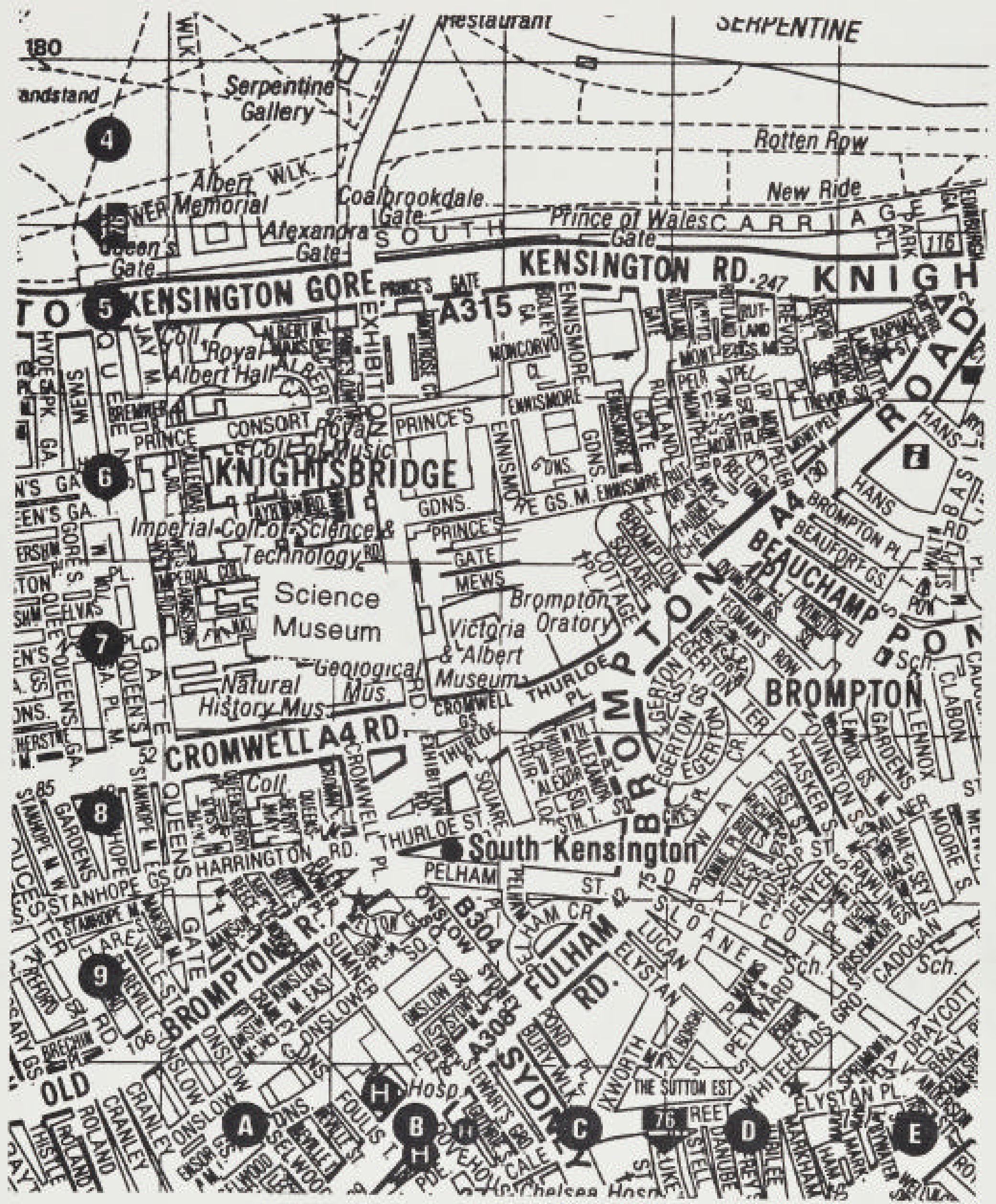
The British Transplantation Society

Autumn Meeting 1996

Queen's Tower and New Biomedical Sciences Building
Imperial College London

October 21st and 22nd 1996

The Royal College of Surgeons, Lincoln's Inn Fields, London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Serpentine Gallery
Rotten Row
New Ride
Albert Memorial
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SOUTH CARRIAGE
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KENSINGTON RD.
KNIGH
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A315
EXHIBITION
ENNISMORE
Royal Albert Hall
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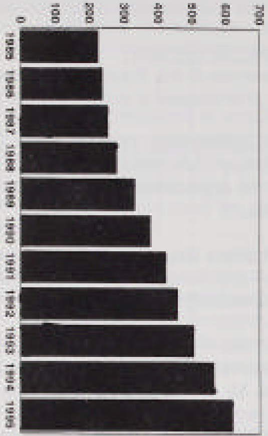
British Transplantation Society Membership



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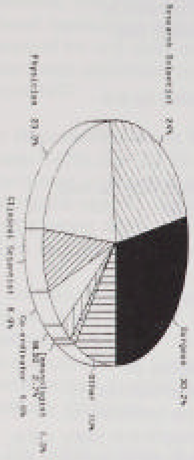

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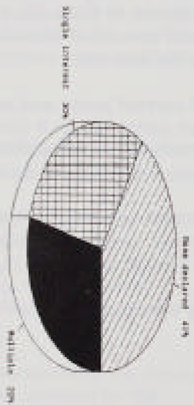

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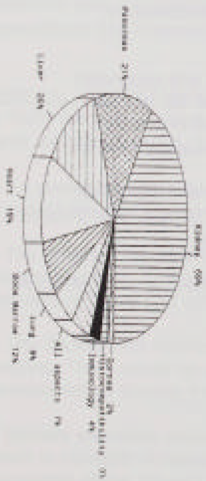

ABSTRACTS SELECTED FOR PRESENTATION

BLOCKADE OF THE CD40 / GP39 PATHWAY AUGMENTS THE CAPACITY OF SMALL RESTING B CELLS TO INDUCE UNRESPONSIVENESS TO ALLOANTIGENS IN VIVO.

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Small resting B cells (srBC) have been shown to induce T cell anergy *in vitro* and to prolong the survival of skin grafts mismatched for the single minor histocompatibility (miH) antigen, H-Y. It has therefore been suggested that they might be potent inducers of tolerance *in vivo*. To test this hypothesis, we examined the ability of srBC to induce tolerance to MHC and miH antigens *in vivo*.

First we confirmed that srBC isolated from the spleen were: (1) MHC class II^+, Ig^+, $B7\text{-}1^-$ and $B7\text{-}2^-$ by FACS analysis; (2) unable to stimulate T cell proliferation in mixed leukocyte culture and (3) able to induce prolonged survival of H-Y mismatched skin grafts when delivered intravenously (iv) one week before transplantation (median survival time (MST) > 100 days, compared to untreated controls, MST = 31 days).

Next, we examined the ability of srBC to prolong the survival of vascularised hetertopic cardiac grafts mismatched for multiple miH, single MHC and multiple miH and MHC antigens. Recipient mice were pretreated with 1×10^7 donor srBC iv on day -14 relative to transplant on day 0.

