The British Transplantation Society

April 11th and 12th, 1995

UNIVERSITY OF MANCHESTER
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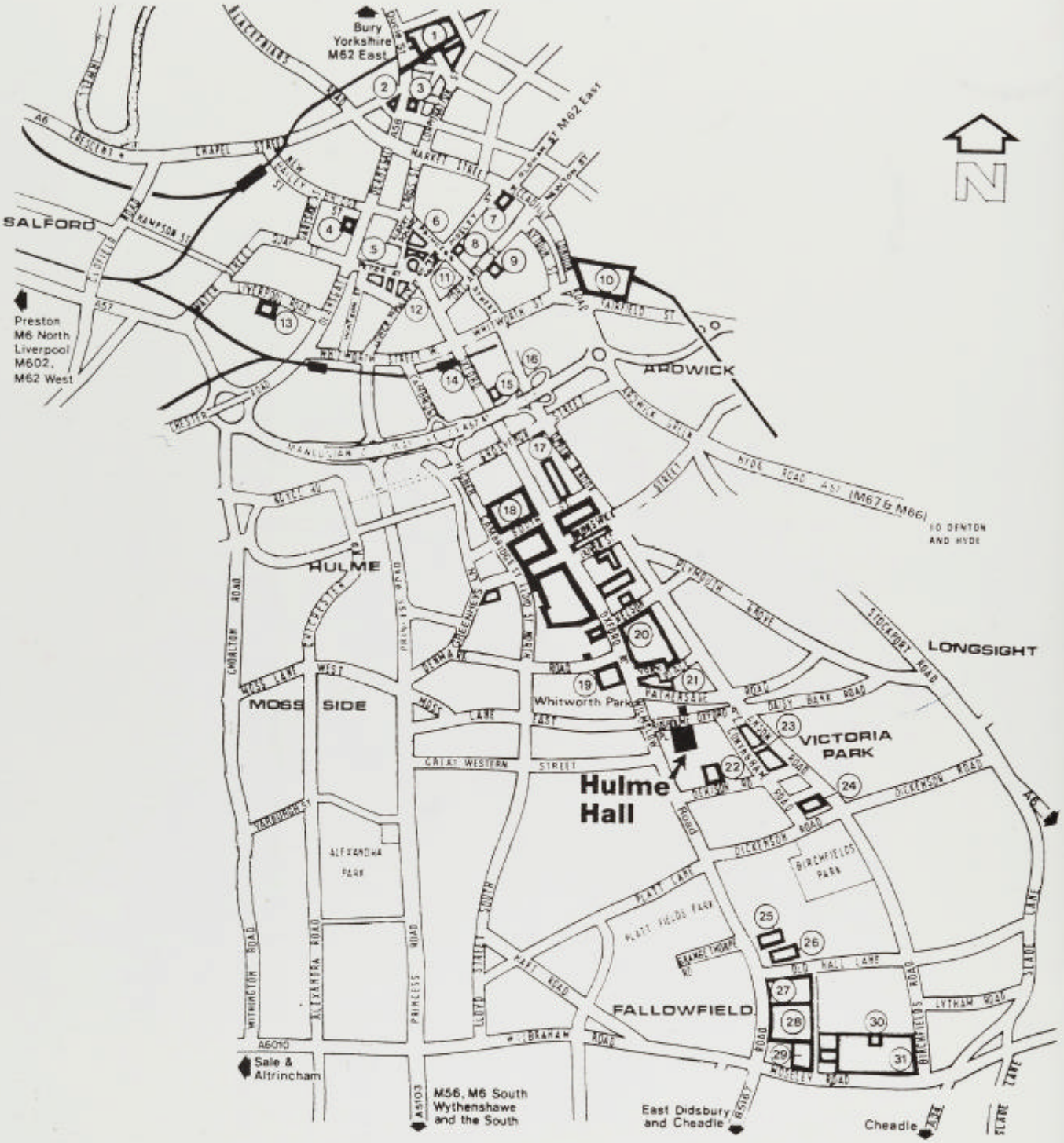

TRAVEL DETAILS INSIDE BACK COVER

BRITISH TRANSPLANTATION SOCIETY

STATEMENT OF ACCOUNTS FOR THE YEAR ENDED 30 SEPTEMBER 1994

RECEIPTS	1993 £	1994 £	PAYMENTS	1993 £	1994 £
Subscriptions received	7692	7244	Postage / stationery	686	1944
Bursaries received	-	6000	Bursaries	8195	9900
			Secretarial honoraria	1000	1000
			Audit fee	40	50
MEETINGS			MEETINGS		
Donations Spring 1994	-	5000	Spring 1994	-	8638
Donations Autumn 1994	-	4200	Advance for Spring 1995	-	1000
Surplus from meetings	5867	1800			
Bank interest	3198	2047	Bank charges	71	269
Working Party	-	31100	Working Party	-	24236
Miscellaneous	700	400	Miscellaneous	615	230
				10607	47267
			Excess receipts over payments	6850	10524
	17457	57791		17457	57791

		Financed by:	
Balance as at 30 September 1993	52485	Ulster Bank Ltd Business Reserve	49027
Add excess of receipts over payments	10524	Current account	13982
	63009		**63009**

The statement of Accounts and Balance are in accordance with information and records provided.

Honorary Treasurer Honorary Auditor

ABSTRACTS SELECTED FOR PRESENTATION

EFFICACY OF MYCOPHENOLATE MOFETIL IN PREVENTION OF ACUTE REJECTION IN RENAL ALLOGRAFTS. A PLACEBO CONTROLLED STUDY.

S A SADEK
On Behalf of the European Mycophenolate Mofetil co-operative Study.

Academic Unit of Surgery, St. James University Hospital, Leeds

Mycophenolate Mofetil (MMF) is a selective inhibitor of both T and B lymphocyte proliferation.

A multicentre European randomized double blind placebo-controlled study recruited 491 patients undergoing first or second cadaveric renal transplants. It compared Cyclosporin (Cya) and steroids with Cya, steroids, and MMF in two doses. There were three arms to the study: 1) Cya, Steroids + placebo n= 166, 2)Cya, steroids, MMF 2 grams/day n=165, 3)Cya, Steroids, MMF 3 grams/day n=160. All patients were followed up for a minimum of one year. The study end point was rigorously set as treatment failure (defined as premature withdrawal from the study for any reason) , or biopsy proven rejection.

Analysis was conducted on intent-to-treat basis. All three groups were demographically similar. The following results are for the early phase encompassing the first 6 months of the study.

	Placebo n=166	MMF 2g n=165	MMF 3g n=160
Treatment failure or Biopsy Proven Rejection.	93 (56.0%)	50 (30.3%)*	62 (38.8%)*
Biopsy proven rejection	77(46.4%)	28 (17.0%)	22 (13.8%)
Patients requiring OKT3, ATG or ALG	35 (21.1%)	9 (5.5%)	5(3.1%)
Mean Serum Creatinine (mg)	1.61 n=114	1.43 n=126	1.48 n=108

* p<0.001 each MMF dose Vs Placebo

The addition of MMF to Cya and Steroids significantly reduces the incidence of acute rejection and substantially curtails the use of biological agents in the treatment of rejection..

TRANSFUSION INDUCED TOLERANCE IS EFFECTIVE AGAINST AN MHC CLASS II BUT NOT A CLASS I DISPARITY IN HIGH RESPONDER RATS

EB Bell, IA Cossens, C-p Yang, SL Hayes and PJ Wood

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Previous investigations demonstrated that DA ($RT1^a$) cardiac allograft survival in the low responder PVG ($RT1^c$) strain was significantly prolonged (MST, 38-44 days) by injecting recipients with 1.5 ml DA blood 1, 2 or 4 weeks before transplantation. By giving PVG recipients a prior blood transfusion from PVG congenic strains of rat before DA heart grafting we show that both MHC and non-MHC antigens contribute in an additive fashion to the extended survival, but unequally. In order of importance MHC class I (A^a) > MHC class II (B/D^a) > MHC class "I-like" (C^a)> non-MHC antigen.

Attempts were made to prolong DA cardiac allografts in the high responder PVG-$RT1^u$ strain by donor specific blood transfusion (DST) but without success. DA hearts were rejected acutely with accelerated kinetics. We asked, therefore, whether this failure to induce tolerance was shared equally between MHC class I and II. PVG-$RT1^u$ recipients given a transfusion of blood from DA (a full mismatch) or PVG-R8 rats ($RT1A^a$ B/D^u C^u) rejected MHC class I disparate PVG-R8 heart grafts acutely. However, PVG-$RT1^u$ rats given a DA or PVG-R23 ($RT1A^u$ B/D^a C^a) blood transfusion and allografted with PVG-R23 hearts (a class II plus class "I-like" disparity) retained their heart allografts indefinitely (>150 days). Recent work (Morton et al, Eur J Immunol. 1993, 23: 2078) has demonstrated that rejection of PVG-R8 hearts in the PVG-$RT1^u$ strain can be induced by anti-A^a alloantibody. However, we found that alloantibody against rat MHC class II (anti-B/D^a) was unable to induce rejection of PVG-R23 allografts by passive transfer in PVG-$RT1^u$ athymic nude recipients.

