time (MST) after semiallogeneic transfusion 29 days, after fully allogeneic MST: 12 days).

In addition, the prolonged, (7 days), persistence of donor derived MHC class II^+ cells in the recipient and reduced levels of anti donor MHC class I specific antibody formation was observed after transfusion of semi-allogeneic blood compared to these responses after fully allogeneic blood transfusion.

These results demonstrate that pretreatment with a semi-allogeneic blood transfusion is more "tolerizing" and less sensitizing than pretreatment with a fully allogeneic blood transfusion.

These findings may be explained by the sharing of MHC antigens between recipient and transfusion donor.

THE ROLE OF APOPTOSIS IN ANTI-TCR INDUCED TRANSPLANT TOLERANCE

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The mechanism of induction of transplantation tolerance by monoclonal antibodies directed at the α/βTCR is unclear. R73, a mouse IgG1 mAb which binds to the α/β heterodimer of the rat T-cell receptor, has been shown to induce T-cell activation *in vitro* when immobilised and T cell depletion *in vivo*. In this study we have shown that a two day pre-operative course of R73 induces tolerance in DA ($RT1^a$) recipient rats to Lewis ($RT1^l$) cardiac allografts (MST >100 days). Animals treated with IL-12 post transplant, however, rejected their grafts (MST 22 days). A potential mechanism for the tolerance seen in this model is TCR mediated apoptosis. LNC prepared from R73 treated allograft recipient on days 4,7,21 and 100 post-transplant, and from untreated graft recipients and naive DA rats were stained with propidium iodide and analysed for PI fluorescence levels by flow cytometry. Cells displaying hypodiploid levels of fluorescence were regarded as apoptotic. Higher levels of apoptotic cells were found in R73 treated graft recipients at days 7 (20%) and 21 (35%) compared with naive (11%) and rejecting controls (10% at day 7).

These findings suggest that R73 induced transplantation tolerance is maintained by apoptosis of alloreactive T cells and that this can be abrogated by IL-12 possibly by inhibiting apoptosis in stimulated T cells.

ANTAGONISM OF INTERFERON-γ BY BLOCKADE OF ITS INTERACTION WITH ENDOTHELIAL GLYCOSAMINOGLYCANS: A NOVEL STRATEGY FOR IMMUNOSUPPRESSION?

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Interferon-γ (IFN-γ) plays a central role in inflammation. Specific effects include augmentation of endothelial immunogenicity by upregulation of class I MHC antigens and adhesion molecules, and by induction of class II MHC antigens and chemokines; these are all deleterious to allograft survival. Heparin, a heavily-sulphated glycosaminoglycan (GAG), can antagonise the biological activity of IFN-γ. In this study we have investigated the molecular basis for this effect.

Radioligand binding assays demonstrated that total binding of ^{125}I-labelled IFN-γ to the EAhy.926 endothelial hybridoma cell line was reduced in the presence of heparin at 125μg/ml ($p<0.001$); the structurally dissimilar GAG chondroitin sulphate had no effect. This suggests that soluble heparin competes to bind IFN-γ with heparin-like, cell-surface GAGs.

Treatment of EAhy.926 cells with 25mM chlorate was non-toxic but inhibited the incorporation of [^{35}S]-sulphate into GAG chains and the expression of class II MHC antigens by IFN-γ stimulated cells ($p<0.001$); this was paralleled by a small decrease in the capacity of chlorate-treated cells to bind [^{125}I]-IFN-γ ($p<0.01$). This indicates that sulphated, negatively charged regions on cell-surface GAGs are involved in both the sequestration and biological activity of IFN-γ.

A cationic 10 amino acid peptide sequence (-AKTGKRKRSG-) from the C-terminal region of IFN-γ was also found to competitively reduce binding of [^{125}I]-IFN-γ ($p<0.001$) to the cell line. This provides evidence for interaction between a specific region of the cytokine molecule and cell-surface GAGs.

These results indicate that IFN-γ is specifically sequestered onto the surface of endothelial cells by binding to sulphated, heparin-like domains on GAG molecules such as heparan sulphate. This interaction appears to be essential for optimal cytokine activity and provides a potential target for clinical immune modulation.

CYTOKINE GENE POLYMORPHISMS PREDICT HEART TRANSPLANT REJECTION

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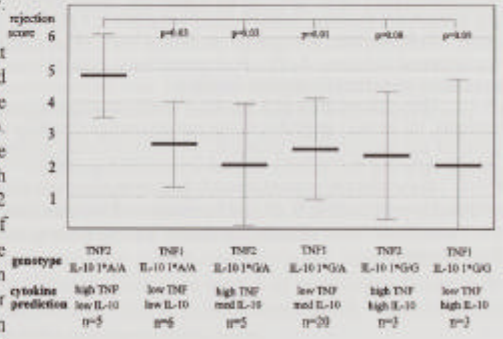
We have analysed promoter region polymorphisms in the genes coding for the pro-inflammatory cytokine TNF-α and the regulatory cytokine IL-10, which has been shown to downregulate Th1 driven inflammatory responses, in order to study their effect on heart transplant rejection.