Mismatch	Donor	Recipient	MST Resting B	MST Control
single miH	B10 male	B10 female	>100 d	42 d
multiple miH	C3H	CBA	>50 d	25 d
single MHC	CBK	CBA	25 d	13 d
multiple miH & MHC	B10	C3H	22 d	10 d

Small resting B cells were found to be unable to induce indefinate (>100 days) survival of grafts mismatched for MHC antigen(s). We hypothesised that this might be due to the activation of the srBC *in vivo*. The CD40/gp39 pathway has been shown to play an important role in B cell activation. Therefore to prevent activation of the srBC after iv injection, recipient mice (C3H/HeJ, (H-2k)) were pretreated on day -14 with anti-gp39 monoclonal antibody (MR1) (250 ug) and either : (1)none (2)donor specific C57BL/6(H-2b) or (3) third party (BALB/c (H-2d) srBC (1×10^7) relative to transplant of a C57BL/6 heart on day 0.

Pretreatment	Graft survival	MST
B6 resting B cell & MR1	23, 29, 44, 50, 52, >100 x4	52 d
B6 resting B cell alone	7, 11, 11, 11, 11, 17, 20	11 d
MR1 alone	10, 12, 14, 18, 24, 24, 24	18 d
BALB/c resting B cells & MR1	9, 12, 15, 18, 18, 25, 28	18 d
BALB/c resting B cells alone	11, 14, 17, 17, 21	17 d

In conclusion, blockade of CD40/gp39 pathway increased the potency of srBC to induce unresponsiveness to MHC antigens in vivo.

A MUTANT CIITA MOLECULE INHIBITS EXPRESSION OF HUMAN MHC CLASS II

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The expression of polymorphic donor MHC class II molecules is of fundamental importance in graft rejection processes. Some cells express MHC class II constitutively, e.g. interstitial dendritic cells, whereas others are susceptible to MHC class II induction following lymphokine stimulation. The transcription of MHC class II genes requires the assistance of a specific nuclear transcriptional activator termed CIITA. It is believed that the CIITA protein consists of at least two domains, the amino-terminal of which acts as a transcriptional activator and the carboxyterminal of which carries the specificity for MHC class II by binding to proteins of the promoter region of class II genes. We have deleted the first 567 bp of the human CIITA cDNA and replaced the initiation of translation with a synthetic variant and/or a nuclear localisation signal. The constructs were transfected into human HeLa cells. Transient as well as stable transfections were followed by MHC class II induction using recombinant human IFN-gamma. Cells transfected with the constructs showed drastically reduced MHC class II expression (down to 10% or less of normal expression) as assessed by FACS analysis of MHC class II cell surface expression as well as MHC class II DRA RT-PCR. Similarly, a B-cell line (DoHH2) that expresses MHC class II constitutively at a high level was transfected with the same constructs transiently. The same assays indicated more than 50% downregulation of MHC class II expression in these cells. Transfection of an empty expression vector, or the deletion construct without initiation codon revealed no reduction of MHC class II expression. We believe the most likely explanation for our results is a successful competition between our construct and the endogenous CIITA. Such downregulation of MHC class II expression in donor organs may drastically reduce their immunogenicity after transplantation.

RETROVIRAL GENE TRANSFER OF A DONOR CLASS I MHC GENE TO RECIPIENT BONE MARROW CELLS INDUCES OPERATIONAL TOLERANCE TO ALLOANTIGENS IN VIVO

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Gene therapy has been used to treat a variety of genetic disorders. This approach may also be used to transduce recipient cells with donor antigen(s) as a means of pretransplant alloantigen delivery to prolong graft survival. This will eliminate the need for the availibity of donor cells before transplantation and the associated risk of graft versus host disease.

Bone marrow cells (BMCs) were harvested from recipient strain mice CBA.Ca ($H2^k$) after intravenous injection of 150mg/Kg of 5-Fluorouracil (5-FU) to deplete the more mature clongenic cells and to recruit primitive stem cells into cycle. Flow cytometry was used to determine the optimal time for 5-FU pretreatment: BMCs were stained for stem cells using c-kit as positive and Cd4, Cd8, B220, Mac-1 and GR-1 as negative markers and with propidium iodide to determine the proportion of cells in cycle.

Intravenous administration of 5-FU increased the bone marrow stem cell population from 0.6% to 1.5% after 12 days and the proportion of cells in cycle from 18% to a peak of 34% after 6 days. 5-FU treatment 7 days before bone marrow harvest was chosen as a comprise, as retroviral vectors only transduce cells in cycle.

The mouse class I MHC gene, K^b, was inserted into a replication defective LNSX retroviral vector. CBA BMCs were cultured with this retroviral vector (K^bYF) for different periods of time and at different concentrations to determine the optimal conditions for gene transduction. Transduced recipient type CBA BMCs were injected into CBA mice together with 2 doses of depleting anti-Cd4 monoclonal antibody 28 days before transplantation of a heterotopic cardiac graft from a fully allogeneic donor, C57BL/10 ($H2^b$), expressing full complement of allogeneic major and minor histocompatibility antigens including the class I molecule K^b.

When $5x10^5$ transduced recipient BMCs that had been cultured for 2 days with the retroviral vector K^bYF were used, long term graft survival (LTGS) was achieved in 44% of recipients. This increased to 58% when $5x10^6$ transduced BMCs were used. Interestingly, shortening the time during which BMCs were incubated with K^bYF in vitro to 4hrs resulted in 100% LTGS in recipients treated with transduced BMCs.

Treatment of the recipient with syngeneic BMCs transduced with a single donor class I MHC molecule before transplantation can induce long term survival of a fully allogeneic cardiac allograft, demonstrating that gene therapy can be used to induce operational tolerance to alloantigens in vivo.

COMPARISON OF ADENOVIRUS GENE TRANSFER TO VASCULAR ENDOTHELIAL CELLS IN CELL CULTURE, ORGAN CULTURE AND *IN VIVO*

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Genetic manipulation of grafts to reduce their capacity to evoke rejection responses is an attractive experimental and clinical objective. We have studied the potential of adenovirus vectors for gene delivery to vascular endothelial cells, because these cells are an immunologically important component of organ grafts. A replication-defective Adenovirus 5 vector carrying the β galactosidase reporter gene was used, and tested for its efficiency for gene delivery to vascular endothelial cells in various situations. Both porcine and human primary vascular endothelial cell cultures were very efficiently transduced (>90%) at adenovirus concentrations of 10^{10} pfu/ml or higher. Cultured rat fibroblasts and keratinocytes were even more readily transduced, with >90% transduction with adenovirus titres of 10^8 pfu/ml or higher. However, non-dividing vascular endothelium *in situ* was very poorly transduced. Pieces of aorta from adult pigs, sheep, rabbit and rat, and pieces of human umbilical artery and vein were studied in organ culture. These showed only occasional positive vascular endothelial cells when exposed to the adenovirus vector at concentrations up to 5×10^{11} pfu/ml. Kidney perfusion studies were also performed. Four rat kidneys, perfused with the adenovirus vector at titres of up to 2×10^{10} pfu/ml and transplanted into syngeneic rats for 2 days, showed only occasional positive vascular endothelial cells. Similar results were obtained with 2 pig kidneys perfused *in situ* with the adenovirus vector at 5×10^{11} pfu/ml and examined 2 days later.
Our data suggest that adenovirus vectors will not be of valua for gene delivery to uninjured vascular endothelium *in situ*, and are therefore unsuited for *ex vivo* genetic manipulation of vacular endothelium in organs for transplantation.

GENETIC MANIPULATION OF CORNEAL ENDOTHELIUM: POTENTIAL FOR MODULATING GRAFT REJECTION.

