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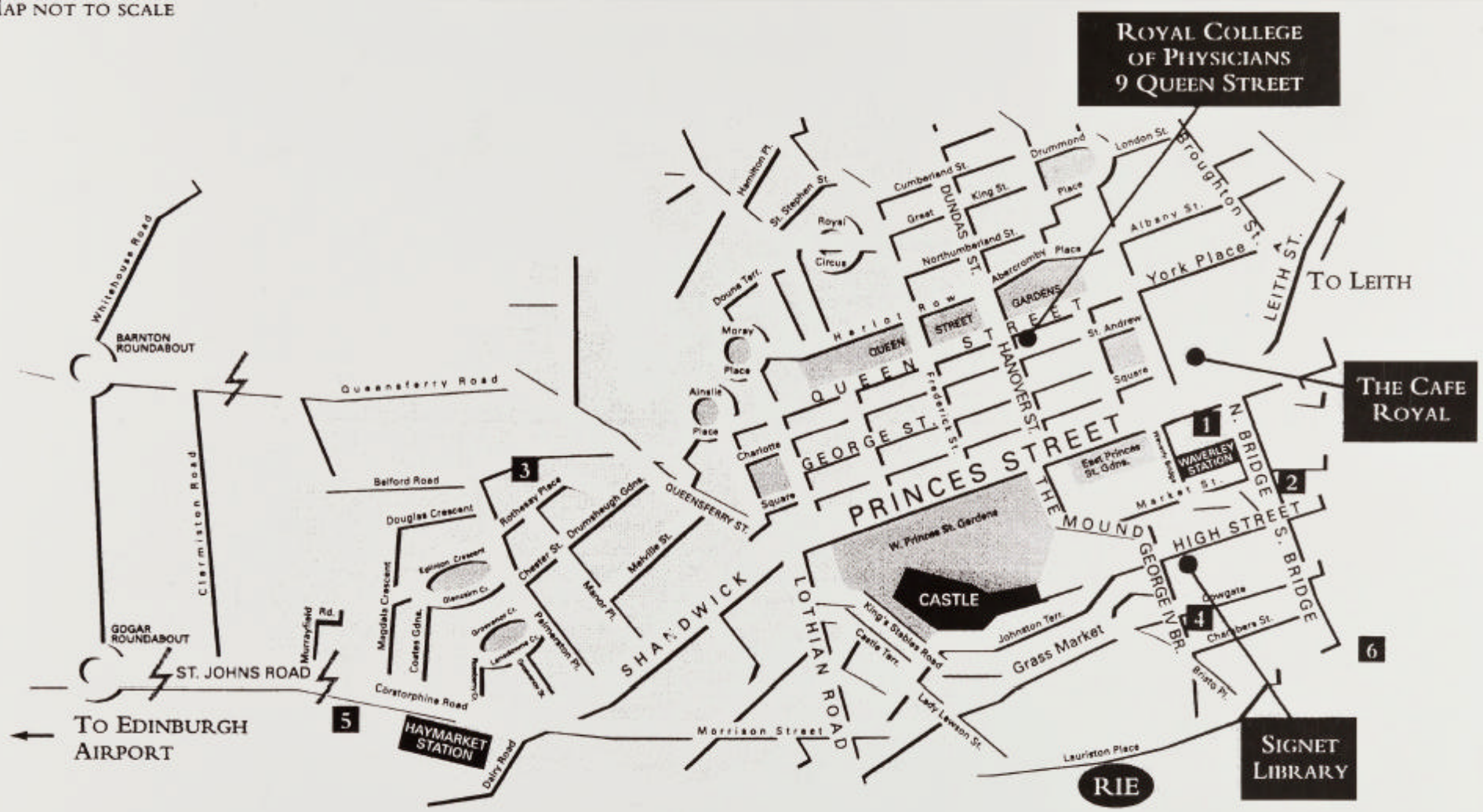

1 THE BALMORAL
2 CARLTON HIGHLAND HOTEL
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Plenary Session 1

Monday 29th March

INDUCTION OF LIVER ALLOGRAFT TOLERANCE USING SITE-DIRECTED GENE THERAPY.