This study may help to define the circumstances (e.g. when transplants are matched at MHC class I) where DST is most likely to have a beneficial clinical effect.

FK506 AND GLUCOSE METABOLISM: A PROSPECTIVE STUDY IN LIVER TRANSPLANT RECIPIENTS

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We evaluated changes in glucose metabolism in FK506-treated liver graft recipients during the first year after transplantation(OLTx).
Serial 75g oral glucose tolerance tests(OGTTs) were performed in 16 consecutive patients at 2, 6 and 12 months after OLTx. Ten matched healthy volunteers acted as the control group. Steroids were tapered off and discontinued by the end of the 3rd postoperative month. The mean daily FK506 dose (mg/kg) was progressively reduced during the study period(0.1 vs 0.08 vs 0.06; 2, 6 and 12 months; P<0.01). At 2 months OGTTs were diagnostic of diabetes mellitus(DM) in 3 patients and of impaired glucose tolerance(IGT) in 5(WHO diagnostic criteria). Overall glucose concentrations during OGTTs were significantly higher in OLTx group than in the controls(P<0.001). After steroid withdrawal OGTTs at 6 and 12 months demonstrated sustained improvement in patients overall response to glucose challenge(2 vs 6 vs 12 months, P=0.01). At 12 months only one patient had DM and 3 others had IGT. However overall glucose concentrations during OGTTs at 12 months were still significantly higher in the OLTx group than in the controls (P<0.001). Plasma insulin concentrations during OGTTs in transplant recipients didn't change during the study period(2 vs 6 vs 12 months, P>0.1). Significant fasting and overall hyperinsulinaemia were observed at each time point (P<0.001 vs control at 2, 6 and 12 months) implicating resistance to insulin action.
We conclude that steroid withdrawal is associated with improved glucose tolerance in FK506-treated liver transplant recipients. However persistent derangement of glucose tolerance and insulin response suggests that the long-term adverse metabolic effect of FK506 is steroid-independent.

Evidence for deletion of antigen reactive T cells after intrathymic injection of alloantigen

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We have established an intrathymic model of tolerance induction across a full major and minor histocompatibility antigen difference in mice. Recipient CBA ($H\text{-}2^k$) mice were treated IV with a depleting anti-CD4 monoclonal antibody (YTA3.1) on two consecutive days (day -28, -27 relative to heart transplantation on day 0). Coincident with the second dose of mab, $5x10^7$ C57BL/10 ($H\text{-}2^b$) spleen leukocytes were injected into the thymus ($2.5x10^7$ per lobe). Operational tolerance to C57BL/10 cardiac allografts was induced in 85% of recipients treated using this protocol (MST > 100 days (n=8) compared to 32 days in mice treated with YTA 3.1 alone (n=5)). Tolerance induction was antigen specific; third party BALB/c allografts were rejected at a rate comparable with the anti-CD4 control group.

To elucidate the mechanisms of tolerance induction following intrathymic injection of C57 alloantigen we used T cell receptor (TCR) transgenic mice (BM3.6) expressing TCR α and β genes specific for the donor class I antigen, $H\text{-}2K^b$. The clonotypic mab TI98 was used to detect T cells expressing the transgenic TCR. As $CD8^+TI98^+$ T cells were present in the periphery of BM3.6 TCR transgenic mice a depleting anti-CD8 mab (YTS169) was included in the tolerance induction protocol to ensure depletion of peripheral T cells. The effects of intrathymic injection of C57 spleen leukocytes on $TI98^+$ thymocytes was examined.

An 80% reduction in the CD8 single positive thymocyte population ($CD4^-CD8^+$) was observed 10 days after intrathymic delivery of C57 alloantigens (n=3). Double positive thymocytes ($CD4^+CD8^+$) were unaffected (n=3). This reduction in the $CD4^-CD8^+$ thymocyte population was antigen specific as injection of syngeneic ($H\text{-}2^k$) or third party ($H\text{-}2^d$) cells did not result in a comparable decrease (n=3). Mab therapy alone had no effect on the thymocyte populations. We were able to confirm that the reduction in the $CD4^-CD8^+$ cells was due to deletion of this population and not downregulation of either CD8 or TCR. TI98 and CD8 expression on the remaining thymocytes was identical to that found on thymocytes in naive or control BM3.6 mice and tissue sections of injected thymii showed a definite deletion of thymocytes compared to control thymii.

In conclusion, deletion of donor reactive single positive thymocytes may be responsible for the induction of tolerance following intrathymic delivery of donor alloantigen.

CYTOKINE INDUCIBLE ADHESION MOLECULES: AN INDICATOR OF ISCHEMIA-REPERFUSION INJURY IN LIVER ALLOGRAFTS

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Intercellular Adhesion Molecule-1 (ICAM-1) and E-Selectin are cytokine inducible polypeptides involved in leukocyte/endothelial cell interaction. Activated donor Kupffer cells, and endothelial cells may participate in cytokine production.

The aim of this study was to investigate the expression of ICAM-1 and E-selectin on liver biopsies following graft storage and warm ischemia/reperfusion. Change in expression was correlated with degree of graft injury by measuring levels of AST were upon arrival at ITU..

Following cold storage (723 ± 31 mins) and reperfusion, at 90 mins, liver biopsies from 22 grafts were snap-frozen. 5µm frozen sections were stained immunohistochemically for ICAM-1 and E-selectin. Expression, following reperfusion, compared to the stored allograft was analysed by light microscopy, and by computer-aided image analysis, measuring integrated optical density (IOD).

ICAM-1 Expression Following Reperfusion	*AST >1000	*AST <1000
Increase	6	3
No Change	2	11

**Fishers Exact Test $p<0.05$*

Expression ICAM-1 and E-selectin was parallel. Activation of ICAM-1 on sinusoids and hepatocytes occurred following graft storage. A further increase in ICAM-1 expression, following reperfusion, was associated with severe ischemia/reperfusion injury. E-selectin expression on endothelium increased significantly: mean IOD 1.07 after storage, compared to 2.69 ($p<0.05$ t-test) following reperfusion.

Induction of both molecules is probably as a result of ischemic injury rather than an immune mechanism. These results support the concept that Kupffer cell activation and endothelial cell injury occur following reperfusion. There is evidence, however, to suggest that cytokine activity continues during storage and may be enhanced by a period of warm ischemia, resulting in graft injury.

CELL INFILTRATION AND INDUCTION OF MHC CLASS II AND ADHESION MOLECULES ON RAT CORNEAL ENDOTHELIAL CELLS DURING REJECTION

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Purpose. The endothelial cell layer at the back of the cornea is essential for corneal transparency and in humans is easily damaged beyond repair by rejection. The processes involved in its destruction are not well understood. We have therefore examined flat preparations of rat corneal endothelium during rejection for leukocyte infiltration and MHC class II and ICAM-1 expression. ***Methods.*** Penetrating keratoplasty was performed on 81 rats. The animals were divided into two groups- isografts (PVG→PVG, n=25) and allografts (LEW→PVG, n=56). Eight normal corneas were also examined. Animals from each group were killed on days 10 and 15 after transplantation (i.e., just before and during rejection). The corneas were removed, the endothelia were carefully peeled off and mounted on glass slides. They were stained by the avidin biotinylated peroxidase (ABC) method with monoclonal antibodies against T cells (OX34), macrophages (ED2), MHC class II (OX6) or ICAM-1. Samples were coded, the presence of leukocyte infiltrate was determined and the intensity of staining were measured using a Quantimed 500 image analysis system. ***Results.*** There was no cell infiltration, class II or ICAM-1 expression on the normal endothelium. Patchy *de novo* expression of class II and ICAM-1 was evident on endothelial cells of allografts and isografts by day 10, on both donor and host. Two-way analysis of variance revealed a significantly grater expression of both MHC class II ($p=0.004$) and ICAM-1 ($p=0.01$) in allografts compared with isografts in the donor tissue, which increased at day 15. Macrophages and T cells, accompanied by donor endothelial damage, were present during rejection in all allografts. ***Conclusions.*** Flat preparation is a useful method for assessing changes in the endothelium after transplantation, as the whole tissue can be seen. MHC class II and ICAM-1 are not normally expressed on normal corneal endothelial cells. *De novo* expression of these molecules is induced in allografts before and during rejection and this may be important in corneal endothelial cell immunogenicity, because of the low expression of MHC class I antigens by these cells. Cell infiltration may suggests a DTH reaction, although a direct $CD4^+$ cytotoxic T cell response may also occur, especially in cases of MHC class II matched grafts.