Previous *in vitro* studies have shown that a G (TNF1) to A (TNF2) polymorphism at position -308 in the TNFA gene is associated with a 6-7 fold increase in transcription. We have also recently described a G to A polymorphism at position -1117 in the IL-10 gene which leads to differential IL-10 production following *in vitro* stimulation of T cells. We have analysed these polymorphisms in heart transplant recipients to see whether cytokine genotypes can predict transplant outcome directly.

Forty two heart transplant recipients were grouped according to their cytokine genotype (see figure). Patients who would be predicted to produce high levels of TNF-α (TNF2 positive) and low levels of IL-10 (IL-10 1*A/A) have significantly more rejection episodes graded equal to or greater than 2 (rejection score) in the first three months post transplant.



TNF-α and IL-10 genotypes predict transplant rejection

In this preliminary study we have shown that cytokine genotypes may be used to predict transplant outcome. However we have only studied IL-10 and TNF-α. The description of polymorphism in other cytokine genes that play a role in transplant rejection, such as IFN-γ, IL-4 and TGF-β, may allow the accurate prediction of a patients cytokine profile, and hence their clinical course, through simple DNA based pre-transplant tests.

PANCREATIC ISLET AUTOTRANSPLANTATION COMBINED WITH TOTAL PANCREATECTOMY FOR THE TREATMENT OF CHRONIC PANCREATITIS

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Islet autotransplantation offers the potential for preventing the surgically induced diabetes that is an inevitable consequence of total pancreatectomy. This paper describes the first islet autotransplant programme in the United Kingdom and the first series in the world to use the spleen as a site for the islet graft.

Between September 1994 and August 1995, 6 patients (median age 48.5 years) underwent total pancreatectomy for chronic pancreatitis. The spleen was preserved in 4 patients. Immediately after resection, the pancreas was transferred to the laboratory where the islets of Langerhans were isolated using a combination of collagenase digestion and density gradient purification. The islets were then transported back to theatre. In all 6 patients, islets were embolized into the liver via the portal vein (median transplanted volume= 8.5 ml) and in addition 3 patients had islets embolized into the splenic sinusoids via a short gastric vein (median transplanted volume= 4 ml). Postoperatively, endogenous insulin production was measured by serum C-peptide levels.

One patient died of a stroke 4 weeks postoperatively. Of the 5 surviving patients, all have C-peptides in the normal range (median = 2.94 ng/ml). All 5 patients are pain-free. In addition, we have recently performed a transplant into the spleen only and this patient is now insulin-independent.

We conclude that when total pancreatectomy is performed for the treatment of chronic pancreatitis, it should be combined with an islet autotransplant. By using the intrasplenic route of transplantation, it is possible to minimise the risk of portal hypertension.

LIVER TRANSPLANTATION IN CHILDREN UNDER 1 YEAR OF AGE

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Hepatic transplantation in children under 1 year represents a considerable surgical, anaesthetic and medical challenge. We report a series of 41 liver transplants performed between January 1989 and December 1995 in 35 children younger than 1 year with a mean weight of 5.7 kg (range 2.6-10).

The commonest indication was biliary atresia (18 out of 22 transplants for chronic liver disease). Twelve infants were transplanted for fulminant hepatic failure (FHF) due to nonAnonB hepatitis (4), neonatal haemochromatosis (3), tyrosinaemia (1), giant cell hepatitis (1), congenital hepatitis B (1) and drug induced hepatitis (2). The standard immunosuppression regimen was Cyclosporine A based triple therapy, rejection being treated with pulsed high dose steroid therapy.

In 36 cases (88%) a reduced size graft was used: right lobe (2), left lobe (9), left lateral segments (22), monosegment III (3). One graft was from a split liver and 2 from living related donors. Arterial reconstruction was achieved using an iliac arterial conduit from the infrarenal aorta in 85% of the cases, and a Roux loop was used for bile duct reconstruction in 95%.

The main post-operative complication was infection and almost all the patients in the neonatal and retransplant groups experienced at least one serious infectious episode. This is usually chest or line related bacterial infections initially, with viral infections such as CMV and EBV occurring later. Rejection is usually not a major problem in this age group. The following vascular complications were observed; hepatic artery thrombosis 3 (7%), portal vein thrombosis 2 (5%) and venous outflow obstruction 1 (2.5%). Small bowel obstruction, perforation and bile leaks occurred in almost 25% of the patients.

Currently 78% of the patients are alive with functioning grafts (mean follow up of 17 months (range 1-54). Eight (22%) children have died; 5 of multi-organ failure following poor graft function, 2 of infection and 1 due to an intra-cranial bleed. Survival in FHF and the retransplant group (both 50%) was significantly lower.

From our experience we conclude that liver transplantation can produce good short and long term results in children less than 1 year of age who would otherwise die of their severe liver disease.