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One attractive approach to preventing or controlling allograft rejection is to genetically modify the donor tissue prior to transplantation. We have chosen the cornea as a suitable target organ to develop this novel approach as (i) the well defined anatomy of the cornea simplifies the introduction of therapeutic genes (ii) the cornea can be cultured *ex vivo* for up to a month, allowing *in vitro* assessment of the kinetics and efficacy of gene transfer and (iii) it is relatively simple to monitor the effects of gene transfer by direct observation through the cornea and by measurement of corneal thickness.

We have concentrated on the corneal endothelium, as this is a critical target of the rejection response, and damage to the endothelium is a major reason for loss of grafts. In initial experiments we investigated the efficacy of gene transfer using either adenoviral or liposome delivery of the β galactosidase marker gene. We have shown that adenovirus is capable of highly efficient gene delivery to the corneal endothelium, with up to 100 % of the cells expressing the marker gene. No significant expression is seen in the stroma or epithelium. Analysis of the kinetics of gene expression following *in vitro* culture indicate that only transient expression is seen, with levels of β galactosidase becoming undetectable 21-28 days after infection (the exact kinetics varying according to the nature of the promoter or the species studied). Similar kinetics are seen *in vivo* following transplantation of genetically modified rabbit corneas. The genetic modification of corneas prior to transplantation does not induce any significant inflammatory responses, nor does it affect the function of the endothelial cells in controlling corneal clarity and thickness.

Liposomes are less efficient than the adenoviral vectors at introducing the marker gene. Again gene expression is only seen in the corneal endothelium, but the efficiency is very variable, with between 1-10% of cells expressing β galactosidase. Clearly, while the non-immunogenic nature of liposomes is appealing, at present they are only suitable in settings where relatively low expression of the therapeutic gene is needed.

We are now examining the expression of two potential therapeutic genes in the cornea, soluble forms of CTLA4 and the tumour necrosis factor receptor (TNF-R). Both constructs are made as fusion proteins with the Fc of IgG (CTLA4-Ig and TNF-R-Ig). The CTLA4-Ig construct inhibits B7.1/B7.2 interactions with CD28/CTLA4, thus interfering with the initial phase of the rejection response. TNF-R-Ig blocks the action of TNF, which is found in high levels in the anterior chamber of the eye during graft rejection. Thus it will act later, modulating the effector arm of the response. We have shown that, following infection of human, rabbit and rat corneas with adenoviral vectors encoding CTLA4-Ig and TNF-R-Ig, high levels of the fusion protein are secreted by the endothelial cells. These molecules are active, as determined by binding to B7 expressing transfectants or by blocking the cytotoxic action of TNF in a bioassay.

These data suggest that modification of donor cornea prior to transplantation is a feasible strategy for preventing or controlling allograft rejection. This is important not only in the context of corneal transplantation, but also in the development of similar gene-based approaches to modulating rejection of other transplanted tissues or organs.

GRAFT AND AQUEOUS HUMOUR INFILTRATING CELLS IN RAT CORNEAL ALLOGRAFT REJECTION

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Purpose To compare the phenotype of cells infiltrating the cornea and aqueous humour in the anterior chamber at intervals following clinically observed onset of rat corneal allograft rejection.

Methods F344 ($RT1^{lv1}$) rats received orthotopic corneal isografts or Wistar-Furth ($RT1^{u}$) donor allografts. No immunosuppressant was given. Graft recipients were examined daily for clinical signs of rejection. At days 1-3 or 5-8 following observed onset of rejection, 2-4μl of aqueous humour was removed from the anterior chamber for flow cytometry, the animal killed, and the anterior segment of the eye (including graft) snap frozen for immunohistochemistry. Similar specimens were taken from control isograft recipients at equivalent intervals following transplantation.

Results Signs of rejection were observed in 25 of 26 allografts at a median time of 18 days. No isografts were observed to undergo rejection. Flow cytometric analysis of aqueous humour identified large numbers of infiltrating cells during rejection, in contrast to the almost acellular aqueous in isograft recipients. Aqueous infiltrates were predominantly lymphocytic, $CD8^+ > CD4^+$ at days 1-3 and $CD4^+ > CD8^+$ at days 5-8. Immunohistochemistry identified infiltrating cells in allograft epithelium, stroma and as focal aggregates on donor endothelium. Earliest infiltrating cells were macrophages, NK cells and neutrophils; later infiltrates were predominantly lymphocytes, $CD4^+ > CD8^+$ at all timepoints examined. ICAM-1 expression was found on endothelial cells of iris and corneal vessels, and donor but not recipient corneal endothelium.

Conclusions Corneal allograft injury at the time of rejection is mediated by cells migrating to the donor epithelium and stroma from recipient corneal stroma/limbus and migrating also through the anterior chamber to the donor endothelium from the recipient iris vessels. Although higher proportions of $CD4^+$ cells were found in the stroma at all timepoints following onset of rejection, higher proportions of $CD8^+$ cells were found in aqueous at earliest examination times.

A RAPID MOLECULAR METHOD TO GENOTYPE ABO BLOOD GROUP AND SECRETOR STATUS.

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It is our hypothesis that kidneys from blood group A^2 donors may be used for transplant into non-A recipients where the anti-A titre is low and ATG is used as induction therapy. In addition, we believe that organs from non-secretor A^2 donors may be used in any non-A recipient. The problem of donor-recipient imbalance in terms of blood group was defined by the Birmingham group at the spring 96 BTS meeting. Positive "social" suggestions were made to readdress this imbalance, which would be complemented by the redirection of HLA matched A^2 donor organs.

ABO blood groups have until recently been defined by traditional serological methods, with the A^2 phenotype being characterised by a weaker expression of A antigen compared to A^1. Furthermore, it has been shown that there is significantly reduced renal expression of A antigen in A^2 individuals and this forms the basis of our hypothesis. We believe that renal expression would be further decreased if the individual was a non-secretor A^2, as expression of the ABH antigens within the kidney is controlled by both the *H* gene and the *Se* gene.

The elucidation of the ABO and secretor transferase nucleotide sequences has enabled the ABO blood groups and secretor status to be genotyped, with the majority of methods in the literature based on PCR-RFLP. Since low A antigen red cell expression is not always be due to the mutation of the transferase sequence we have devised a rapid molecular method to define ABO and secretor status utilising the PCR-SSP methodology. This can be incorporated into the PCR-SSP method used to HLA-type organ donors within this laboratory to allow the ABO genotype, secretor status and HLA-type to be defined simultaneously.

This molecular method allowed the retrospective genotyping of 150 consecutive cadaver renal donors for ABO and secretor status to assess the potential of transplanting non-secretor A^2 donor kidneys into non-A recipients. Preliminary results indicate phenotype frequencies of the blood group A donors are 77% and 23% for A^1 and A^2 subgroups respectively, whilst 22% of the donor sample population are non-secretors. These encouraging results coupled with a detailed analysis of HLA type and antibody status of our panel suggests that use of A^2 donors would also be a useful adjunct to our current strategy for transplanting highly sensitised patients.

OKT_3 IN RENAL ALLOGRAFTS : SURVIVAL AND COMPLICATIONS

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Previous reports have suggested that the use of OKT_3 may increase the risk of life-threatening infection and/or malignancy, particularly of early lymphoproliferative disorders (LPD). We retrospectively examined the records of 40 patients who had received OKT_3 for steroid-resistant rejection between 1987 and 1995, and compared the outcomes of these patients with age, sex, HLA-matched kidney allograft recipients who were transplanted contemporaneously in the same period. We used dual therapy, cyclosporin and prednisolone, as our primary immunosuppressive therapy.