THE IDENTIFICATION OF NON-HLA SPECIFIC ANTIBODIES BY FLOW CYTOMETRY

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The flow cytometry crossmatch (FCXM) is a highly sensitive technique which detects the binding of both complement fixing and non-complement fixing antibodies to donor cells. A positive FCXM is associated with increased numbers of rejection episodes and early graft failure. However some centres have reported "false positive" FCXMs suggesting that flow cytometry is too sensitive or is detecting antibodies irrelevant to transplant outcome.

Cytotoxic studies which have inhibited alloantibody binding to HLA class I by blocking target cells with a class I specific monoclonal antibody, showed that a positive crossmatch due to non-HLA specific antibodies was not associated with graft failure. However cytotoxic blocking assays are limited by the availability of suitable non-complement fixing monoclonal antibodies. Flow cytometry does not involve complement fixation and can therefore use any monoclonal antibody of appropriate specificity as a blocking agent.

We have used the monoclonal antibodies W6/32 and MCA477 to block antibodies to HLA class I and class II respectively. By incubating donor cells with these antibodies prior to three colour flow cytometry crossmatching we have been able to demonstrate the presence or absence of HLA specific antibodies in 48 sera from 12 cytotoxic negative, FCXM positive donor/recipient pairs. Of the 12 pairs tested 7 patients were shown to have HLA specific antibodies to their respective donors. By performing a flow cytometry autocrossmatch on the 5 patients with no evidence of donor specific HLA antibodies we have identified 3 patients with low levels of IgG autoantibodies. It is possible that the presence of non-HLA antibodies in positive FCXMs may account for a significant proportion of the "false positive" results that have been reported. Using this method to define antibodies giving a FCXM positive cytotoxic negative result, patients with non-HLA antibodies can be successfully transplanted whilst avoiding graft failure due to low levels of HLA specific antibodies.

CELLULAR LOCALISATION OF COMPLEMENT C3 SYTHESISED IN HUMAN RENAL ALLOGRAFTS

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The role of complement in renal allograft injury still remains to be defined. Earlier we found that C3 mRNA was expressed in human renal allografts and that expression of C3 mRNA was increased during renal allograft injury both in ischaemic kidney and during the cellular allograft response. It was clear from the determination of the allotype of C3 mRNA expressed in renal allograft biopsies that the C3 synthesised was in part of donor type, but it was not clear which cells in the graft expressed C3 or whether the expression of C3 mRNA resulted in protein synthesis. To investigate this further we took advantage of the two main C3 allotypes: C3S and C3F. These can be distinguished on western blots using the C3F selective monoclonal antibody HAV 4.1. Using this antibody as an histological reagent we analysed the expression of C3F in the kidneys of 12 C3F donors transplanted into C3S recipients. This revealed almost exclusively renal tubular cytoplasmic staining of C3, indicating that the tubules were the prominent source of tissue protein synthesis during allograft dysfunction. There was no staining of mononuclear cells, indicating that donor C3 synthesis was not attributable to passenger leukocytes. In contrast, staining with polyclonal anti-C3 antibody, detecting both donor and recipient C3 allotypes, identified a predominantly glomerular capillary and peritubular pattern of staining. With donor C3F kidneys transplanted into C3F recipients, HAV 4.1 produced a similar pattern of staining to polyclonal anti-C3, because in this case both donor and recipient C3 types were detected with both reagents.

The conclusion from this study is that renal tubules are the main source of C3 synthesised within transplanted kidney, whereas extrinsic C3 produced by the recipient seems to be deposited in a capillary and peritubular distribution. The synthesis of complement within renal grafts displaying ATN and cellular rejection may represent an important adaptation to, or mechanism of, allograft injury, and may play a different role from plasma C3 fixed in renal transplant tissue.

AN IN VITRO MODEL OF HYPERACUTE REJECTION OF XENOGENEIC ISLETS

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We have recently shown that freshly isolated rabbit islets transplanted into untreated cynomolgus monkey recipients undergo extensive destruction within 6 hours with histological features typical of hyperacute rejection of vascularised organs, namely binding of natural antibody, infiltration by neutrophils and cell lysis. Interestingly, incubation of the same islets with fresh human serum containing both natural antibody and complement did not result in decreased viability (as assessed by FDA/EB scoring) after culture for up to 2 days, suggesting a further factor was necessary to initiate islet destruction. Since early neutrophil infiltration was a constant feature of our in vivo model, we added human neutrophils to freshly isolated rabbit islets in the presence of fresh human serum and saw a significant decrease in islet viability after 2 hours incubation. This finding was repeatable using both human and cynomolgus monkey serum and neutrophils. We then demonstrated that the damage process was dependent on the presence of active complement, and was specific for the xenogeneic serum but not for the source of neutrophils: thus rabbit, human or cynomolgus neutrophils will destroy rabbit islets only if fresh primate serum is present. However, human neutrophils have no effect on rabbit islets in the presence of rabbit serum. Dilution experiments showed that the number of neutrophils required was remarkably small (<5 neutrophils/islet).

We believe that this simple assay amounts to an in-vitro model of hyperacute xenograft rejection (arguably the first ever described), and we have now used the model to develop a tissue-culture based strategy for preventing hyperacute rejection of xenografted islets. Preliminary transplantation studies in primates indicate that this strategy does indeed significantly reduce the destruction of xenografted islets in-vivo.

T CELL RESPONDING TO ALLOGENEIC HEART AND KIDNEY CELLS USE DIFFERENT T CELL RECEPTOR REPERTOIRES

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Background & Aim: Alloantigens can be recognised by the T cell heterodimeric antigen receptor either "directly" on the surface of allogeneic cells, or "indirectly" as antigen-peptide fragments bound to MHC molecules . We have shown recently the predominant usage of certain TCR V genes by alloreactive T cells. Established lymph nodes T cell lines and clones predominantly used the Vβ 6.0 gene in 80.0 - 85.7% and 60.0 - 64.3% of cases respectively. Only one joining region, Jβ 2.1, was used by these T cells and a common motif could be discerned in the TCR 'N' region. No Dβ gene segments could be identified.

The aim of this study was to compare the TCR β chain repertoire of T cells responding to alloantigens on different organs namely kidney and heart.

Methods: DA rat T cell lines were generated in culture by stimulation with isolated Lewis rat kidney and heart cells. Total RNA was extracted and the reverse transcription-polymerase chain reaction (RT-PCR) performed using a universal Vβ and a Cβ primer. The PCR product was cloned into PCRscript vector and the TCR β chain was characterised by direct sequencing of the plasmid DNA using dideoxy chain termination method.

Results: Several different Vβ genes (6.0, 8.3, 4.0, 5.2) were expressed by the kidney alloreactive T cell lines. Vβ 6.0 was most commonly used, present in 40 - 45% of lines, but Vβ8.3 also accounted for a significant proportion Vβ usage (26% of lines). Jβ2.1 accounted for 55 to 60% and Jβ2.5 for approximately 26% of the total Jβ segments used by these T cells.

In contrast, a more heterogeneous pattern of Vβ gene usage was seen in T cell lines reactive against allogeneic heart. Vβ 4.0, 6.0, 5.2, 5.1 and 16.0 were all detected. Of these, the most frequently used Vβ was Vβ4.0 (33%) followed by Vβ6.0 (31%). The Jβ gene segments 2.1 and 2.5 accounted for approximately 95% of Jβ segments used and Jβ1.3 was detected in the remainder of the T cell lines.

Conclusion: The pattern of Vβ gene usage in kidney alloreactive T cells was similar to that observed for lymph node-stimulated cells. This suggests that the T cell response to both allogeneic lymph node and kidney cells is directed toward a single immunodominant epitope and that this epitope reacts with a restricted range of T cell receptors. Vβ6.0 T cells also accounted for approximately 30% of heart alloreactive lines. However, almost 33% of heart alloreactive T cells used Vβ4.0 and over 25% of kidney-reactive T cells use Vβ8.3, suggesting that organ specific peptides also play a role in determining the alloreactive T cell repertoire. The limited Vβ and Jβ usage along with a common N region motif still points to a very restricted response. Selective immune intervention directed at specific TCR structures would appear to a viable strategy in preventing allotransplant rejection.

HEPARIN MODULATES EXPRESSION OF HLA-DR AND ICAM-1 BY CYTOKINE STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC).

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Endothelial expression of Class II MHC antigens and of the adhesion molecule ICAM-1 may play an important role during initiation of the rejection process following allogeneic renal transplantation. The proinflammatory cytokines IFN-γ and TNF-α upregulate expression of these molecules. Many cytokines are sequestered prior to engaging their specific receptor by interaction with specific domains on cell-surface or matrix glycosaminoglycans such as heparan sulphate. Soluble heparin may modulate this initial sequestration.

HUVEC were cultured in medium supplemented with varying concentrations of heparin and were stimulated with IFN-γ and TNF-α. After culture for 72 hours the expression of Class II MHC antigens and ICAM-1 were assessed by quantitative flow cytometry. A sensitive lymphocyte adhesion assay was then used to measure the ability of these cells to bind activated lymphocytes.