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Background The fusion protein CTLA4-Ig binds B7 proteins, preventing activation of the CD28/B7 signaling pathway. Since this signaling pathway is required for T cell activation, CTLA4-Ig can induce T cell unresponsiveness. Systemically administered CTLA4-Ig has been shown to increase graft survival, in some cases indefinitely, in animal models of transplantation. Whilst animals showing long term graft survival are tolerant specifically to the donor antigens of the graft, they will inevitably experience a period of non-specific immunosuppression following administration of the fusion protein. In an attempt to address this issue we have investigated the use of a recombinant adenovirus containing the CTLA4-Ig cDNA as a system for the delivery of fusion protein direct to the transplant in a rat liver allograft model.

Methods Orthotopic liver transplants were performed in the high responder DA ($RT1^{av1}$) to Lewis ($RT1^{l}$) rat strain combination. A recombinant adenovirus containing a CTLA4-Ig cDNA expression cassette (AdRSVCTLA4-Ig) or an adenovirus containing no cDNA cassette (Ad0) was delivered *in vivo* to the donor DA rat one day prior to transplantation to allow for maximal fusion protein expression 2 days post transplantation. Following transplantation serum AST levels were measured as a marker of liver function and the level of circulating serum CTLA4-Ig was determined from binding assays using Cd80 transfected CHO cells (CHOB7.1 cells). Recipients of AdRSVCTLA4-Ig livers which maintained their grafts for over 100 days were regrafted with donor-specific (DA) and 3rd party (PVG) skin grafts.

Results Recipients receiving untreated livers or Ad0 infected livers uniformly rejected their grafts by day 11. Recipients receiving livers infected with AdRSVCTLA4-Ig demonstrated prolonged graft survival ranging from 15 days to over 1 year (15, 16, 23, 46, >63, >211, >212, >376 days). Liver dysfunction at the time of rejection in recipients of both untreated and Ad0 infected grafts was reflected in high serum AST levels (uninfected day 8: n=7: mean 856U/L, Ad0 day 10.5: n=2: mean 790U/L). The animals receiving AdRSVCTLA4-Ig infected livers also displayed elevated AST levels (week 6: n=3: mean 529U/L) yet surprisingly maintained a level of liver function compatible with survival. No rats displayed the good liver function seen in syngeneic (Lewis to Lewis) untreated liver transplanted rats (week 6: n=3: mean 102U/L). Binding assays revealed a high level of circulating CTLA4-Ig (10µg/ml) in animals receiving AdRSVCTLA4-Ig infected livers and this was maintained in some animals for up to 3 months after transplantation. Serum taken from animals grafted with untreated or Ad0 infected livers displayed no binding to CHOB7.1 cells. No sera bound to untransfected CHO cells. Recipients of AdRSVCTLA4-Ig infected livers which were re-challenged with skin grafts promptly rejected their 3rd party (PVG) graft, but retained the donor-specific (DA) graft indefinitely.

Conclusions We have demonstrated that in this high responder liver transplantation model, donor-specific tolerance can be achieved with site-directed gene therapy. Importantly we were able to deliver the adenovirus to the donor prior to organ harvest allowing maximal expression of the fusion protein at an appropriate time following transplantation. However, the high circulating levels of CTLA4-Ig observed in the recipient for prolonged periods of time suggest that recipients given this type of therapy would be subject to systemic non-specific immunosuppression.

DONOR CYTOKINE GENE POLYMORPHISM INFLUENCES THE DEVELOPMENT OF ACUTE ALLOGRAFT REJECTION

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Acute allograft rejection is a major cause of morbidity after renal transplantation, and is an important determinant of both chronic rejection and longterm graft function. The severity of the rejection response may be affected by cytokines produced by recipient lymphocytes that infiltrate the graft but do graft cytokines play a role in recruitment of these cells or in the inflammatory process itself?