	OKT_3-treated (n=40)	**Control (n=40)**	**p-value**
Age mean±SD(range)	43±12 (17-67)	46±10 (24-66)	NS
Sex	19F 21M	19F 21M	NS
Follow-up (months)	34±31 (0-105)	52±26 (1-91)	<0.001
HLA:total mismatches	4.2±1.2	4.2±1.2	NS
HLA:DR mismatches	1.2±0.7	1.2±0.7	NS
Rejection episodes	2.7±1.4 (1-6)	0.4±0.6 (0-3)	<0.001
Creatinine at 3 months (μmmol/l)	282±178	170±89	<0.004
Creatinine at 12 months (μmmol/l)	219±119	141±46	<0.002
Graft survival at 12 months	26/40	38/40	<0.001
Patient survival at 12 months	36/40	39/40	NS
Severe infection in first 6 months	11/40	2/40	<0.01
Malignancy	2/40	0/40	NS
Deaths after 12 months	8/36	3/39	NS

The surprise finding is the increased number of deaths, although not statistically significant, in the OKT_3-treated patients during the follow-up period due to a predicted increase in severe infections as well as an unexplained increase in cardiovascular and cerebrovascular events . There was no significant increase in the occurrence of malignancies; no case of LPD was observed despite 14 patients receiving both OKT_3 and ATG. Not unexpectedly, both graft survival and creatinine levels are significantly better in the control patients. Kaplan-Meier survival analysis also showed significant difference in the long term graft survival in the OKT_3 patients with a median graft survival of only 35 months. However, one has to take into consideration of the adverse circumstances of refractory acute rejection in the interpretation of these less than satisfactory findings.

PROLONGATION OF ALLOGRAFT SURVIVAL BY ANTI-IL-12 ANTIBODY

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Interleukin-12 is a key immunoregulatory cytokine which promotes a TH 1 type immune response and is likely, therefore, to play an important role in allograft rejection. We investigated the role of IL-12 in graft rejection by using a neutralising polyclonal antibody against IL-12 at the time of grafting C57Bl/6 mice with semi-allogeneic (C57Bl/6 x DBA/2) F1 skin grafts (0.5mg anti-IL-12 injected i.p. at day -1, 2, 5, and 8 with grafting on day 0). Mice treated with anti-IL-12 alone did not show prolonged graft survival compared to untreated controls (MST 11 days in both groups). Interestingly, however, when grafted mice were pre-treated with a combination of anti-IL-12 and intravenous donor strain splenocytes ($5x10^7$ cells at day -7, and anti-IL-12 at day -8, -5 -2 and 1), skin graft survival was significantly prolonged (MST 17 days). Survival of grafts in mice treated with i.v. splenocytes alone was no different from controls (MST 10 days).

Splenocytes from graft recipients were cultured with irradiated donor splenocytes and supernatants harvested and analysed for their cytokine content by ELISA. Cells from unmodified graft recipients produced high levels of both IL-2 and gamma interferon (γIFN). Grafted mice pre-treated with donor strain splenocytes showed high levels of γIFN but little IL-2. In contrast, animals treated with both splenocytes and anti-IL-12 showed markedly reduced IL-2 and γIFN production together with increased production of the TH 2 cytokine IL-5.

Conclusion: pre-treatment with anti-IL-12 and donor splenocytes enhances murine skin graft survival and is associated with a decrease in TH 1 and an increase in TH 2-type cytokine responses.

HEPATIC CYTOKINE mRNA EXPRESSION IN TOLERANT LIVER RECIPIENTS :

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Background and aims: Clinically, a proportion of liver transplant recipients are tolerant to their allografts off immunosuppression. In experimental models of liver transplantation elevated levels of IL-4 and IL-10 (Th2 type cytokines) are believed to play a major role in graft acceptance. In contrast, IL-2 and IFN-γ (Th1 type cytokines) are reported to be up-regulated during graft rejection. The aims of the present study were i) to identify cytokine mRNA expression in liver allograft from 17 long-term recipients, ii) to establish whether an alteration in the balance between Th1/Th2 type cytokines may be responsible for the maintenance of tolerance in clinical transplantation.

Patients and Methods: liver biopsies from 6 *tolerant*, 6 time-matched *partial tolerant*, 5 acute cellular rejection and 4 normal donor liver were investigated. RNA was extracted using RNAzol, cDNA was synthesised using oligo-dT, MMLRT and the levels of IL-2, IL-4, IL-10, IFN-γ and GAPDH (a house keeping gene) mRNA expression were detected using semi-quantitative RT-PCR. The PCR products were run on an agarose gel and visualised under UV. Three μl of products were transferred into a nylon membrane and hybridised using ^{32}P labelled specific probes for each cytokines and dot blotting. The blots were autoradiographed and the dots were counted using a scintillation β-counter.

Results:

	IFN-γ	***IL-2***	***IL-4***	***IL-10***
Donor Liver	3/4	0/4	0/4	0/4
Acute Rejection	5/5	3/5	0/5	0/5
Partial tolerant	4/6	1/6	1/6	1/6
Tolerant	5/6	4/6	3/6	3/6

(p = ns)

Conclusion: i) The results of the *in situ* cytokine expression of IFN-γ mRNA in the groups studied herein suggest this cytokine is constitutively expressed in the liver tissue. ii) The pattern of IL-2 mRNA post liver transplantation is overlapping. iii) The absence of IL-4 and IL-10 mRNA in the donor liver and acute cellular rejection are in favour of the immunosuppressive effects of the latter cytokines and their possible involvment in the graft acceptance and tolerance in long term liver recipients.

LUNG TRANSPLANTATION WITH AND WITHOUT CARDIOPULMONARY BYPASS.

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Lung transplantation may require the use of cardiopulmonary bypass (CPB). Many units have avoided its use where possible due to fears of severe pulmonary reperfusion injury, early graft dysfunction and increased blood loss.

We have examined these effects in 85 consecutive single lung transplants, of which 21 were performed on cardiopulmonary bypass. The mean CPB time was 140.2 minutes in these cases.

Donor organ ischaemic time was not significantly different in bypass (CPB) and non-bypass (NCPB) groups (275.5 mins vs 257.3 mins respectively ;p=0.31).

Pre-operative inspired oxygen (FiO_2)/arterial oxygen content (PaO_2) ratio was not significantly different between NCPB and CPB single lung transplant groups.

At 1 hour and 24 hours post operatively the FiO_2/PaO_2 ratio was no different (mean 3.3 and 3.42 in NCPB cases; 3.55 and 3.73 in CPB patients, p=0.44 and p=0.71 respectively). Similarly, extubation times were not influenced by the use or otherwise of CPB.

Mean blood loss, however, was greater post operatively in those cases performed on bypass (1252.2ml vs 989.3ml), although this was not statistically significant (p=0.29).

Although the use of fresh frozen plasma (FFP) and platelets was similar in the two groups (p=0.6 and 0.5 respectively), more blood was transfused during the post operative care of patients undergoing single lung transplantation performed on bypass (p=0.016).

In conclusion, fears of poor post operative lung graft function after operation involving cardiopulmonary bypass appear unfounded. We could detect no difference in function at 1 or 24 hours, nor was there any difference in extubation time.