GLUCOSE INTOLERANCE AND SENSITIVITY TO INSULIN IN LIVER TRANSPLANT RECIPIENTS RECEIVING FK506 OR CYA IMMUNOSUPPRESSION

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The aim of this comparative study was to evaluate derangements in glucose metabolism and insulin action in liver transplant recipients under FK506 and CyA immunosuppression.
Responses to 75 g oral glucose tolerance tests (OGTTs) and low-dose incremental insulin infusions (0.005-0.05 U/kg/h) were examined 12 months after transplantation in 2 matched groups of stable patients receiving FK506 (n=8) or CyA (n=8). Steroids were withdrawn 3 months postoperatively. Eight healthy volunteers acted as the controls. In the fasting state glucose concentrations were similar in the three groups (P>0.1), but insulin concentrations in both transplant groups were significantly elevated relative to the controls (P=0.01 and P=0.005, CyA and FK506 respectively). During OGTTs overall glucose concentrations in both transplant groups were significantly higher than in the controls (P<0.0001 for CyA and P=0.0005 for FK506). A similar trend was observed for insulin response with significant overall hyperinsulinaemia in both groups (P<0.0001 for CyA and FK506). Glucose and insulin concentrations did not differ significantly between FK506 and CyA patients (P>0.1). OGTTs were diagnostic of impaired glucose tolerance in 4 patients receiving CyA and of diabetes mellitus in one FK506-treated subject (WHO criteria). Relative to the controls insulin-glucose regression lines for both transplant groups were displaced significantly to the right (P<0.0001 for CyA and P<0.005 for FK506), suggesting resistance to insulin action on glucose metabolism. CyA group regression line was also displaced significantly to the right of that for FK506 group (P<0.001) implying greater impairment of insulin action in CyA-treated patients.
Results of this study demonstrate that derangement of glucose metabolism after liver transplantation is associated with resistance to insulin. This effect is steroid-independent and appears to be more pronounced with CyA than FK506 immunosuppression.

CHANGES IN GLUCOSE METABOLISM SENSITIVITY TO INSULIN IN FK506-TREATED LIVER TRANSPLANT RECIPIENTS

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It has been suggested that resistance to insulin may be responsible for FK506-associated glucose intolerance. In this study we examined the sensitivity of glucose metabolism to insulin in FK506-treated liver transplant recipients during the first year after transplantation. Serial low-dose incremental insulin infusions (0.005-0.05 U/kg/h) were performed in 8 patients at 2, 6 and 12 months postoperatively. 8 matched healthy subjects acted as the control group. In addition to FK506 all patients also received tapering dose of steroids which were withdrawn after 3 months. The mean daily FK506 dose decreased during the study (0.11, 0.08 and 0.06 mg/kg; 2, 6 and 12 months) but the overall difference fell short of statistical significance level (P=0.06). With insulin infusion there were highly significant negative correlations between plasma insulin (log) and blood glucose concentrations in the transplant group on all 3 occasions, and in the control subjects. 2 months postoperatively regression line for the transplant group was significantly displaced to the right of that for the control group (P<0.0001) suggesting resistance of glucose metabolism to insulin. Follow-up infusions demonstrated sustained improvement in patients response to insulin with a significant shift to the left of the regression line between 2 and 12 months (P<0.001). However, regression lines at 6 and 12 months were still significantly displaced to the right relative to the control group (P<0.0001 and P<0.005 respectively) indicating persistence of insulin resistance in transplant recipients.
This study shows that FK506-treated liver transplant recipients are resistant to the action of insulin on glucose metabolism. The sensitivity to insulin improves with time after transplantation, possibly as the result of steroid withdrawal and reduction in FK506 dosage.

THE "ASYSTOLIC DONOR SYNDROME": TRANSAMINITIS AND THROMBOCYTOPOENIA FOLLOWING NON-HEART-BEATING RENAL TRANSPLANTATION

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We report a characteristic syndrome of elevated alanine aminotransferase (ALT) and thrombocytopoenia in recipients of kidneys from non-heart-beating (NHB) donors.

Data from 38 NHB transplants were examined, transplanted from 1988-1994. All recipients received prednisolone, azathioprine, cyclosporin, and prophylactic ALG. Daily records of ALT and platelet counts were correlated with the length of delayed primary function and graft survival. Results were compared to two control groups of heart-beating (HB) grafts, treated with (n=32) or without (n=32) ALG.

ALT was elevated in 84% of NHB recipients, peaking at two days (172 ± 20u/l; NR<35 u/l; p<0.001), and normalising by five days post-transplantation. In contrast, ALT was rarely elevated in control groups (peak 41±6 and 44±7 respectively; both NS). Elevated ALT predicted delayed graft function within all three groups, but particularly in NHB donors. Especially high values were found in 9/38 NHB donors requiring graft nephrectomy (240±40; p<0.01).

Thrombocytopoenia typically occurred with nadir day five, and recovery by day 14. Mean platelet count in NHB donors was 113±10 $x10^9$/l, compared to 128±9 in HB donors receiving ALG, and 164±9 in HB donors without ALG (p=NS; p<0.05 respectively). The nine NHB recipients requiring nephrectomy had nadir 80±11 (p<0.05).

We propose that the observed increase in ALT results from enzyme release by damaged renal tissue, and that the thrombocytopoenia is caused in part by intragraft platelet consumption. These changes may be of predictive value regarding graft management and prognosis.

RANDOMISED CONTROLLED TRIAL OF THREE NEEDLE SIZES FOR RENAL TRANSPLANT BIOPSY

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The size and quality of the biopsy material are important factors in the interpretation of renal transplant histopathology. Needle core biopsy has been shown to be safe and effective but a number of different needle sizes are available. The aim of this study was to examine the success and complication rate of three different needle sizes for renal transplant biopsy.