It was shown that the upregulation by IFN-γ of both Class II MHC antigens and and ICAM-1 was reduced in the presence of increasing concentrations of heparin (Table). A similar effect was not seen following the activation of HUVEC by TNF-α.

Cell Stimulation	Flow Cytometric Antigen Quantification (MESF ± SD; n = 5)	
	MHC Class II Antigens	ICAM-1
Resting	10,342 ± 182	59,850 ± 13,017
50U/ml IFN-γ; no heparin	588,516 ± 37,073	417,799 ± 19,439
50U/ml IFN-γ; 100μg/ml heparin	185,722 ± 19,357	156,267 ± 14,872
50U/ml IFN-γ; 500μg/ml heparin	18,506 ± 206	93,855 ± 12,952

The adhesion between IFN-γ stimulated HUVEC and mitogen activated lymphocytes was reduced significantly when the HUVEC were treated with IFN-γ in the presence of heparin. Heparin also reduced the synergistic activation of HUVEC produced by mixture of IFN-γ and TNF-α.

These results suggest an explanation for the immunomodulatory properties that have been reported for heparin. It is known that IFN-γ has a higher affinity for purified heparin than for physiological glycosaminoglycans. Therefore, heparin may competitively displace this important pro-inflammatory cytokine from the endothelium and reduce its biological activity by allowing gross dilution into the blood.

ALLOANTIGEN PRESENTATION BY B LYMPHOCYTES: REQUIREMENT FOR ACTIVATION.

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The role of B cells in antigen presentation is controversial. In this study the ability of B cells to present alloantigen was studied in MLR. Lymphoproliferation and IL-2 production were measured. EBV-transformed B cell lines (EBV-LCL) and purified resting B cells were capable of initiating a primary MLR. However only EBV-LCL retained this ability after fixation. Preactivation of resting B cells with a $F(ab')_2$ anti-human IgM enabled these cells to induce lymphoproliferation and stimulate cytokine production after fixation. Flow cytometry revealed that resting B cells constitutively express Class II MHC antigens but not the ligands B7-1 (CD80) and B7-2 (CD86). These molecules were induced by activation and peaked after 72 hours but were expressed constitutively at high levels by EBV-LCL. Serial fixation where B cells were trapped at various times during activation demonstrated a correlation between costimulatory ligand expression and APC function.

Period of B cell activation with $F(ab')_2$ anti-IgM	Ligand expression (flow cytometry; MESF)		5 Day MLR with stimulation by fixed B cells (n = 5)	
	B7-1 (CD80)	B7-2 (CD86)	^{3}H-thymidine (cpm)	IL-2 (U/ml)
0	810	1,212	886 ± 107	0.18 ± 0.05
3 days	4,150	11,881	28,775 ± 4,617**	0.33 ± 0.08**

The importance of these ligands was further evidenced by inhibition of MLR with mAb to B7-1, B7-2 and Class II MHC and CTLA4-Ig. Furthermore, the hyporeactivity observed in MLR with resting B cells can be overcome by stimulation of CD28 on lymphocytes using an anti-CD28 mAb. This study suggests that only activated B cells have the ability to stimulate naive CD4+ T cells as they can provide both antigen presentation and the necessary costimulatory signals.

A MULTI CENTRE STUDY OF FLOW CYTOMETRIC CROSSMATCHING IN THE U.K.

A.W. Harmer[1], B.K. Shenton[2], A.E. Bell[2], S. Garner[3], S Martin[4] on behalf of The British Society for Histocompatibility and Immunogenetics (BSHI) Flow Cytometry Group

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Flow cytometric crossmatching (FCXM) is a highly sensitive method for the detection of HLA specific antibodies in patients' serum and positive recipient-donor crossmatches are considered a contraindication to renal transplantation by some transplant centres. However, the clinical significance of positive crossmatches is not clear as the association with transplant outcome differs between centres. The BSHI flow cytometry group initiated a study to determine whether differences in techniques and interpretation of results affects the definition of a positive crossmatch and thus accounts for apparent variation in clinical significance.

Five cell/serum exchanges have been carried out with 12 centres participating in the crossmatching exercises. A total 17 sera and 6 cells, giving 25 cell/serum combinations, were tested for IgG antibodies. With each laboratory using their own criteria for positivity there was complete agreement for 10 crossmatches and over 85% agreement for a further 8. Seven crossmatches did not produce clear agreement between centres. Based on the fluorescence data obtained for positive and negative results where there was agreement, and the use of calibration beads, an independent definition of positivity was determined. When this was applied to the unclear results the concordance increased from 51% to 77%. In addition tests on doubling dilutions of 4 sera also showed differences in sensitivity between centres.

Cytotoxic testing of 18 combinations was carried out at the same time as the FCXM by 4 centres, confirming the greater sensitivity of flow cytometric assay. Three sera collected from patients with previous transplant failure were negative by cytotoxicity but were reported as FCXM positive by the majority of laboratories. This indicates the clinical significance of the greater sensitivity of the flow cytometric assay.

In conclusion this study demonstrated that there is agreement on the FCXM result for a majority of undiluted sera. There were however apparent differences in sensitivity between centres shown by 27% of sera tested at neat and by dilutions of sera. The BSHI group has shown that some of these differences can be attributed to the definition of positivity used by individual centres. The group is continuing to work towards optimising the FCXM.

POLYMORPHISM WITHIN THE HUMAN IgG ANTI-PIG REPERTOIRE

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It is accepted in pig-to-human xenotransplantation research that a dominant proportion of human IgG and IgM anti-pig is against epitope(s) carried on the pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc [the 'Galili' antigen(s)]. The development of two possible therapeutic strategies (antigen-specific immunoadsorption and soluble antigen inhibition) will depend on the extent to which individuals share the same anti-pig specificities. We have tested sera from individuals from all 4 ABO groups to assess whether IgG and IgM anti-Galili specificities within the population are monomorphic. 20 sera (5 from each ABO group) were tested by ELISA for IgG and IgM binding to 3 separate HSA-conjugated antigens derived from the parent Galili molecule: the terminal disaccharide Galα1-3Galβ1-HSA ("di-HSA"), the terminal trisaccharide Galα1-3Galβ1-4GlcNAcβ1-HSA ("tri-HSA"), and the whole pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-HSA ("penta-HSA"). All sera had equivalent binding of IgM to the three antigens. However, for IgG there were 3 distinct patterns of binding:

Antigen binding pattern	Number of sera (20)	%
di-HSA alone	2	10
di- and penta-HSA only	7	35
di-, tri- and penta-HSA	11	55

45% of sera therefore, did not bind to tri-HSA. In keeping with this, it was found in 8 sera tested that whilst in 4 soluble tri-HSA readily inhibited IgG binding to penta-HSA by ELISA (50% inhibition point at c.100nM tri-HSA), the other 4 sera were resistant to inhibition by tri-HSA. There are therefore distinct subgroups of IgG anti-Galili antibodies. Nevertheless, absorption using a disaccharide-Sepharose column showed that IgG binding to penta-HSA by ELISA can be at least partly removed by disaccharide in the 6 sera tested. This study shows for the first time that human IgG anti-Galili specificities are polymorphic, but that disaccharide immunoadsorbents may still be capable of removing anti-Galili activity.

APPLICATION TO TRANSPLANTATION OF HLA CLASS I DNA TECHNIQUES

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We have previously, in joint studies with the Collaborative Transplant Study (CTS), shown the benefit of the detection of HLA-DR alleles by DNA techniques (i) in assessing the differences in DNA and serological determination and (ii) in the improved graft survival (GS) found using DNA techniques to determine the HLA-DR allele. We have now developed techniques to determine HLA-A and -B alleles by amplification of DNA by PCR and detection by sequence-specific oligonucleotide probes.

We have applied the system to test 77 renal donors and 18 recipients used at this unit. Four differences in SSO typing from serological typing for donors were found as follows: HLA-A74 instead of HLA-A31; HLA-B58 on two occasions instead of HLA-B57 and HLA-B70 was confirmed when HLA-B70 had been queried by serology. One recipient had been designated as positive for HLA-B47 but was negative by SSO.

In addition this new procedure has been applied to serologically homozygous donors on our Bone Marrow Registry of 5,000 donors. The homozygous antigen was detected on all occasions. However for HLA-B 135 of 695 serologically homozygous donors were found to have an extra allele. For HLA-A we only tested donors homozygous for the less common antigens (and excluded HLA-A1,-A2,-A3). We found 36 of the 114 homozygous donors had an allele not detected by serology.

The results are not surprising. The sera used to type potential bone marrow donors differs in quality from the sera we use for solid organ transplantation. Most of the alleles missed have a low frequency in this population. It is important they are detected in order to have available bone marrow donors with these alleles.

At present we are testing zero mismatched (HLA-A,-B,-DR) renal transplant pairs from the CTS study in order to verify the class I serological type and to determine if detection of class I alleles by DNA techniques would improve graft survival.