To test our hypothesis that donor cytokine profile could influense the incidence and severity of acute graft rejection, we analysed 20 polymorphisms in 11 functionally related cytokine and cytokine receptor polymorphisms under identical PCR conditions. Single nucleotide polymorphisms in IL1 alpha, IL1 beta, IL1 receptor, IL1 receptor antagonist, IL6, IL4, IL4 receptor, lymphotoxin, TNF receptor, TGF beta were analysed in first group of 105 cadaveric renal transplant donors, and a second group of 76 donors. These donors were selected on the basis of outcome of the kidney after transplantation, and were divided into four groups: no rejection within the first three months after transplantation (68 donors); mild/moderate acute rejection (44), severe rejection (33), and late rejection (37). Recipients of all grafts received cyclosporin, azathioprine and prednisolone as initial immunosuppression. Associations were assessed using Chi-square and Fisher's exact tests, and were initially defined in the first set of donors. Any significant findings were then examined in the second set. Only those associations significant in both cohorts are reported.

An intronic polymorphism of the IL6 gene was shown to be strongly associated with development of acute rejection after transplantation (p no rejection vs acute rejection within one month =0.0003), and this was most significant in the severe rejection group (p no rejection vs severe rejection =0.000007). We have not yet investigated the functional effect of this polymorphism, but it is a reasonable hypothesisis that it is associated directly or indirectly with variability in IL6 production. No other associations of donor cytokine polymorphisms with acute rejection were demonstrated.

We hypothesise that the incidence and severity of acute renal allograft rejection may be influenced by the amount of IL6 produced by renal mesangial cells after transplantation. Analysis of donor IL6 genotype may predict the development of acute allograft rejection after renal transplantation.

IgV-Cd80 HAS A NEGATIVE REGULATORY ROLE IN ALLOGRAFT REJECTION

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Background: The B7 costimulatory proteins play a crucial role in eliciting immune responses. They are expressed on APCs and interact with Cd28 on T cells thereby triggering an elevation of cytokines which leads to clonal expansion. CTLA-4 ligation by B7 proteins subsequently provides a negative signal to T cells and terminates the response. Blockade of costimulation with CTLA4-Ig can lead to indefinite survival of an allograft in some models. There are two naturally occurring Cd80 proteins in mouse - full length Cd80 and the splice variant, IgV-Cd80. In order to elucidate the role of Cd80 and IgV-Cd80 in transplantation, we generated a mutant mouse which only expressed Cd86 and IgV-Cd80, but not Cd80.

Methods: Cd80 mutant mice were produced by gene targeting and backcrossed onto both C57BL/10 (H-2^b) and C3H/He (H-2^k) backgrounds. C57BL/10 LPS stimulated splenocytes were used as APCs and C3H/He purified T cells as responders in MLR experiments while C57BL/10 mice (wt and mutant) were used as donors and C3H/He (wt and mutant) as recipients in heterotopic heart transplantation. Anti-Cd80/IgV-Cd80 mAb (1610A1), anti-Cd86 mAb (GL1) and CTLA4-Ig fusion protein were used in in-vitro and transplantation experiments.

Results: In MLR experiments, both wt and mutant T cells responded less well to mutant than to wt APCs. Addition of anti-Cd80/IgV-Cd80 mAb (5µg/ml) in wt to wt MLR partially reduced proliferation. However only a marginal effect was seen when mutant APCs were used, suggesting only a small contribution from IgV-Cd80 to the MLR. Taken together, the in-vitro results indicate that Cd86 cannot fully compensate for the lack of Cd80 and that IgV-Cd80 does not play a crucial role in proliferation in the primary MLR.

The table below shows the results from transplantation experiments. No prolongation in graft survival was seen when mutant hearts were transplanted to wt recipients (mean 9.6 days) as compared to wt to wt control (mean 9.6 days). In contrast, when mutant recipients were used, prolongation was observed of both wt and mutant grafts. This was more so for mutant donor hearts (mean 29.6 vs 12.6 days days), with one graft achieving survival beyond 100 days. Anti-Cd86 mAb treatment (200 µg) given on day 2 resulted in indefinite graft survival in mutant to mutant transplantation in almost all animals (mean 86.5 vs 29.6 days) but when anti-Cd80/IgV-Cd80 mAb (200 µg) was given, graft survival was decreased (mean of 7.5 vs 29.6 days).