A consecutive series of 100 renal transplant biopsies performed under ultrasound guidance were studied. Patients were randomised to undergo biopsy using either a 14, 16 or 18 gauge 'Bioptycut' needle. All histological material was examined by the same consultant histopathologist. The length and diameter of each biopsy was measured and the presence/absence of renal tissue, renal cortex and medulla and the number of glomeruli were recorded. The pathologist scored the diagnostic value of each specimen and the ease of use of each needle was graded by the operator. Post-biopsy pain was assessed using verbal response and linear analogue scales.

14G biopsy cores (n=33) were longer than both 16G (n=33) and 18G (n=34) cores (p<0.05) and contained more glomeruli (mean number for 14, 16 and 18G = 14, 11 and 9 respectively). Scores for diagnostic usefulness were higher for 14G v 16G (p=0.04), 14G v 18G (p=0.001) and 16G v 18G (p=0.018). There were no significant differences in the ease of use between the 14G, 16G and 18G needles. Post-biopsy pain levels were not different between the 3 groups using verbal response data but more pain was recorded for 14G v 16G needles using a linear analogue scale (p=0.025). Macroscopic haematuria occurred in 8 patients (no significant differences between the 3 groups) and there were no other complications.

All 3 needle sizes are safe for use in renal transplant biopsy but the larger needles provide more tissue, more glomeruli and are more useful diagnostically. A 14G needle may be associated with more pain and the 16G needle would appear to offer the best compromise between diagnostic usefulness and patient acceptability.

THROMBOCYTOSIS IN PANCREATICO-RENAL TRANSPLANT RECIPIENTS

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Pancreas transplant recipients have an increased risk of post-operative thrombosis involving coronary, cerebral, retinal and graft vessels. Furthermore, fine control of blood sugar by CSI infusion in non-transplanted diabetics has been associated with initial deterioration in retinopathy (Kroc Collaborative Study, N. Eng. J. Med 1984, 3311, 365-372). Could euglycaemia in angiopathic diabetics alter platelet numbers or function and thereby raise the risk of thrombosis?

Method: Platelet counts were performed in the following patient groups: **Group 1 (n=45) euglycaemic pancreaticorenal transplants; Group 2 (n=23) renal transplant alone in diabetics; Group 3 (n=25) renal transplant in non-diabetics; and Group 4 (n=26) major surgery in diabetics.**

Results: (censored for pancreas transplant failure): Platelet count $x10^{-3}/mm^3$

	Day 0	Day 1	Day 7	Day 14	Day 28	6 wks	3 mths	6mths	12 mths
Group 1	314	239	326	457	410	381	320	292	285
Group 2	289	238	268*	395	288**	276**	301	274	248
Group 3	216***	180**	312	294	294***	266**	302	250	262
Group 4	282	296*	321	412	412	257*	275	265	289

* $p < 0.05$
** $p < 0.01$
*** $p < 0.0001$

Two-tailed Mann Whitney Test comparing Group 1 with other groups.

Conclusion: Euglycaemic pancreas graft recipients exhibit a marked relative thrombocytosis which persists longer (up to 6 weeks) than in other groups studied. Routine Aspirin prophylaxis could well reduce the incidence of post-operative thrombosis in these patients.

THE TRANSPLANT LOTTERY - AN UNFAIR GAME FOR ASIAN PATIENTS

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Our unit serves a large community originating from the Indian subcontinent. This significantly affects our renal transplant waiting list (RTWL) on which 25% of listed patients are of Asian descent. By contrast, Asian patients have accounted for <12% of renal transplant recipients in any year. Several factors favour this imbalance.

Firstly, 44% of Asian RTWL patients have blood group B found only in 8.9% of UK donors. Secondly, significant heterogenicity exists, particularly at DR and B loci, between HLA profiles of Asian patients and those encountered in the donor pool. Thirdly, during the past decade <1% of local donors have been Asian individuals. Surprisingly, despite more pregnancies, female Asian RTWL patients do not appear more prejudiced by high sensitisation than Caucasian counterparts.

Whilst only limited numbers of Asian patients have been transplanted/year the number joining the list has grown by 24%/year – four times the growth of the total RTWL. Hence, if present circumstances persist approximately 200 Asian patients (50% of the list) may be listed by the year 2000 – many with little chance of receiving a kidney. This increasing imbalance between recruitment and transplantation has serious ethical and economic implications but could be eased by reducing the stringency of HLA matching. However, it can only be fully redressed when more donors originate from the Asian population. This will require the education and cooperation of the whole Asian community and hopefully the Fatwa on donation, recently negotiated by our Transplant Co–ordinators, will give impetus to this process.

EVALUATION OF THE EFFECTIVENESS OF TRANSPLANT PRESERVATION SOLUTIONS IN PREVENTION OF CELL SWELLING BY USE OF A NEW CELLULAR MODEL TO SIMULATE WARM ISCHAEMIA AND REPERFUSION

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During both cold and warm ischaemia the Na/K pump is not operating and the resultant ionic shift causes the cells to swell. Transplant preservation fluids act to limit such swelling by osmotic retention of fluids outside the cells. These fluids are also required to maintain the cell composition in such a way as to prevent swelling at reperfusion. We have developed a model that facilitates the measurement of cell volume in real time. Experiments are carried out at 37°C and make use of strophanthidin (rather than lack of oxygen) to block selectively, the Na/K pump, to provide a simulation of warm ischaemia.