It is clear, however, that the use of CPB, appears to increase post operative bleeding and the need for transfusion.

RENAL TRANSPLANTATION IN CHILDREN; REPORT OF THE UNITED KINGDOM TRANSPLANT SUPPORT SERVICE AUTHORITY 1995

Verrier Jones K

On behalf of the UKTSSA Users' Kidney Advisory Group, UK Transplant Support Service Authority, Bristol, UK.

The United Kingdom Transplant Support Service Authority (UKTSSA) provides a national computerised database for the distribution of cadaveric organs to the most appropriate recipients based on agreed rules. Data on outcome are collected annually. This report describes the results of renal transplants in children aged 0 to 18 between 1984 and 1993.

During the ten year audit 16606 renal transplants were registered including 1406 children (8.5%). Follow up data were available on 914: 0-4 yrs 119 (13%), 5-14 yrs 489 (53.5%), and 15-18 yrs 306 (33.5%). The commonest causes of renal disease were pyelonephritis/interstitial nephritis (28%), congenital renal dysplasia/hypoplasia/malformation (17%) and glomerulonephritis (17%). The number of children receiving renal transplants has remained constant over the study period. The majority have been carried out in 14 paediatric nephrology centres. One year graft survival was 73%, 72% and 80% for 1983-85, 1986-89 and 1990-93; five year survival was 58% and 57% for 1983-85 and 1986-89. Death was recorded in 48 children.

The relative risks of graft failure decreased with year of transplantation (0.65* for 1990-93 vs 1.0 for 1984-86) and increased with donor age under 10 years (1.4* for 5-10 yrs, 3.04* for 0-1 yrs), non-beneficial matching (1.92*), non-trauma cause of donor death (1.25*), shipping to other units (1.04) and graft number (1.13 for regraft). Recipient age had relatively little effect overall (1.0 for 15-18 yrs vs 0.81 for 5-14 yrs vs 1.06 for 0-4 yrs); however epoch analysis demonstrated a significantly increased risk in the youngest children in the first three months after transplantation corresponding to the increased incidence of renal vascular thrombosis in this age group in the immediate post-operative period.

* $p < 0.05$

COMPETING RISK ANALYSIS OF THE RENAL TRANSPLANT WAITING LIST IN EUROTRANPLANT

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Introduction: This retrospective study was performed in order to identify patient groups with low transplant chances and to evaluate allocation strategies. ***Patients and methods:*** All patients registered for their first renal allograft between 1.1.80 and 31.12.93 (N=40636), in the Eurotransplant area were selected. The influence of patient characteristics like age (children versus adults), HLA phenotype frequency: expressed as the number of possible zero HLA-A-B-DR mismatched donors in the same period and degree of peak immunization, on the outflow towards transplantation was studied. The competing risk methodology was applied as the outflow from the waiting list [WL] was not a single distinct type: some patients died before they were transplanted, others were removed from the WL without being transplanted. ***Results:*** The chances (in percentages) for a transplantation within the next year in relation to the time already spent on the waiting list are given in the table. (NR=newly registered patients N=40636, 2y=patients who waited at least 2 years N=16592, C=children, A=adults).

	AGE		BLOOD GROUP				HLA - FREQUENCY					%PRA			AV
	C	A	A	AB	B	O	0	1	2-4	5-13	14+	0-5	6-84	85+	
NR	61	29	34	49	35	13	19	24	28	34	40	34	21	30	29
2Y	24	21	24	22	25	13	16	18	21	26	28	25	15	14	21

Once patients had spent some time on the waiting list, their chances for being transplanted within the next year decreased. These chances were different for the distinct patient groups: children, blood group AB patients, patients with a frequent HLA phenotype and non immunized patients had a significant higher chance of transplantation. ***Conclusion:*** Competing risk analysis allows us to characterize the long waiting patients and to evaluate, post factum, the allocation strategies. The aim of an equitable allocation policy is to strive for balance among the distinct patient groups.

COMPARISON OF THE RESULTS OF RENAL TRANSPLANTS FROM CONVENTIONAL AND NON-HEART BEATING CADVERIC DONORS

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In an attempt to address the shortage of conventional kidney donors a non-heart beating donor (NHBD) organ retrieval programme has been established. This paper evaluates the renal function and graft survival of NHBD renal transplants (n=30) in comparison to conventional heart beating donor (HBD) cadaveric kidneys (n=114) performed during the same 3 year period.

The principle donor sources were patients found to be dead on arrival or dying following failed attempts at resuscitation in the Accident Department or ward based patients dying as a result of intracerebral haemorrhage. After death in-situ kidney perfusion and cooling was achieved using an intra-aortic catheter inserted via femoral artery cut down. The maximum acceptable period without cardiac massage (absolute first warm time) was 40 minutes.

Forty-four NHBD kidneys were retrieved over 3 years; 30 were transplanted locally, 8 were used in other UK centres and 6 kidneys were not used. Post-operative dialysis was required following all NHBD transplants for a median interval of 22 days (range 7-63 days). Twenty-six NHBD transplants (87%) functioned and there were four primary non-functions (PNF=13%); in HBD kidneys the PNF rate was 2.6% ($P=0.034$ vs NHBD). The median (95% CI) serum creatinine at 24 months was 227 (178-276) μmol/l in NHBD kidneys compared to 175 (147-203) μmol/l for HBD kidneys. The actuarial 2 year graft survival rate for NHBD kidneys (first warm time < 40 mins) was 82% compared to 89% for HBD kidneys ($P=0.07$). During the period under study NHBD organs accounted for 21% of the total programme.

NHBD kidneys have a significantly higher rate of primary non-function but yield acceptable renal function and graft survival at two years follow-up and have proved a valuable additonal source of transplant kidneys.

BENEFICAL EFFECT OF HLA MATCHING IN PAEDIATRIC RENAL TRANSPLANTATION

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Prior to 1995 this unit allocated paediatric kidneys on the basis of negative crossmatch and waiting time. In 1995 a matching policy was introduced such that each patient was allocated individualised HLA minimum matching criteria. The number of cadaveric transplants performed, A,B,DR matching achieved and graft survival for the years 1991 - 1996 are shown below.

	1991	1992	1993	1994	1995	1996 Jan-June
No.transplant	19	11	17	13	14	10
Mean no. mismatches	4.0 +/- 1.3	3.6 +/- 1.3	3.2 +/- 1.5	3.4 +/- 1.7	1.9 +/- 0.9	2.5 +/- 1.2
6 month graft survival	68%	56%	82%	46%	93%	(100%) at 2 mths

When HLA DR matching was analysed it was found that in 1995/96 50% of grafts were fully DR matched compared to 13% of grafts performed between 1991-1994. Cumulative graft survival and the level of antibody production resulting from graft failure was determined for each category of DR match.

DR mismatches	graft survival	panel reactivity post graft loss
0	88 %	20 %
1	67 %	55 %
2	67 %	67 %

The results show that it has been possible to improve the HLA matching achieved in cadaveric renal transplantation without a decrease in the number of transplants performed. The benefits of using HLA matching as a factor in the allocation of kidneys are demonstrated by the improved graft survival in patients receiving 0DR matched grafts and by the lower levels of sensitisation which result from the loss of DR matched grafts.

HLA COMPATIBILITY IN HEART TRANSPLANTATION: A DIFFERENTIAL ROLE IN SHORT AND MEDIUM TERM PATIENT SURVIVAL.