ANTITHYMOCYTE GAMMAGLOBULIN INDUCTION THERAPY IN THE MANAGEMENT OF HIGHLY SENSITISED RENAL TRANSPLANT RECIPIENTS.

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Between 1st November 1990 and 1st October 1993, we performed 35 cadaveric renal transplants in 35 highly sensitised patients with a measured peak panel reactive antibody levels (PRA) of greater than 80%. Eight were primary transplants, 19 were second, 6 were third and 2 were fourth transplants. The mean number of HLA antigen mismathces was 2.8.

Post-operative immunosuppression consisted of polyclonal antibody therapy using intravenous antihymocyte globulin (ATG) Fresenius R 2.5mgs/kg daily and standard triple therapy, (cyclosporine 10mgs/kg orally, azathioprine 1 - 2 mgs/kg orally and prednisone 20mgs/day orally, tapered over three months to 7.5mg/daily). The ATG doseage was adjusted to maintain the absolute lymphocyte count between 0.1-0.2 x 10^9/L. The mean duration of ATG treatment was 8.4 days (range 2 - 14 days; median 10 days).

Three allografts were lost due to technical reasons in the immediate post-operative period and two patients suffered accelerated acute vascular rejection, necessitating allograft nephrectomy within the first week. Two patients have suffered graft loss due to acute rejection on days 202 and 601 post-transplant and a further graft was lost on day 1155 due to chronic rejection. If those allografts that have suffered technical loss or failed within one week of transplantation are excluded from analysis, the one year actual patient and allograft survival was 100% and 96.55% respectively. Mean post-transplant serum creatinine at 1 month, 3 months and currently is 165mmol/L, 162mmol/L and 159mmol/L respectively.

Ten patients (29.4%) developed serological evidence cytomegalovirus infection, with 6 patients manifesting evidence of active clinical disease. All cases responded to intravenous gancylovir treatment.

In conclusion, we fell that excellent results can be safely achieved in highly sensitised renal transplant recipients using prophylactic inductiontherapy with ATG in combination with cyclosporine based triple therapy instituted immediately post-transplantation.

KIDNEY TRANSPLANTATION IN PATIENTS OLDER THAN 60 YEARS OF AGE - IS IT WORTH IT?

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With recent increase in the age of the population, there is a rising number of dialysis patients who are older than 60 years, and many of these are being considered for transplantation, particularly when their survival results on dialysis are inferior to those of younger individuals. During the past 3 years we have accepted for transplantation 32 patients age 60-76. Before being accepted for for transplantation, all patients underwent thorough medical assessment, particularly with regard to pulmonary and cardiac functions, which included thallium stress tests and sometimes coronary angiography. Many patients were found to have additional risk factors besides renal failure, which included hypertension (54%), cardiac problems (38%), diabetes (21%), previous surgery (20%), and past history of malignancy (13%). Three patients had received previous failed transplants. Two patients received kidneys from living related donors, and 30 from cadaveric donors aged 10-74 years with a cold ishchemia time of 19-41 hours. Whenever possible, kidneys from older donors were given to these recipients. After transplantation there was no primary nonfunction, but 15% of the grafts showed delayed function. In living donor recipients, patient and graft survival was 100% at 1-3 years. In recipients of cadaveric donors, patient survival was 93.7%, at 1, 2, and 3 years, and graft survival 75%, 66%, and 66% at the same time intervals, respectively. One patient was lost to cerebral lymphoma with a functioning kidney, and another to overwhelming CMV infection. Five other grafts were lost to rejection. A number of non-fatal post-transplant complications were seen in these patients which included cardiac arrythmia, pulmonary and urinary tract infections, de novo malignancy, late onset diabetes mellitus, and wound dehiscence. All patients with functioning grafts are active and enjoying a good quality life. From this study and from the known lower survival rates and poor quality of life in elderly patients on dialysis therapy, we believe that kidney transplantation in the elderly recipient is a very valuable, cost effective and worthwhile effort.

ONE THOUSAND LIVER TRANSPLANTS: SURGICAL COMPLICATIONS AND OUTCOME.

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The marked improvement in results of liver transplantation have been attributed to better recipient selection, refinements in organ preservation and procurement, improved operative and anaesthetic techniques aided by technological advances, superior immunosuppressive regimens and better post-operative management.

The results of 1000 consecutive orthotopic liver transplants(OLT) performed in this centre (721 adults; 160 children) were evaluated, comparing early (1982-1987; 133 OLT) with more recent (1988-1994; 867 OLT) experience. Changing trends in indications (primary tumours, fulminant hepatic failure, alcoholic liver disease and retransplantation) and in vascular, biliary and infective morbidity and mortality were analysed. Thirty day mortality improved from 28% to 10%. Operative time (7 hours (range 3-12) vs 6 hours(range 2-11)), blood replacement (17 units (0.2-141) vs 5 units (0-54)), hospitalisation (26 days (0-153) vs 18 days (0-142)) were all significantly reduced ($p<0.05$). One and five year actuarial survival improved from 51% and 41% to 79% and 74% in adults and from 47% and 33% to 78% and 73% in children.

Despite liberalisation of recipient selection criteria, use of more marginal donors, and major surgical complications, patient survival continues to improve.

FUNCTION OF ALVEOLAR EPITHELIAL ICAM-1 AND LFA-3 IN THE ADHESION OF ALLOGENEIC LYMPHOCYTES.

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The lung contains a large pool of lymphocytes which have a phenotype of chronically activated cells. During rejection, lymphocytes are observed to infiltrate a transplanted lung. The aim of this study was to investigate the function of epithelial adhesion molecules in lymphocyte binding.

Monolayers of primary human alveolar epithelial cells (AEC; type II pneumocytes) were cultured in vitro, and incubated with ^{51}Cr labelled lymphocytes. Non-adherent lymphocytes were removed; the adherent cells lysed and the released ^{51}Cr measured by γ-spectrometry. The effects of antibody blockade of LFA-1 or CD2 on lymphocytes, or of their ligands on the AEC were determined.

The effect of in vitro activation of lymphocytes resulted in a significant increase in binding ($p = 0.0026$). Lymphocytes activated with PHA for 2-3 days bound to AEC with a mean of 19.6% ($n = 10$), whereas 43.7% of lymphocytes cultured more than 6 days with PHA bound to similar monolayers ($n = 9$). The binding capacity of morphological variants of cultured alveolar epithelial cells was different: monolayers consisting of flat, thin cells (type I pneumocyte-like) reproducibly bound a greater proportion of lymphocytes than those with a dense classical type II pneumocyte-like morphology. The results of 3 separate experiments demonstrated a significant increase ($p = 0.0005$) in binding of the same populations of lymphocytes to flat, thin alveolar epithelial cells (mean binding of 35%) compared to those with a dense type II-like morphology (mean binding of 4%).

Antibodies to CD18 reproducibly inhibited lymphocyte adherence ($p < 0.05$), whereas the CD2 antibody failed to modulate binding. Antibodies specific to the epithelial adhesion molecules ICAM-1 and LFA-3 also inhibited the binding of lymphocytes($p < 0.05$).

In conclusion, AEC express functional ICAM-1 and LFA-3, and the adherence of allogeneic lymphocytes is affected by their activation state, and the differentiation state of the epithelial cell.

CYTOMEGALOVIRUS INFECTION AND COLONIC PERFORATION IN RENAL TRANSPLANT PATIENTS

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Cytomegalovirus (CMV) infection in immunocompromised patients is a major cause of morbidity and mortality. In both AIDS and transplant patients, it can induce life threatening organ specific disorders such as pneumonitis, hepatits and gastrointestinal disease. A well documented manifestation of gastrointestinal CMV infection is gastrointestinal haemorrhage but, in contrast, CMV-associated intestinal perforation has only been reported on one previous occasion after transplantation, although it is well documented in AIDS patients.

Here we report three cases of CMV-associated colonic perforation in three renal transplant patients, all of whom developed active CMV infection within 3 months of transplantation.

Three patients received their first cadaveric renal transplant in 1994 and were managed with triple immunosuppression without adjunctive antibody therapy. All had excellent renal function and only one had any evidence of rejection (2 mild acute episodes). All three developed proven CMV infection (2 primary, 1 reactivation/reinfection), diagnosed by CMV antigenaemia testing associated with typical symptoms, and ganciclovir therapy was initiated in the two with primary infection. During the course of their CMV illness each developed abdominal pain with clinical suspicion of peritonitis and proceeded to laparotomy. All three were found to have sigmoid colon perforations with histological evidence of ulceration with many CMV inclusions in both macrophages and endothelial cells. Following bowel resection and defunctioning two made an uneventful recovery and have had the continuity of their bowel restored, but one died within hours of surgery secondary to faecal peritonitis. In the patient with reactivation/reinfection ganciclovir therapy was started postoperatively once the diagnosis of CMV was established.