Transplantation	Graft Survival (days)	Mean
wt to wt	9,10,10,9,10	9.6
mutant to wt	9,10,10	9.6
wt to mutant	8,8,12,12,24,12	12.6
mutant to mutant	13,14,24,13,14,>120	29.6
mutant to mutant + GL1	46,>100,>100,>100	86.5
mutant to mutant + 1610A1	8,7,7,8	7.5

Conclusion: Our in vivo experiments showed that blocking of IgV-Cd80 using the mAb 1610A1 curtailed the survival of mutant to mutant transplants. This was not reflected in the MLR studies. Although the mechanism by which IgV-Cd80 operates is as yet unclear, the results suggest that it could play an important role in negative regulation of the costimulatory pathway in transplantation.

MONITORING PERIPHERAL T-CELL IL-5 AND IL-13 GENE EXPRESSION – EVIDENCE OF THEIR ROLE IN ACUTE RENAL ALLOGRAFT REJECTION.

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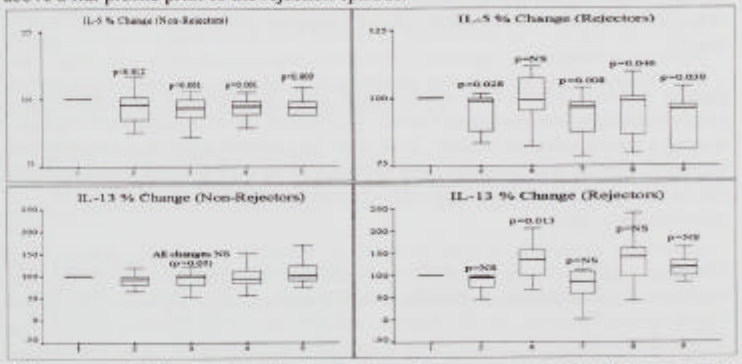
Cytokines play a pivotal role in the interactions between immune cells orchestrating acute allograft rejection. Many "single time point" gene expression studies have produced varied and often conflicting results. We monitored sequential changes in the level of IL-5 and IL-13 gene expression in peripheral blood T cells in the early post renal transplant period to assess the role of these cytokines in acute rejection.

Methods: 27 recipients without rejection and 15 recipients with histologically proven acute rejection were studied. Peripheral blood T-cells were extracted at various time points: [1] pre-Transplant (Tx), [2] early post-Tx, [3] 1 week post-Tx, [4] 2 weeks post-Tx, [5] 4 weeks post-Tx. In rejectors, additional samples were taken:- [6] before anti-rejection therapy (Th), [7] early post-Th, [8] 1 week post-Th and [9] 4 weeks post-Th. RT-PCR of IL-5 and IL-13 cDNA sequences followed by ELISA detection of digoxigenin-labelled PCR products were performed.

Results: Both cytokines exhibited an increase in expression at the time of rejection [6], and following anti-rejection therapy, showed a similar profile to that seen in non-rejectors. For IL-5, the increased expression was relative to the generally reduced level of expression prior to the rejection episode, while for IL-13, there was a significant rise above a flat profile prior to the rejection episode.



Wilcoxon matched-pairs signed-ranks test. p values compare each time point with baseline. NS= not significant

Conclusion: Sequential monitoring of peripheral IL-5 and IL-13 expression suggests that these cytokines play an active role in the process of allograft rejection with reversible surges at the time of acute rejection. IL-5 expression is substantially reduced in the non-rejector group and may play a more active part in the rejection process. These results are in agreement with IL-5 and IL-13 acting as immune modulators of eosinophils, which are commonly associated with acute allograft rejection.

SELECTIVE RETROSPECTIVE DONOR CROSSMATCHING IN CADAVERIC RENAL TRANSPLANTATION; EFFICACY, SAFETY AND EFFECTS ON COLD STORAGE TIME

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Introduction Ensuring a negative pre-transplant lymphocyte crossmatch caused by HLA specific antibodies has been mandatory to avoid the risk of hyperacute rejection. However, the necessity to perform the crossmatch on lymph-node and spleen taken at the time of organ retrieval results in increased cold storage time and a higher incidence of delayed graft function. Following recent refinements in antibody screening protocols, with increased sensitivity and specificity, it is now often possible to predict the crossmatch result. In the past year our centres have adopted a policy of omitting the pre-transplant crossmatch in carefully selected cases.