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Recent studies demonstrating a beneficial role of HLA matching in cardiac transplant outcome has led some centres to advocate prospective HLA matching policies. We have performed a univariate and multivariate analysis, examining the short and medium term influence of HLA matching on 556 consecutive primary heart transplants performed at Papworth Hospital between 1983 and 1994. Overall graft survival at one, three and five years was 80%, 74% and 67% respectively. Sixteen grafts failed within five days and were not considered in the analysis of the HLA matching and graft survival data.

Complete HLA-A, -B and -DR typing data was available on 477 transplant pairs. The results demonstrate a 12% one year survival advantage for 31 patients with 0-2 HLA antigens mismatched compared to 3-6 mismatches. The influence of each individual locus was 6.1%,8.4% and 5.4% for zero HLA-A,-B or -DR mismatches compared to two mismatches respectively. However, when considering outcome from one to five years, analysis of the role of each locus revealed marked differences. HLA-A matched grafts had a 24% lower survival compared to two antigens mismatched (p=0.009). Furthermore, 34% of HLA-A matched grafts failed between one and five years compared to only 5% of HLA-B matched grafts (p=0.013, Table 1).

Table 1. Relationship of HLA-A and HLA-B matching combinations with five year actuarial graft survival for transplants which function beyond one year.

HLA-A match	HLA-B match	No. at risk	No. of deaths	5 year actuarial survival (95% CI)
Yes	No	43	12	66% (50-82) [a]
Yes	Yes	2	1	
No	No	328	39	86% (81-90) [b]
No	Yes	21	1	95% (85-100) [c]

[a] vs [b] p=0.001, [a] vs [c] p=0.013, [b] vs [c] p=0.212.

Our findings indicate that although HLA matching is effective at reducing acute graft loss, in the longer term HLA-A matching impairs survival. We postulate that HLA-A may serve as a restriction element for indirect presentation of allopeptides or tissue specific minor histocompatibility antigens, facilitating chronic graft loss. A blanket approach to prospective matching for heart transplants may be premature for optimal long term survival.

EVALUATION OF CIRCULATING αGST, sICAM-1 AND sE-SELECTIN AS INDICATORS OF REJECTION EPISODES IN LIVER TRANSPLANT RECIPIENTS.

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Background Histology examination of liver biopsies remains the gold standard for diagnosis of acute rejection. However there is an associated risk which may limit the repeated use of biopsy and less invasive (ie serological) diagnostic tests are required where it is contraindicated because of impaired coagulation/thrombocytopenia. An increase of transaminases has often been used routinely for detecting hepatocellular damage but these tests are insensitive and non-specific. On the basis of its plasma half life (about 1hr) and homogeneous distribution in the liver the isoenzyme alpha glutathione transferase (αGST) has been suggested as a sensitive marker of liver damage during hepatic allograft rejection. The local expression of adhesion molecules is crucial for recruitment, localisation and subsequent movement of leucocytes from the blood into sites of inflammation. Elevated levels of adhesion molecules such as soluble intercellular adhesion molecule-1 (sICAM-1) and soluble E-selectin have been reported in acute and chronic inflammatory disorders including allograft rejection. The aim of the study was to evaluate the utility of αGST, sICAM-1 and sE-selectin singly and in combination as markers of liver allograft rejection.

Results During 25 biopsy proven acute rejection episodes in 19 patients, αGST, sICAM-1, and sE-selectin were elevated by 5.1, 2.2 and 2.3 fold compared to time matched non-rejectors with means(±se) 50.8±13.6μg/l vs 10.1±1.6μg/l, p=<0.001; 1566±282μg/l vs 714±112μg/l, p=<0.002; 269± 28μg/l vs 119±45μg/l, p=<0.04. In 8 of 19 rejection episodes (42%) αGST decreased towards baseline before subsequent rises associated with rejection. Following successful anti-rejection therapy with high dose steroids, αGST fell rapidly towards baseline compared to conventional liver function tests (LFTs). Values >50% above the upper limits of normal for sICAM-1, sE-selectin and αGST were detected 24 to 48 hrs before diagnosis of rejection or treatment in 100%, 92.3% and 76.2% of acute rejection episodes respectively. Performance of assays using a cut off 50% higher than control values showed predictive accuracies of 76.7% (αGST), 77.3% (sE-selectin) and 61.5% (sICAM-1). These values were not improved using combinations of test results. Only αGST discriminated between patients with acute and chronic episodes (50.8±13.6μg/l vs 10.8+2.3μg/l, p=<0.001) respectively and between patients with acute rejection or infectious episodes (50.8±13.6μg/l vs 6.5±1.1μg/l, p=<0.001 respectively).

Conclusions

1. GST may be more sensitive and specific for detecting acute rejection episodes than conventional LFTs such as AST.
2. Its predictive accuracy in acute rejection (76.7%)) is not improved by combining test results with those measuring the release of cell adhesion molecule markers eg sICAM-1 and sE-selectin.

DONOR-SPECIFIC HYPORESPONSIVENESS IN CARDIAC TRANSPLANT PATIENTS

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Objective
To quantify the direct anti-donor alloresponse in recipients of heart grafts at one year or more following transplantation

Methods
8 patients receiving maintenance cyclosporin and azathioprine immunosuppression were investigated. All had developed severe chronic rejection within their first post-transplant year.
Limiting dilution analysis (LDA) is the most sensitive and quantitative technique for measuring the frequency of alloreactive T cells. The frequencies of recipient T helper and cytotoxic cell precursors with direct anti-donor allospecificity were determined following transplantation. Limiting numbers of recipient T cells were cultured with fixed numbers of irradiated donor derived splenic antigen presenting cells (APC's). Plates were irradiated prior to the addition of an IL-2 sensitive indicator cell line (CTLL) or ^{51}Cr labelled targets. Frequencies were calculated from the proportion of wells negative for IL-2 (HTLf) or ^{51}Cr release (CTLf) at each recipient cell dilution utilising a standard mathematical model.
Frequencies were compared to those generated between the recipient T cells and a third party stimulator with at least equivalent HLA mismatch. As an additional control, third party responder T cells were cultured with donor-derived and third party spleen cells. Stimulator cells were used only if they exhibited equivalent immunogenicity to third party cells (data not shown).

Results
Following transplantation the direct anti-donor alloresponse is substantially reduced in 5 out of the 8 patients studied.

Patient (Years post Tx)	HTLf Patient vs donor APC	HTLf Patient vs 3rd party APC	CTLf Patient vs donor APC	CTLf Patient vs 3rd party APC
Patient A (1.5)	1/27223	1/31484	1/59442	1/38892
Patient B (3.5)	1/13880	1/25289	1/33773	1/39475
***Patient C (4.0)**	**1/102218**	**1/11829**	**<1/500000**	**1/30392**
***Patient D (4.0)**	not performed	not performed	**<1/500000**	**1/10359**
Patient E (6.0)	1/64974	1/62747	not performed	not performed
***Patient F (6.0)**	**1/206294**	**1/22105**	**1/118365**	**1/41880**
***Patient G (6.5)**	**1/169104**	**1/49966**	1/66982	1/20319
***Patient H (7.0)**	**1/178500**	**1/10455**	**1/254697**	**1/37200**

*** Exhibit donor specific hyporesponsiveness**

Conclusions
These are the first data utilising LDA to demonstrate and quantify donor specific hyporesponsiveness in cardiac transplant patients. These findings may have implications for monitoring, long term graft outcome and adjustment of immunosuppression. Our study continues to recruit patients in order to characterise more completely the incidence of hyporesponsiveness, the time of its occurrence and its relationship to chronic rejection.