The kidneys of anaesthetized NZW rabbits (1.4-2.0kg) were flushed with Phosphate Buffered Sucrose (PBS140) or UW and stored at 4°C doe 0-4 hours. Isolated proximal tubules were set up unperfused on micropipettes and bathed in oxygenated physiological saline (containing NaCl 114, $NaHCO_3$ 25, K_2HPO_4 2.5, $MgSO_4$ 1.2, $CaCl_2$ 2.0, glucose 5.5, alanine 6.0, Na lactate 4.0 and Na citrate 1.0 mmol/l, pH 7.4) at 37°C for 15 minutes to equilibrate cell volume. The bathing fluid was then exchanged for 10^{-3} molar strophanthidin, dissolved in physiological saline, PBS140 or UW for 35 minutes and then returned to physiological saline for a further 20 minutes. Outside tubule diameter (μm) was measured at 5 minute intervals and is shown as mean + SEM (n=6) for the end of each of the three periods.

Storage solution	Start diameter (in saline)	simulated warm isch. (10^{-3} M strophanthidin)	simulated reperfusion (in saline)
PBS	33.2 ± 1.2	42.8 ± 2.8 (in saline)	34.4 ± 1.8 μm
PBS	33.4 ± 1.2	31.4 ± 1.4 (in PBS)	35.0 ± 1.6 μm
UW	33.7 ± 1.2	30.5 ± 1.4 (in UW)	34.9 ± 2.0 μm

Blockade of the Na/K pump with strophanthidin at 37°C caused the cells to swell by approximately 66%. Both UW and PBS140 provided effective protection against such swelling. We are using this model to measure the efficacy of various preservation solutions in preventing cellular swelling.

EURO-COLLINS AND HYPEROSMOLAR CITRATE DO NOT PREVENT CELLULAR SWELLING IN PRESERVED KIDNEY TUBULES: DEMONSTRATION USING A SIMULATED WARM ISCHAEMIA MODEL

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Using simulated warm ischaemia in a cellular model (by use of strophanthidin to inhibit selectively the Na/K pump) we compared the relative efficacy of Euro-Collins (EC), Hyperosmolar citrate (HOC), University of Wisconsin Solution (UW) and Phosphate buffered sucrose (PBS140) in the prevention of cell swelling in preserved kidney tubules.

Kidneys of anaesthetized NZW rabbits (1.4-2.4kg) were flushed with and stored in test preservation solution for up to 72 hours. Isolated proximal tubule segments were then set up unperfused on micropipettes and bathed in oxygenated physiological saline at 37°C for 15 minutes to equilibrate cell volume. The bathing fluid was then exchanged for 10^{-3} molar strophanthidin, dissolved in the test solution for 35 minutes and then returned to physiological saline for 20 minutes. Outside tubule diameter (μm) was measured at 5 minute intervals and is shown as mean±SEM (n=6) for the end of each of the three periods after 0-4 hour hypothermic storage.

Test solution	Starting diameter (in saline)	Simulated warm isch. (10^{-3}M strophanthidin)	Simulated reperfusion (in saline)
EC	36.7 ± 2.5	46.4 ± 1.9	42.0 ± 3.0 μm
HOC	35.2 ± 1.0	36.1 ± 1.1	41.1 ± 1.0 μm
UW	33.7 ± 1.2	30.5 ± 1.4	34.9 ± 2.0 μm
PBS140	33.4 ± 1.2	31.4 ± 1.4	35.0 ± 1.6 μm

Euro-Collins caused marked cell swelling during simulated warm ischaemia recovering partially upon reperfusion. With HOC, although swelling was not significant during warm ischaemia the cells became swollen at reperfusion. Both UW and PBS140 caused a reduction in cell volume during warm ischaemia recovering to their original volume at reperfusion. After 72 hours cold storage, similar changes to those shown above were seen with EC, PBS140 and UW (results will be presented). Cells stored in HOC, exhibited a larger starting diameter which increased further at reperfusion.

These observations may explain improved clinical results with UW and PBS140

INTRATUBULAR T LYMPHOCYTE PROLIFERATION AND CYTOTOXICITY IN RENAL ALLOGRAFT REJECTION

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The precise role of lymphoid cell invasion of tubules during renal allograft rejection remains undefined. One may speculate that the cytokine-rich microenvironment within an actively-rejecting graft would allow proliferation and activation of intratubular cytotoxic T lymphocytes, resulting in tubular cell damage.

This hypothesis requires the demonstration in situ of actively-proliferating, intratubular T lymphocytes (ITL). Thus, we have developed, and applied, a double immunohistochemical labelling procedure in paraffin-embedded renal tissue, in which CD3 was used as the T cell marker and Ki67 as the nuclear, cell proliferation-associated antigen. Proliferating and quiescent T cells were unequivocally identified and their precise location clarified by PAS counterstaining to outline basement membranes. Proliferating tubular epithelial cells (TEC) were also clearly identified. A study of 25 renal transplant biopsies showed significant correlation between % proliferating TEC and % proliferating ITL ($p = 0{\cdot}000$).