The perforations reported here occurred within a six month period and are the only cases seen out of nearly 1300 renal transplants performed in our unit. The explanation for the apparent clustering of this rare condition in transplant patients is uncertain.

QUANTIFICATION OF ADHESION BETWEEN CULTURED HUMAN INTRAHEPATIC BILIARY EPITHELIAL CELLS (HIBEC) AND ALLOGENEIC LYMPHOCYTES

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HIBEC are major immunological targets during acute and chronic liver allograft rejection. It is known that cytokine-stimulated HIBEC express potentially immunogenic Class I and Class II MHC antigens and the adhesion molecules ICAM-1 and LFA-3. Our previous studies have shown that comparable levels of these molecules are expressed by HIBEC and immunocompetent 'professional' antigen presenting cells.

Cell-mediated immune responses are dependent on initial establishment of stable cell-cell contact between lymphocytes and their targets. For this reason it was decided that the adhesive interaction between cultured HIBEC and mitogen-activated T lymphocytes should be studied. Static adhesion assays were used to measure the adhesion of activated T cells to monolayer-cultured HIBEC under basal conditions and after stimulation of HIBEC with pro-inflammatory cytokines. Studies were also performed to examine modulation of this adhesion using monoclonal antibodies to block the interaction between specific adhesion molecules.

A novel flow cytometric assay was designed to compensate for variations in HIBEC confluence after cytokine stimulation. This assay allowed evaluation of the mean number of lymphocytes rosetted to each epithelial cell. Each resting HIBEC bound 2.2 ± 0.3 lymphocytes, IFN-γ stimulated HIBEC bound 2.6 ± 0.3 lymphocytes, TNF-α stimulated HIBEC bound 4.2 ± 0.9 lymphocytes and HIBEC treated with both cytokines bound 6.0 ± 1.1 lymphocytes. These values represent an increase in cell-cell binding of 33% following IFN-γ treatment, 63% following TNF-α treatment and of 110% following stimulation with the cytokine combination.

Antibody blocking experiments showed that pre-treatment of the activated T cells with antibodies to CD18, CD11a and CD2 and with ATG (Merieux) significantly blocked their binding to cytokine-stimulated HIBEC (51% with CD18 and CD11a, 21% with CD2, and 44% with ATG; all $p \leq 0.02$). These results demonstrate the importance of the LFA-1→ICAM and the CD2→LFA-3 adhesion systems in stabilising binding between lymphocytes and HIBEC.

The increased adhesion between activated T cells and cytokine-stimulated HIBEC may be related to the susceptibility of these cells to immunological damage during hepatic allograft rejection.

MONOCLONAL ANTIBODY THERAPY IN SMALL BOWEL TRANSPLANTATION

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An attractive immunosuppressive strategy in highly immunogenic transplant models is to combine monoclonal antibodies (MAb) in order to block more than one inter-cellular interaction. In this study we used a combination of an anti-CD4 MAb (OX38) and an anti-LFA-1α MAb (WT.1) in an attempt to prolong survival following small bowel transplantation (SBT) in the rat.

Fully allogeneic vascularised heterotopic SBT was performed between PVG ($RT1^c$) donors and DA ($RT1^a$) recipients. The recipients were given the following daily doses of MAb by intravenous injection on days -1, 0 (day of SBT) and +1.

	OX38	WT.1	Survival (days)	Median
Control	-	-	7, 7, 7, 8, 9	7
Group A	1mg/kg	-	6, 6, 6, 8, 8, 9	7
Group B	5mg/kg	-	5, 11, 12, 13, 14, 17, 20	13 *
Group C	-	1mg/kg	6, 10, 14, 15, 16	14
Group AC	1mg/kg	1mg/kg	7, 7, 7, 13, 16	7
Group BC	5mg/kg	1mg/kg	6, 9, 9, 12, 13	9

* $p = 0.05$ *v.* Controls and *v.* Group A (Wilcoxon rank sum test)

The effects of these protocols on circulating cell populations was investigated in parallel groups of untransplanted rats (u) receiving the same MAb regimens. Regular blood samples were taken for total and differential leucocyte counts and for analysis by flow cytometry. The absolute CD4+ count of group B(u) fell by more than 75%, but by less than 50% in group A(u). Maximal OX38 binding site blockade was approximately 40% and 25% respectively. In contrast, WT.1 blocked more than 80% of its binding sites and appeared to reverse the effects of OX38 because the CD4+ counts of groups AC(u) and BC(u) both increased by more than 50%.

In this study, therefore, combination MAb therapy was not beneficial, possibly because of the increase in circulating CD4+ cells induced by WT.1.

T AND B CELL RESPONSIVENESS TO DONOR CLASS 1 MHC MOLECULES AND PEPTIDES IN LONG SURVIVORS OF KIDNEY ALLOGRAFTS

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Nineteen LEW rats with long-surviving (>100 days) (DA x LEW)F_1 kidney allografts were generated by treating the recipients with cyclosporin A for 10-14 days after grafting. Cyclosporin A completely suppressed the early antibody response to intact DA MHC class 1 molecules in all 19 LEW rats, but 17 of the 19 rats developed antibodies between four and six weeks after grafting, i.e. between two and four weeks after the cessation of cyclosporin A immunosuppression. Thus, in these 17 recipients, T cells responsive for indirect recognition of donor class 1 molecules (for provision of help to B cells) were obviously functional.

Two of the 19 long survivors did not produce antibodies to donor class 1 molecules at any stage. These 2 rats, and one of the 17 rats which did produce antibodies, were immunised with synthetic peptides corresponding to regions of the DA class 1 molecule know to be recognised by LEW CD4+ T cells. None of these long-survivors suffered rejection episodes as a consequence of the peptide immunisation. However, all 3 developed self APC-dependent CD4+ T cell proliferation to the immunising donor peptides, and strong antibody responses to these peptides. In one of these long-surviving rats, the peptide priming resulted in the prompt development of strong antibodies to intact donor class 1 molecules. This long-survivor therefore had potentially responsive, but quiescent, T helper and B lymphocytes to the donor class 1 molecule. In the other long-surviving rat without antibodies to intact donor class 1, peptide priming did not stimulate an antibody response to the intact donor class 1 molecule. This suggests B cell anergy or tolerance in this long survivor. Thus in this model of kidney allograft tolerance, B cell and indirect CD4+ T cell reactivity to donor antigens is the norm, in spite of long-term exposure of the recipient to donor antigens without evidence of rejection. None of the animals develop tolerance for the indirect T cell recognition of donor class 1 antigen. In occasional animals, potentially reactive B cells are present but quiescent because of the absence of T cell help, perhaps as a consequence of suppressor phenomena. And in other occasional animals, B cell non-reactivity (anergy or tolerance) develops.

RAPAMYCIN PREVENTS THE DEVELOPMENT OF CHRONIC REJECTION IN RENAL TRANSPLANTS IN THE F344-LEW MODEL

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Transplantation of kidneys from F344 to Lewis rats (which are MHC antigen identical, non-MHC antigen disparate strains) results in the development of clinical (proteinuria, hypertension, slowly rising creatinine) and histological features (glomerulosclerosis, tubulo-interstitial fibrosis and arterial intimal thickening) of chronic rejection. We have previously used this model to show the importance of immunoglobulin, macrophages and growth factors in the development of these features, confirming that chronic rejection is an immunologically mediated process.

To test this hypothesis, we used drugs with differing effects on the immune system to block various steps in these pathways. **Cyclosporin**, which is immunosuppressive by virtue of its ability to block Il-2 receptors on T-lymphocytes, failed to prevent the development of chronic rejection at any dosage. **Rapamycin**, which as well as inhibiting T-cell function, diminished endothelial IgG1 deposition and growth factor receptor expression and prevented the development of chronic rejection. Prevention was dependent on pre-treatment with rapamycin and administration after the development of the disease did not produce remission. Blockade of growth factor receptors alone using high doses of **angiopeptin** (a non-immunosuppressive somatostatin analogue) also prevented the development of chronic rejection.

Identification of the pathogenesis of chronic rejection explains why current immunosuppressive regimens have been ineffective in reducing the continuing rate of long term graft loss. The use of immunosuppressive agents which inhibit immunoglobulin production, or which modulate growth factors offers hope of a treatment for this condition for the first time.

THE RISK OF NEOPLASIA IN THE EUROPEAN RENAL TRANSPLANT POPULATION HAS BEEN UNDERESTIMATED

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Renal allograft recipients are at an increased risk of neoplasia, although the extent of the problem has not been established in the typical European transplant population. To assess this risk, a comprehensive retrospective study was performed of 918 recipients transplanted at one centre over 24 years. Eight local, regional, national and international record sources were accessed for each patient in order to attempt maximum yield.

The search revealed 70 patients (7.6%) who had developed a neoplastic lesion with 10 having more than one type. More than half (42) were cutaneous lesions: 71% squamous cell carcinoma, 29% basal cell carcinoma.