CTLA4Ig and anti-CD2 monoclonal antibody synergise to prolong cardiac allograft survival in the DA to Lewis high responder rat strain combination

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T cell activation requires engagement of the T cell receptor as well as provision of costimulatory signals through T cell surface molecules such as CD28 and CD2 whose respective ligands are B7 and CD48 on antigen presenting cells. In this study we have examined the effect of *in vivo* blockade of CD28/B7 and CD2/CD48 receptor/ligand interactions, both individually and simultaneously, on cardiac allograft survival in low- and high-responder rat strain combinations.

In the low-responder Lewis ($RT1^l$) into DA ($RT1^a$) strain combination, administration of anti-CD2 mAb OX34 (5mg/kg on days -1 and 0) produced long-term graft survival (MST >100 days). Similarly treatment with CTLA4Ig (0.5mg on day 2) led to long-term survival of Lewis heart grafts in DA recipients (MST >100 days). However, in the high responder DA into Lewis combination treatment with either anti-CD2 mAb or with CTLA4Ig alone led to a modest increase in graft survival (MST 20 days and 23 days respectively) and failed to ensure long-term graft acceptance. In contrast, when Lewis rats were treated with a combination of both anti-CD2 mAb and CTLA4Ig (given as above) prolonged survival of cardiac allografts was obtained (MST 60 days) with permanent engraftment in some cases. The mechanisms maintaining long-term survival in such animals is currently being studied; preliminary analysis has shown that T lymphocytes obtained from Lewis rats with a long-standing DA heart graft display normal *in vitro* proliferation to DA stimulator cells suggesting that active suppression rather than deletion or anergy may be responsible.

These results suggest that simultaneous blockade of the B27/CD28 and CD48/CD2 pathways at the time of transplantation has a synergistic and potent effect in promoting long-term allograft survival.

NHS EXECUTIVE "GUIDANCE ON THE MICROBIOLOGICAL SAFETY OF HUMAN TISSUES AND ORGANS USED IN TRANSPLANTATION" - WHAT DO THE ORGAN RETRIEVAL CENTRES ACTUALLY DO?

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In March 1996 the NHS Executive issued a document entitled "Guidance on the microbiological safety of human tissues and organ used in transplantation" to all U.K. transplant centres. It was created by a 13-strong working party which included two transplant surgeons. Conclusion 5 states: "All donors must be tested for antibody to HIV 1 and 2, antibody to HCV, HBsAg and syphilis; any positive donors should not be used. The immune status against CMV and toxoplasma should be known".

In our unit we had not routinely tested donors for syphilis or toxoplasma. We had not considered HBsAg to be an adequate test for hepatitis B and have also done anti-HBc since 1993, following transmission of hepatitis B from HBsAg negative donors in our programme. Therefore, we decided to circulate the 25 U.K. kidney retrieval centres to ask their policy pre-guidelines and again 5 months later; 23 responded.

All units tested for HIV 1 and 2, HCV, HBsAg and CMV both before and after the guidelines were issued. Before the guidelines 5 units screened for syphilis and 3 for toxoplasma. Five months later 10 units had initiated screening for syphilis and 6 for toxoplasma whilst the rest had not altered their previous policy. Anti-HBc was not included in the guidance document but 4 units test for this routinely.

We conclude that as few units have altered their donor screening policy the significance of syphilis and toxoplasma testing should be reviewed. Some thought should be given to anti-HBc as it is our current practice not to retrieve from donors who are HBsAg negative but anti-HBc positive.

NON-INVASIVE ASSESSMENT OF METABOLIC PERTIBATIONS OF HARVESTED PIG LIVERS FOLLOWING HYPOTHERMIC REPERFUSION.

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Introduction- Assessment of donor liver viabilty is critical to transplantation outcome and is becoming more important as there is an increased use of organs previously rejected due to age criteria and harvesting complications. Methods currently employed to assess the liver status prior to transplantation include standard biochemical liver function tests, macroscopic appearance and the clinical history of the donor. Some of the biochemical tests involve invasive techniques and results may not be available prior to transplantation. In this study we have attempted to assess the relative merits that ^{31}P-MRS may have in a pig model by examining 3 storage buffers and their effects on the metabolism post harvest. This technique allows for a rapid assessment of the biochemical integrity of the liver when accompanied with oxygenated hypothermic reperfusion (HtR) of the liver. **Methods-** Fifteen Land Race Large White cross pigs (30kg) (3 groups of 5) were fasted for 12h. Livers were retrieved using standard clinical techniques and were perfused with 1l of ice-cold citrate buffer plus one litre of ice-cold University of Wisconsin (UW) solution. Two groups had either adenosine (Ad) (1.34g/l) or a prostacyclin derivative (PD) [ZK 36374] (10^{-8}M) added to the solutions. Following storage on ice for 2h the liver was positioned in a 1.6 Tesla Picker Prototype MRS/MRI machine and ^{31}P spectra collected every 2 minutes. The liver was then reperfused with ice-cold Na-gluconate (100mM), raffinose (30mM), glycine (5mM), $MgSO_4$ (5mM) $CaCl_2$ (0.5mM) made up with Hespan (1l) and dd.H_2O to a total volume of 3l; pH 7.30-7.40. Livers treated with Ad and PD had these same compounds in their respective reperfusion buffer. **Results-**Prior to HtR there were no detectable ATP levels. Following HtR, ATP levels increased maximally to 4.49±0.52% (UW), 5.82±0.27% (UW+Ad) & 6.79±0.40% (UW+PD) with initial rates of $9.7 \times 10^{-3} s^{-1}$, $9.5 \times 10^{-3} s^{-1}$ & $14.2 \times 10^{-3} s^{-1}$, respectively. These changes represent an increase from the UW solution of 30% with added Ad ($p<0.05$) and 51% with added PD ($p<0.02$).Concurrently, there were decreases in Pi of 36% and PME of 31% and an increase in PDE of 29% for both UW+Ad and UW+PD, repectively. There were no changes in Pi but decreases in PDE and PME of 22% and 40% in the UW group. All pH values decreased by approximately 0.20, however starting values were 7.4, 7.1 and 7.3 for UW, UW+Ad and UW+PD, respectively. **Conclusion-** From the results presented it is clear that ^{31}P MRS can provide important information on the bioenergetics of the harvested liver with minimal intervention in a time period of approximately 1 hour. Pharmacological agents with predicted protective effects demonstrated improved metabolic recovery by ^{31}P-MRS. By analysing the changes in ^{31}P-MRS spectra it may be possible to assess relative viability of donor livers, non-invasively during the window of opportunity.