A considerable proportion of ITL in irreversibly-rejected kidney was CD8+, as demonstrated by immunoperoxidase staining in cryostat sections. The cytotoxic potential of infiltrating intratubular cells was demonstrated by identification of perforin mRNA and protein by in situ hybridisation and immunohistochemistry, respectively. Individual cells expressing perforin mRNA were localised in severely damaged tubular areas in close association with TEC. Perforin protein was identified in individual cells in similar locations.

These results show that T lymphocytes proliferate within the tubular compartment during acute renal allograft rejection and that there is associated TEC turnover. Our findings also confirm that cells within the intratubular infiltrate can be fully activated with cytotoxic potential suggesting that TEC turnover is a response to local cytotoxic damage.

DETECTION OF ANTIENDOTHELIAL ANTIBODIES: A COMPARISON OF WESTERN BLOTTING AND FLOW CYTOMETRY.

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Previous studies using Western blotting have shown a significant correlation between the development of transplantation-associated coronary artery disease (TxCAD) and the presence of anti-endothelial antibodies (AEA), with IgM AEA reactive with a doublet of polypeptides of approximately 56 and 58 kDa being of particular importance. The aim of this study was to further investigate the role of AEA after transplantation and to develop a quick and reliable method for screening sera for AEA. Sera from 30 cardiac transplant patients was collected before and at 1, 3, 6, 9 and 12 months after transplantation, and tested for AEA using Western blotting and flow cytometry. No correlation was seen in the results for IgG AEA. However, a strong correlation was found in the results for IgM. 18/30 patients had detectable IgM AEA post-transplantation by Western blotting and of these, 16 were also found to be positive by flow cytometry. Of particular interest was the strong correlation seen between the presence of IgM antibodies detected by flow cytometry and the presence of AEA reactive with the 56/58 kDa doublet. 8/30 patients developed AEA to this doublet post-transplantation and all 8 had strong IgM AEA detected by flow cytometry. The patients are now approaching their first annual angiography and, therefore, we will be able to correlate the production of AEA with the development of TxCAD. Results from this study suggest that flow cytometry is a quick and reliable method for detecting AEA. This technique may provide a useful tool for screening patients to identify those at an increased risk of developing TxCAD.

THE UK MULTICENTRE STUDY OF NEORAL vs SANDIMMUN IN NEW RENAL TRANSPLANT RECIPIENTS : INTERIM RESULTS AT 12 WEEKS

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First or second cadaveric renal transplant recipients from 17 UK units were openly randomised (2:1) at transplantation to Neoral (n=191) or Sandimmun (n=97) formulations of Cyclosporin A (CyA), both used according to existing local protocols. Antibody induction protocols were not permitted. Apart from a 10% excess of males in the Neoral group, the treatment arms were evenly matched for demographics, medical history and HLA matching of the current transplant. At week 12, patient and graft survival were 96.8% versus 97.9% and 90.1% versus 90.7% for Neoral and Sandimmun respectively. One Neoral (0.5%) and 3 Sandimmun patients (3.1%) discontinued for reasons other than graft loss/death. Therapy for episodes of suspected rejection, and the incidence of clinically confirmed acute rejection were lower for Neoral (41.4% versus 54.6%, p=0.03 and 31.9% versus 46.4%, p=0.016). More Sandimmun patients had multiple acute rejections (9.3% versus 5.2%) and steroid resistant rejections/antilymphocyte antibodies (14.4% versus 9.4%). Analysis of survival functions using the intention-to-treat definition (acute rejection + graft loss + death) showed fewer early treatment failures for patients receiving Neoral (39.8% versus 51.5%,Wilcoxon p=0.02). CyA trough levels rose faster in the Neoral group reaching therapeutic levels (>240µg/l) by day 2, 48 hours earlier than Sandimmun, and remained consistently higher during the first 14 days despite lower Neoral doses. There was no associated increase in reports of renal toxicity or prolonged ATN. Over 12 weeks, the incidence and frequency of known CyA side-effects were similar and there were no differences between treatments in the number, type or severity of infective episodes reported. These results strongly suggest that Neoral improves the prevention of early acute rejection without incurring the penalties of increased toxicity or overimmunosuppression.

DIFFERENTIAL IMMUNODOMINANCE OF INDIVIDUAL MHC LOCUS PRODUCTS: IMPLICATIONS FOR THE INDUCTION OF IMMUNOLOGICAL TOLERANCE TO ALLOGRAFTS

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Background: Immunodominant molecules are those that when compared to a whole range of related molecules, are able to induce an immune response more effectively. The understanding of this phenomenon has important implications in the therapy of cancer and autoimmune diseases. Likewise, a better understanding of molecular immunodominance in alloreactivity may allow the development of tolerance-inducing strategies.

Methods: C3H/He mice ($H2^k$) were pretreated iv with blood from C57BL/10 mice ($H2^b$) or with recipient L cells expressing K^b or D^b molecules. FACS assays to assess antibody formation and CTLp frequency assays both directed against the donor class I MHC molecules K^b or D^b were performed 28 days later. C3H/He mice also received the same L cell treatment under the cover of an anti-CD4 antibody, and, 28 days later, received a heart from a C57BL/10 donor.