The risk of developing neoplasia in the first 10 years following transplantation was 14%. By 20 years this had risen to 40%, compared to a 6% cumulative risk of neoplasia in an age-matched control population ($p<0.005$, Kaplan-Meier analysis, Kologorov-Smirnov test).

The full extent of this problem in the European transplant population has been underestimated and, as recipients and grafts survive longer, there is a clear need for both life-long surveillance and closer investigation of these patients.

ANTISENSE INHIBITION OF THE MAJOR PIG EPITOPE FOR HUMAN NATURAL ANTIBODIES

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Human natural antibodies (NAb) which can bind to carbohydrate antigens of pig endothelial cells invariably initiate the destruction of pig tissues. This currently constitutes the main barrier to the use of pig organs in transplantation. The pig Galα1,3Gal epitope has been suggested to be the major target for these NAb. We have used affinity purified human Galα1,3Gal antibodies and anti-human IgM and IgG secondary reagents in FACs analyses to determine the target density of this and other epitopes on pig endothelial cells. Our results show that over 90% of the human IgM antibody targets consist of the Galα1,3Gal epitope whereas less than 5% consist of other epitopes. Human IgG antibodies also bind to less than 5% of epitopes other than Galα1,3Gal.

The pig Galα1,3Gal epitope is made by a specific α1,3galactosyltransferase. We have previously isolated cDNA and genomic clones coding for pig α1,3galactosyltransferase. Expression of the cDNA clone in green monkey COS cells reconstitutes the expression of the Galα1,3Gal epitope in these cells as indicated by binding of IS-B4 lectin as well as affinity purified human anti-Galα1,3Gal NAb. Antisense oligonucleotides designed to bind to the region for initiation of translation of the pig α1,3galactosyltransferase mRNA markedly inhibit the expression of the Galα1,3Gal epitope on pig endothelial cells. Since this epitope appears to constitute almost all of the target structures for human NAb on pig endothelial cells efficient inhibition of this epitope using antisense technology *in vivo* or *ex vivo* should obliterate the hyperacute response to pig tissues.

THE POSITIVE INFLUENCE OF MULTI ORGAN PROCUREMENT ON RENAL GRAFT SURVIVAL

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Purpose: In view of the increasing tendency towards multi organ procurement (1988: 38% ; 1993: 61%), this observational study was undertaken to evaluate whether the renal graft survival was affected by the procurement policy. **Methods:** All first renal cadaveric transplants performed between 1988 and 1993 within Eurotransplant (Austria, Belgium, Germany, Luxembourg, The Netherlands) were analysed (N=9875). **Results:** MOD (Multi Organ Donor) kidneys came from younger, mainly male, donors who more often died of a trauma capitis than the SOD (Single Organ Donor) kidneys. The MOD kidneys were transplanted into younger recipients with a lower pre-transplant immunization, within a shorter cold ischemic time period, over a less good HLA-DR mismatch gradient and were more often preserved with the UW solution compared to SOD kidneys.

TXP	N	DONORS			RECIPIENTS			TRANSPLANTS			
		Median age	Sex Male	Death due to trauma	≤ 5% PRA	Median age	Sex Male	Solution UW	Solution EC	CIP	0-1 HLA-DR mismatch
MOD	4781	29 yr	64%	57%	64%	44 yr	60%	69%	23%	22 hrs	87%
SOD	5094	44 yr	59%	39%	60%	47 yr	61%	17%	66%	24 hrs	90%

The univariate analysis showed that kidneys from MOD's had a significantly better graft survival than kidneys from SOD's. The 1 year and 3 years graft survival for MOD kidneys was 89% and 81% respectively; for SOD kidneys, 83% and 73% respectively. Results of the multivariate analysis demonstrated that the relative risk of graft failure for a SOD kidney versus a MOD kidney was 1.13 (p=0.04).

Conclusion: A statistically significant better outcome of a MOD versus a SOD kidney transplant is currently present, irrespective of the observed differences in donor-patient-transplant characteristics. It is anticipated that a better and more attentive global management of the potential MOD donor at the IC unit and the broader expertise of multi-organ explantation teems may contribute to this benefit.

OUTCOME OF KIDNEYS FROM OLDER DONORS IN OLDER AND IN YOUNGER RECIPIENTS

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While the demand for renal transplant grows, the potential donor pool is diminishing as deaths from head injury and intracranial haemorrhage decrease. This has led to the need to use kidneys from older donors and in the UK 12% were over 60 years in 1993, compared with 3% in 1984.

This analysis looks at 10233 cadaveric transplants using adult donor (19+ years) kidneys which were performed in 33 centres in the UK and Republic of Ireland between 1984 and 1993. Exploratory analysis identified three study age groups, 19-45 years, 46-60 years and 61+ years.

Multifactorial analyses of 3 year transplant survival indicated generally that the risk of failure increased with donor age and with recipient age. However, percentage transplant survival estimates at 3 years suggest a change in trend with kidneys from donors over 60 years faring better in recipients over 60 years than in recipients aged 46-60 years.

Donor Age (years)	Recipient age (years) 19-45	46-60	61+
19-45	76%	75%	71%
46-60	68%	65%	58%
61+	65%	58%	62%

Among 5160 recipients with grafts functioning at 1 year for whom serum creatinine values are available, these values correlated directly with donor age and inversely with recipient age. Mean creatinine values (μmol/l) at one year for the 3 age groups (in ascending order) were 166, 220, 274 for donors and 198, 182, 172 for recipients.

This analysis provides support for age matching in recipients over 45 years so that kidneys from older donors are used preferentially for older recipients.

A SUCCESSFUL STRATEGY TO IDENTIFY ACCEPTABLE HLA MISMATCHES IN HIGHLY SENSITISED PATIENTS AWAITING A RENAL TRANSPLANT.

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Highly sensitised patients (HSP), by definition, have antibodies to a wide range of HLA specificities. Within the UK a scheme has been established whereby HSP are prioritised and kidneys are exchanged if a donor has only one HLA-A or -B mismatch (MM) with the patient and the MM has been previously defined as acceptable. An acceptable mismatch (AMM) is an HLA-A or -B specificity to which the patient does not have antibodies. One accepted strategy for the identification of AMM is to screen HSP against a selected panel of lymphocytes having only one HLA-A or -B MM with the patient. A negative result indicates that MM as being acceptable. Such a strategy involves cytotoxic screening and requires a larger cell panel than is usually available at one centre. In addition when sensitised patients are crossmatched using flow cytometry (FC) that must also be incorporated into the screening programme.

Our protocol involved initial cytotoxic screening of one or two sera with peak antibody reactivity from each of the HSP (Screen 1). Patients known to have only IgM autoantibodies were excluded. An 80 cell panel was selected to include all common and some rare HLA specificities and to avoid common haplotypes. If a patient serum was negative against a panel cell then that serum and up to 10 others from that patient were tested against that cell using FC (Screen 2). When a patient was negative by FC then all available sera were cytotoxic crossmatched against that panel cell (Screen 3). Only after a negative result in Screen 3 were the HLA-A and -B MM between the panel cell and the patient defined as AMM.

Of 35 HSP entering Screen 1, 26 went on to Screen 2 but only 6 of those were then negative and went onto Screen 3. The 6 patients negative by FC had been tested against 8 to 16 cells but were negative only with between 1 and 5 cells. At the end of Screen 3, only 3 of the 6 patients had negative results with 2, 4 and 5 cells respectively and have now been registered in the HSP scheme with 1, 6 and 7 AMM. This was a rigorous but successful strategy for the definition of AMM. However, the workload and hence cost implications were considerable which is an issue that must be addressed when HSP are being considered for transplantation.

INTENSIVE INITIAL QUADRUPLE IMMUNOSUPPRESSION IN THE MANAGEMENT OF SIMULTANEOUS PANCREAS KIDNEY RECIPIENTS

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Between December 1992 and December 1994, we performed 12 consecutive simultaneous pancreas kidney (SPK) transplants in 7 male and 5 female recipients. All patients had suffered long-term insulin dependent diabetes mellitus IDDM (mean duration 21 years) and were in established renal failure (mean duration of dialysis 13 months) prior to transplantation. Post-transplant, intensive immunosuppression consisting of antithymoglobulin (ATG Fresenius R) cyclosporine (CsA), asathioprine (AZA) and prednisone was instituted in all cases. ATG was continued for a total of 10 - 14 days and a CsA whole blood level (Incastar R of $\geq$ 400ng/ml was maintained for the first 30 days post-transplantation. The CsA dosage was then adjusted to maintain whole blood levels in the range of 250-350ng/ml. No patient required dialysis following transplantation and all allografts functioned immediately. All patients received anti-cytomegalovirus (CMV) prophylaxis in the form of intravenous gancyclovir in the immediate post-operative period followed by high dose oral acyclovir. Only 1 patient developed clinical CMV infection which responded to intravenous gancyclovir therapy.