AN ANALYSIS OF TIME FACTORS INFLUENCING THE DURATION OF COLD ISCHAEMIA FOR CADAVERIC KIDNEYS

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Background: Previous retrospective studies have shown that prolonged cold ischaemia time (CIT) contributes to late graft loss. An association between delayed graft function (DGF) and late graft loss has also been documented. This study was designed to evaluate any relationship between CIT and DGF and to document factors influencing prolonged CIT.
Design: Data was collected prospectively from 60 consecutive cadaveric kidney transplants commencing March 1996.
Patients and Methods: The following data was collected; time back to base (BTB), time to crossmatch (BCROSS), time until patient prepared (CRREAD) and time patient prepared until commencement of operation (READTH). Locally retrieved and imported kidneys were analysed separately. Statistical testing was by Wilcoxon or Kruskall-Wallis testing, stratified multiple tables and univariate analysis.
Results: Mean CIT was 1411 min; 1501 min for imported (I) and 1378 min for local kidneys (L) (no statistical difference). Mean BTB differed between I and L kidneys ($p<0.001$). All other factors analysed did not show any difference between the groups. Mean READTH was 212.5 minutes but this is considered to be artificially low because patients were not called up to the transplant unit until probable theatre access times were arranged. In contrast to some previous studies CIT did not predict DGF (Kruskall-Wallis $p=0.46$). A stratified analysis of donor gender, recipient gender, L or I kidney did not influence the overall result. In all univariate analyses no significant risk factors for DGF were found.
Conclusion: We remain unable to predict DGF by CIT or any of the other variables we examined. The BTB time, although different between local and imported kidneys did not result in a difference of CIT between L and I and did not impact on DGF. We conclude that the present system for allocating "beneficially" matched kidneys should be continued since moderately prolonged CIT resulting from organ exchange does not influence DGF. In our unit we could significantly reduce CIT if we had better access to theatre time.

EFFECT OF PRESERVATION - REPERFUSION INJURY ON INTERCELLULAR ADHESION MOLECULE - 1 (ICAM -1) EXPRESSION IN HUMAN LIVER ALLOGRAFTS

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ICAM-1 is a cytokine inducible cell adhesion molecule involved in leucocyte trafficking and emigration in inflammation, and thought to play an important role in preservation - reperfusion injury.

We studied ICAM-1 expression in serial biopsies following cold preservation and after reperfusion of liver grafts, and its correlation with the histology of post reperfusion biopsy.

46 liver grafts were studied. Biopsies were taken at the end of cold ischeamic period, and approximately 90 minutes after graft reperfusion. ICAM-1 expression was demonstrated by immunohistochemical techniques. The procedure was performed simultaneously on four identical sections of each biospy. Sections were systematically analysed for ICAM-1 distribution on sinusoidal endothelial cells(SEC), bile ducts, & hepatocytes, and for intensity of stain (no stain, mild stain, moderate stain, and intense staining).

Following cold storage, ICAM-1 was expressed on Kupffer cells lining the sinusoids and on SEC in all grafts. No bile duct staining was noted. In 16/46 biopsies there was mild to moderate ICAM-1 staining on hepatocytes.

In post-reperfusion biopsies ICAM-1 expression was increased on SEC in 23/46 biopsies. Of these 23, 12 had intense and 11 moderate ICAM-1 expression on hepatocytes. In 13/46 biopsies there were no change in the pattern or intensity of ICAM-1. In 5/46 there was decrease in ICAM-1 expression. In the remaining 5/46 patients no post reperfusion biopsies were obtained in 4 and no staining was demonstrated in one.

ICAM-1 expression was correlated with histological findings in the post reperfusion biopsies. Of the 28 such biopsies, standard histological assessment were normal in 10(group 1). 13 had mild reperfusion changes (group 2), and 5 severe (group 3).Moderate or intense ICAM-1 expression was seen in 3 biopsies from group 1 (3/10), 10 from group 2 (10/13), and all 5 from group 3 (5/5).

This study has demonstrated that ICAM-1 expression on the sinusoids is increased over the period of reperfusion in human liver transplantation. Intensity of ICAM-1 expression is correlated with severity of reperfusion injury in standard histological assessment of the graft.

ROLE OF IMPERMEANTS IN THE PREVENTION OF CELL SWELLING IN TRANSPLANT PRESERVATION: SUCROSE VERSUS GLUCOSE AND MANNITOL

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Organ preservation solutions include impermeants, usually sugar, to prevent cell swelling during cold ischaemia, warm ischaemia and re-perfusion. Using simulated warm ischaemia in a cellular model (by the use of strophanthidin to block Na/K pump) we have previously shown that Euro-Collins (EC) and Hyperosmolar Citrate (HOC) provide less than ideal control of cell volume in comparison with phosphate buffered sucrose(PBS140). These may be an effect of sucrose. In these experiments we have investigated the effect of substituting sucrose for the glucose of EC (EC-sucrose) and mannitol of HOC (HOC-sucrose) upon the control of cell volume.

The kidneys of anaesthetised NZW rabbits (1.4-2.2kg) were flushed with one of EC, EC-sucrose, HOC and HOC-sucrose. and stored at 4°C for up to 72 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 the test solution also containing 10^{-3} molar strophanthidin for 35 minutes and finally returned to oxygenated 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 and duration of storage	Start diameter (in saline)	Simulated warm ischaemia 10^{-3}M strophanthidin	Simulated re-perfusion In saline
EC, 0-4 hr	36.6 ± 0.9	52.0 ± 0.8	40.6 ± 2.4
EC-sucrose, 0-4 hr	35.7 ± 0.7	35.7 ± 1.2	37.4 ± 2.9
HOC, 0-4 hr	35.2 ± 1.1	36.1 ± 1.2	41.1 ± 1.1
HOC-sucrose, 0-4 hr	36.4 ± 0.7	39.9 ± 1.0	43.1 ± 0.6
HOC, 72 hr	46.1 ± 2.1	47.5 ± 2.4	51.1 ± 2.7
HOC-sucrose, 72 hr	41.7 ± 1.4	42.5 ± 1.0	43.1 ± 1.2

Replacement of glucose of Euro-Collins with sucrose prevented the cell swelling seen during the experimental period. Replacement of mannitol of HOC with sucrose did not have the same effect on the day of preservation, but prevented the cell swelling at 72 hour preservation. Overall, sucrose seems to be more effective impermeant than either glucose or mannitol in the prevention of cell swelling in our model

MANNAN BINDING LECTIN LEVELS DO NOT IMPACT ON ADULT RENAL TRANSPLANT OUTCOME.

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Mannan binding lectin (MBL) plays an important role in opsonisation defence against microbe infection. Serum levels of MBL are genetically controlled and correlate with increased risk of infection, especially in early years of life. In this study we aimed to investigate retrospectively MBL levels in immunocompromised renal transplant recipients to examine for possible correlations with post-transplant morbidity.

In the 23 months between August 1988 and June 1990 we performed 200 cadaveric renal transplants and serum samples for MBL level assay were available on 96.5% of these. MBL levels were established by a time resolved immunofluorometric assay (TRIFMA) using europium-labelled monoclonal MBL specific antibody. Each transplant recipient had a single serum sample tested and samples were collected at times random to the date of transplant. Post transplant infection events were collected retrospectively from patient notes.

We found that 30% (58) of recipients had MBL levels higher than 1753 ng/ml (MBL-H) and 30% (57) had MBL levels lower than 337 ng/ml (MBL-L) and chose to compare and contrast morbidity data between these groups.

There was no difference between MBL-H and MBL-L groups for HLA mismatch, donor / recipient gender, immunosuppressive therapy, re-transplants, sensitisation or cold storage time. There was no difference between MBL-H and MBL-L for incidence of bacterial or fungal infections. CMV infections were more common in MBL-L (8/57) than in MBL-H (2/58) ($p<0.05$) The incidence of rejection, steroid resistant rejection, chronic loss, quality of function and survival were the same in both groups.

We conclude that MBL serum level does not predispose to significant post-transplant morbidity but that MBL levels in CMV infection should be further investigated.