Results: C57BL/10 blood induced twice as much anti-K^b than anti-D^b IgG antibodies. Likewise, the anti-K^b CTLp frequency varied between 2x and 6x higher than the anti-D^b response. Similarly, L-K^b cells proved to be more immunogenic than L-D^b cells. Finally, administration of L-K^b cells was more effective than L-D^b cells in the induction of transplantation tolerance when given under the cover of an anti-CD4 antibody.

Conclusions: Immunodominant MHC molecules are more effective in the induction of transplantation tolerance. The knowledge of the immunodominant HLA molecules may allow the design of strategies for clinical application with the aid of gene transfer technologies.

REMOVAL OF XENO-ANTIBODIES BY ANTIGEN-SPECIFIC EXTRA-CORPOREAL IMMUNO-ADSORPTION (EIA) PREVENTS PIG HEART-TO-BABOON HYPERACUTE REJECTION.

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Hyperacute rejection of pig organs by Old World primates and humans is initiated by the binding of natural antibodies to pig endothelial antigens, primarily to Galili antigens, i.e. those terminating in Galα(1-3)Gal. Specific depletion of such Galili-reactive antibodies may abrogate such rejection. We have found that an immuno-adsorbent column of appropriate Galili selectivity can remove all Galili-reactive antibody specificities from all human sera tested to date.

We modified the CITEM 10 EIA system (Excorim AB) using columns of the relevant Galili selectivity. Using a 1500 ml pool of human whole blood, there was a >16-fold (93%) reduction in IgM, IgG and IgA anti-Galili titre by ELISA following a 3 plasma volume EIA.

Two baboons were then immuno-adsorbed using this system. EIA of 3 plasma volumes daily for three days without concurrent immunosuppression reduced anti-Galili binding by ELISA to background levels and lymphocytotoxic titres (LCT) by 4-16 fold following each EIA, but antibody rebound was seen between treatments. When subsequent EIA was combined with immunosuppression (IS) (cyclophosphamide, cyclosporin and methyl prednisolone) this rebound was prevented. Following this treatment, a pig heart was transplanted into each baboon. In the first baboon, in which the pre-transplant LCT was 0, there was no hyperacute rejection. Without further EIA but with continuation of IS, the heart continued beating until between 104 and 115 hours. In the second baboon, LCT was 1/4 at transplantation, and the heart was hyperacutely rejected within 10 minutes.

These data show 1) by using appropriate immuno-adsorbents polymorphic anti-Galili antibodies can be removed from a large volume of human blood in a clinically applicable system, 2) IS is effective at limiting anti-Galili rebound, and 3) removal of such antibodies can prevent hyperacute rejection in a pig-to-baboon model, but such removal must be complete.

PAPER 4. Session 2. March 27th 16.00

A UK-WIDE TRIAL OF THE BANFF CLASSIFICATION OF RENAL TRANSPLANT PATHOLOGY

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The Banff Working Classification of renal transplant pathology was published in 1993. This is the first study of its use by diagnosticians throughout a whole country. No training was given beyond provision of a reprint of the original description of the scheme (KI 44:411-22, 1993).

21 transplant biopsies were selected which had all caused some difficulty in the diagnosis or exclusion of acute rejection. We only used cases within 6 months of transplantation, in which a clear 'correct' diagnosis could be deduced from the subsequent clinical course. Sections were circulated to pathologists in 33 laboratories, covering all but one of the renal transplant centres in the UK. 564 responses were received, an average of 26.9 per case, a response rate of 84.1%.

In accordance with published studies by a few 'superspecialist' pathologists, there was greater reproducibility in the diagnoses made using the Banff system.

However, irrespective of where in the Banff classification the cut-off point for the diagnosis of acute rejection is placed, there was no significant difference in the number of 'correct' diagnoses when compared with a conventional diagnostic approach. We suggest that the best balance of false positive and false negative diagnoses results if any grade of Banff category 4 is considered to be acute rejection.

If one of the Banff grades is considered to be 'equivocal' and input of clinical information is permitted, a large increase in the number of correct diagnoses can be achieved. This confirms the importance of clinico-pathological correlation.

One might expect performance with a new system to improve with time, but pathologists who had already adopted the Banff classification in their routine work produced correct diagnoses under the classification no more often that pathologists who had not.

An opinion survey after the trial showed considerable support for the approach represented by the Banff schema amongst the participating pathologists. It provides a method of harmonising reporting styles and for refinement of diagnostic criteria. We support its use in research projects, in teaching and as an 'aide memoire', even for pathologists who choose not to use it as a reporting format.

PAPER 5. Session 2. March 27th 16.15

METHYLPREDNISOLONE VERSUS ATG AS INITIAL TREATMENT FOR ACUTE REJECTIONS AFTER RENAL TRANSPLANTATION

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Anti-thymocyte globulin (ATG) is very effective for the treatment of acute rejections (AR) after renal transplantation (Tx). Due to the frequency and severity of adverse events as well as the high costs of this drug, it is usually applied only after treatment with methylprednisolone (MP) has failed. This policy may result in a delay of effective treatment and ultimately in more immunosuppression in a number of cases.