No patient has suffered any episode of acute rejection. With a mean follow up period of 10 months, all patients have excellent renal function (mean serum creatinine 153mmol/L despite the high dose CsA regime employed. Eleven of the 12 patients are insulin independent. One patient developed Type 11 diabetes, 8 months post-transplantation.

In summary, we believe that initial intensive CsA based immuno-suppression is a safe and highly effective regime in the management of SPK recipients without compromising renal function.

THE DETECTION AND CLINICAL IMPORTANCE OF ANTI-ATG ANTIBODIES IN RENAL TRANSPLANTATION.

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Monoclonal and polyclonal anti-lymphocyte preparations are used in solid organ transplantation, both for the induction of immunosuppression and the treatment of steroid -resistant rejection. In our centre rabbit anti-thymocyte globulin (ATG,Merieux) has been used in 30% of patients over the past year and exposure to the xenogenic protein component of this preparation may lead to the development of antibodies directed against the antilymphocyte globulins. Since the ATG is administered with concomitant immunosuppression the likelihood of an individual patient mounting a significant antibody response has been hard to predict. The aim of the present study was to determine the clinical relevance of the presence of antibodies to ATG using both an ELISA and a flow cytometric assay. 27 sera from patients who had received ATG therapy were analysed with both assays and a hundred fold increased sensitivity of the flow cytometric method was found. The cytometric assay was also rapid and specific.

Sequential testing of serum from a renal transplant patient given a second course of ATG for rejection, showed an initial elevated level of anti-ATG antibodies (titre-1/78000) before treatment which rose to a titre of >1/390000 after treatment. This was accompanied by T (CD3+) cell escape from the immunosuppressive regime. The use of a second ATG preparation raised in the horse permitted successful treatment of the rejection episode to be achieved.

This study has shown that the newly developed flow cytometric assay can detect the presence of extremely low, but clinically relevant levels of antibody to ATG to be detected thus allowing accurate monitoring of patients who require second courses of ATG therapy. Indeed in one patient, ATG sensitisation occurred after a single dose and after a time interval of 20 months a rapid antibody response developed which abrogated the therapeutic effect of further ATG therapy.

LANGERHANS CELL MIGRATION FROM SKIN ALLOGRAFTS

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Immediate and permanent skin replacement with allografts would be desirable in the treatment of conditions involving massive skin loss, for example major burns. At present this is not feasible because skin provides one of the most powerful antigenic challenges of any transplant. The reasons for this immunogenicity are not clear but may include the following: tissue-specific alloantigens; delayed revascularisation; upregulation of major histocompatibility antigens on keratinocytes; and especially the presence of Langerhans cells (LC). Langerhans cells, members of the dendritic cell (DC) family, are highly specialised leukocytes with important functions in the initiation of skin allograft rejection. LC migrate from skin grafts, and are uniquely able to stimulate naive T cells, and initiate immune responses within the recipient. Although skin graft rejection has been shown to be dependant on intact lymphatic connections, the evidence that LC migrate to lymph nodes is largely indirect. Therefore, the aim of this study was to track LC migration directly into the lymphoid tissue of the mouse.

Skin was grafted between different strains of mice. Between 1 and 7 days after grafting, rare cells expressing donor major histocompatibility complex (MHC) class-II molecules were detected by immunocytochemistry in frozen sections of draining lymph nodes (frequency 1 cell / 20 sections ; $p < 0.01$ vs syngeneic grafts) and spleen (frequency 1cell / 2 sections; p 0.001). This low frequency of detection may have been due to low numbers of LC leaving the graft (~ 1-2 x 10^5/cm^2 / day; average graft size = 4cm^2). Therefore, various doses of fluorochrome-labelled, LC-enriched epidermal cells were instilled into an abdominal subcutaneous pocket to mimic cell entry to a skin graft bed. Label was detected in draining but not control nodes 24 hours later providing that a minimum of 1 x 10^5 LC was instilled. Using the polymerase chain reaction (PCR) technique we further demonstrated that cells containing donor MHC class-II mRNA were present in draining, but not control nodes, 24 hours after skin grafting or the instillation of donor LC. We conclude that epidermal LC can enter draining lymph nodes after skin grafting, the probable route by which a recipient may become sensitised to a skin allograft. The significance of donor MHC class-II positive cells in recipient spleens is being evaluated in current experiments.

SURVIVAL OF RENAL RETRANSPLANTS CAN MATCH THAT OF PRIMARY TRANSPLANTS.

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Recent reports show that survival rates for renal retransplantation have improved throughout the 1980's with one year graft survival approaching that of primary transplants. The reasons for this are not clear. We have compared the results of 844 primary and 146 retransplanted adult cadaveric transplants performed in our single centre over two periods from 1984-1988 and 1989-1993 to identify any improvement in survival and determine possible causes. Variables subjected to uni- and multivariate analysis were HLA-A, B, DR mismatch(mm), cold ischaemic time(CIT), delayed graft function(DGF), acute rejection, donor age, previous graft survival time and time on waiting list. Immunosuppressive protocols were the same throughout the study period.

Actuarial 1yr & 5yr patient survival for primary and retransplant groups were unchanged between epochs despite increased mean recipient age in the second epoch (39.24yr v 42.27yr $p=0.0005$). One year & 5yr actuarial graft survival in the first epoch for primary and retransplanted groups was 83.9% & 63% and 73.1% & 58.2% with a significant improvement in the second epoch to 87.5% & 75.8% and 88.6% & 76.4% ($p=0.001$). There was no significant difference in the rate of DGF between primary and retransplant groups within or between epochs despite CIT being significantly increased in the second epoch (20.16h v 25.12h $p<0.0001$) and an increase in older donors (>49yr) during 1989-93($p<0.0001$).

During 1984-88, 0 DRmm transplants comprised 45% of primary and 48% of retransplants increasing to 58% and 61% respectively during 1989-93($p=0.0001$). Second epoch 0 DRmm grafts had significantly fewer rejection episodes than 0 DR mm grafts from 1984-88 (mean 0.93 v 0.72 $p=0.02$). Apparent homozygosity of HLA-DR specificities was reduced from 21.4% in the period 1984-88 to 11.3% between 1989-1993 ($p=0.00001$). From 1984-1993 significant improvements were made in lymphocytotoxic antibody screening, crossmatching and HLA-DR definition.

We have shown that survival for retransplants can match primary transplants and propose that this can be achieved by both intensive pre and post-transplant antibody screening as well as minimising HLA-DR mismatch.

PRE-EMPTIVE CADAVERIC RENAL TRANSPLANTATION: OPTIMAL MANAGEMENT OR UNWISE USE OF SCARCE RESOURCES

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Background and aims: Pre-emptive cadaveric renal transplantation (PCRT) maximises the chance of maintaining high quality of life and may avoid the morbidity of dialysis together with the associated financial costs. These benefits are offset by disadvantages which include the possibility of transplantation many months before the need for dialysis, resulting in wasted organ function, an immediate risk of graft failure with conversion to a dialysis-dependent state, and uncertainty of the safety of PCRT: there is some evidence that the immunosuppressive effects of uraemia may improve graft survival.

We have performed a case-control analysis of the outcome of PCRT in one centre and, with the aid of a computer model, have sought to predict a) the optimal time to place patients with chronic renal failure on a cadaveric transplant waiting list with respect to financial costs and b) the implications of PCRT on the utilisation of cadaveric organs.

Methods and results: Patients who underwent PCRT between 1976 and 1993 were compared to a matched cohort of contemporaneous first cadaveric transplant recipients who were dialysis-dependent when transplanted. 100 patients met the criteria for PCRT and they were well matched to the control group with respect to sex, age, blood group, HLA match, sensitisation, donor age, donor source (local retrieval vs. shipped) and year of transplantation. There were no significant differences in patient survival (5 year: 86.6% vs. 90.7%), graft survival (5 year: 69.2% vs. 57.0%) or graft function as assessed by 1, 2 and 3 year plasma creatinine (139±6, 139±7 & 150±20 vs. 150±8, 146±7 & 144±9) for PCRT and controls respectively.

To examine the implications of placement on a transplant waiting list before the need for dialysis, a Markov-type computer-based model was established to predict the cumulative costs and organ usage associated with PCRT. Throughout a wide range of conditions, chosen to mirror those prevalent in UK transplant units, and encompassing patient and graft survival, transplantation rates and the relative cost of transplantation versus dialysis, inclusion on a transplant waiting list before the predicted date of end-stage renal failure was associated with a financial saving at 3 or 5 years but at the expense of greater usage of kidneys. The optimal timing for placement on the waiting list was dependent upon the accuracy of the prediction of time to end-stage failure, the mode of dialysis selected when required, and the expected period on the waiting list before transplantation.

Conclusion: PCRT appears to be safe and can result in cost savings but may increase the total number of organs required. Early inclusion on a transplant waiting list with a view to PCRT can be justified if it allows better overall HLA matching and graft survival, to compensate for the greater organ usage that would otherwise occur.