We compared MP and rabbit ATG as the initial treatment of AR in a prospective randomised trial. Our study population consisted of patients with a first cadaveric renal allograft who had a first AR within 90 days after Tx during treatment with cyclosporin and prednisone. In 17 patients initial treatment consisted of MP (1000 mg/d on 3 consecutive days), and in 19 patients rabbit ATG (RIVM, Bilthoven, The Netherlands) was administered as initial treatment in a dosage of 200 mg on alternate days for 10 days. The duration of follow-up ranges from 16 to 77 months.

Both groups were comparable with respect to age, sex ratio, interval between Tx and AR, HLA match grade, and serum creatinine at start of therapy. Additional treatment for rejection (within 3 months after Tx) was necessary in 8 patients (47%) of the MP group and in only 3 cases (27%) of the ATG group ($P<0.05$). Graft loss occurred in 7 patients of the MP group and in 4 of the ATG group (NS), the death of one patient in each group being included as a cause of graft loss. The estimated graft survival rate at two years after Tx was 59% in the MP group and 78% in the ATG group (NS).

Conclusion: The choice of MP as initial treatment for AR will avoid costly and more complicated treatment with ATG in about half of the patients. However, this approach, which is currently favoured by many centres, may lead to a delay of effective therapy and even to more graft losses.

PAPER 6. Session 2. March 17th 16.30

SUBSTANTIAL IMPROVEMENT OF LIPID PEROXIDATION AND FIBRINOLYSIS AFTER CONVERSION FROM CYCLOSPORIN A TO AZATHIOPRINE

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Atherosclerotic cardiovascular disease is the most important cause of death after renal transplantation. This complication may be related to hypertension, alterations in the plasma lipid profile, especially the susceptibility of low density lipoprotein particles to oxidation, and impaired fibrinolysis.

We studied the influence of the use of cyclosporin A on these risk factors in 18 renal transplant recipients (12 M and 6F, age 41±13 yr., 24±5 months afterTX) who were switched from cyclosporin A to azathioprine-based immunosuppressive treatment. Before and 16 weeks after conversion blood samples were taken for measurement of LDL-particle size, the susceptibility of LDL to in vitro oxidation, LDL subclass pattern, and the concentration of antibodies against oxidized LDL in plasma. In addition, plasma concentrations of parameters of the fibrinolysis pathway and vasoactive prostanoids were measured, and 24hour ambulatory blood pressure was recorded.

After conversion plasma LDL-cholesterol and triglyceride concentrations were significantly lower, LDL-particle size was larger, and LDL-particles were less susceptible to in vitro oxidation. Moreover, the titer of IgM-antibodies against oxidized LDL decreased and the fraction of patients with LDL subclass pattern A increased. These data indicate that apart from an increased LDL cholesterol and triglyceride level, LDL is more atherogenous during cyclosporin A treatment. Conversion was also followed by an increased fibrinolytic activity, as plasminogen activator inhibitor activity and antigen decreased, tissue plasminogen activator increased, and plasmin-antiplasmin complexes decreased. Plasma levels of prostaglandin E_2 and thromboxane B_2, while 24-hour blood pressure profile had considerably improved.

These data suggest that cycloporin A contributes to the high incidence of cardiovascular disease in transplant recipients, as it is not only accompanied by elevated blood pressure, but also by increased atherogenicity of LDL and impaired fibrinolysis.

PAPER 7. Session 2. March 27th 16.45

EPIDEMIC OF NON-MELANOMA SKIN CANCER IN RENAL TRANSPLANT RECIPIENTS: VIRAL AETIOLOGY & INFLUENCE OF SUN, SEX & DRUGS

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Since the early 1970s an increased risk of non-melanoma skin cancer (NMSC) has been noted in renal allograft recipients (RAR), which increases with time from transplantation. A cohort study of RARs at the Royal London Hospital has established an unusually high prevalence of NMSC: 40% of fair skinned individuals were affected 5 years after transplantation. These cancers occur in sun exposed sites on a background of widespread human papillomavirus (HPV) infection, with atypical warty keratoses. Many of these are locally aggressive and some metastasize. Previous studies of the role of genital and cutaneous oncogenic HPVs in these lesions found very low rates of virus detection, using Southern blotting, reverse blot hybridisation and known HPV probes and primers. However, with the development of degenerate primers for polymerase chain reaction (PCR), together with nested PCR primers for cutaneous oncogenic viruses (EV HPV), a 65-80% positivity has been found in both tumours and warts from RARs. A notable feature has been the detection of 17 novel HPVs, some being EV-HPVs and others a new group of viruses (HPV-4 related), that are closer to genital than cutaneous viruses (functional studies of the transforming ability of the E6 and E7 genes of these viruses are in progress). Independent risk factors for the development of NMSC are the male gender and cumulative UV exposure and now the role of specific oncogenic HPVs. The cohort study showed these results emphasize that cyclosporin containing regimens were associated with a significant increase in squamous cell carcinomas (48 NMSC per 10^3 person years) compared to non cyclosporin containing regimes (29/10^3, adjusted hazard ratio = 8.43 (1.30,54.8), p=0.03). The implication of these findings for the design of immunosuppressive regimens, or their tailoring to particular subgroups of RARs, remain to be clarified. We conclude that regular dermatological surveillance of this high risk group of patients is extremely important.