Methods Patients were selected for transplantation on the basis of blood group identity, HLA match ranked for HLA-DR>B>A, sensitisation status, negative donor HLA specific antibody crossmatch (performed either before or after the transplant operation), time on the transplant waiting list and donor and recipient age. Authorisation for transplantation in the absence of a donor crossmatch was given for non-sensitised recipients (panel reactive antibody negative tested using complement dependant cytotoxicity), and sensitised recipients where the antibody specificities were precisely defined and not donor HLA specific.

Results Between October 1997 and November 1998 70 consecutive cadaveric donor kidney transplants were performed (Table). 41 (59%) of these transplants were carried-out without a pre-transplant crossmatch. In all cases the predicted negative crossmatch was confirmed after the transplant operation. Omission of the pre-transplant crossmatch test resulted in a significant reduction in cold ischaemic time (CIT) for locally retrieved cadaveric donor grafts.

Donor origin	No. Transplants	1st graft / regraft	Crossmatch Pre / Post	mean CIT (hr) Pre / Post	mean 1M Cr Pre / Post
Local	51	47 / 4	23 / 28	19 / 14*	205 / 151
Imported	19	18 / 1	6 / 13	20 / 18	156 / 237

* = P <0.01, Pre / Post = crossmatch performed pre- or post-transplantation.

Conclusion This study shows that with careful patient assessment and accurate antibody screening data, transplantation can be performed safely without the need for a pre-transplant crossmatch. This policy reduces the cold storage time.

SENSITISATION TO HLA ANTIGENS IN PAEDIATRIC RECIPIENTS OF RENAL ALLOGRAFTS

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on behalf of the Paediatric Task Force of the UKTSSA Users' Kidney Advisory Group

The Paediatric Task Force was established by the Kidney Advisory Group (KAG) to investigate factors influencing the survival of renal transplants in paediatric recipients in the UK. The aim was then to provide evidence to be considered when revising the national allocation of kidneys to paediatric recipients.

Sensitisation to HLA antigens following transplantation was one of the factors investigated. A study was performed to determine the frequency with which transplantation resulted in sensitisation and to relate this both to the degree of mismatching between donor and recipient and to transplant outcome.

The Paediatric Task Force sensitisation analysis was based on all recipients receiving first grafts during the period January 1986- 31 December 1995, resulting in a dataset of 1205 transplants.

Evaluation of the screening data by individual laboratories demonstrated that transplantation resulted in sensitisation to HLA antigens in 385 (32%) of recipients, whereas no sensitisation was detected in 320 (27%) and in 500 (41%) recipients the result was unknown. The analysis showed that sensitisation following transplantation was significantly related to the total number of mismatches (0-6) at the HLA -A -B and -DR loci (Chi-squared test, p=0.001). Antibody specificities following transplantation were reported for 254 recipients and in 74%, the specificity was related to the mismatched antigens on the graft.

In analysis of the transplant outcome, 5 year transplant survival was significantly poorer in transplants resulting in sensitisation to HLA antigens, compared to those where no sensitisation was detected in the recipient (Log-Rank test, p=0.0001).

In conclusion, avoiding sensitisation to HLA antigens is considered particularly important in paediatric recipients who are likely to require a regraft at a later stage of their life. These UK data provide powerful evidence that an increased level of mismatching between donor and recipient is related to an increased incidence of sensitisation after transplantation and to a poorer overall transplant survival. Therefore in allocating kidneys to paediatric patients, HLA mismatching been donor and recipient should be avoided whenever possible.

Concurrent Sessions

Monday 29th March

Immunobiology

Monday 29th March

NON-CYTOTOXIC ANTI-MHC CLASS II ANTIBODIES PROLONG PRIMARILY VASCULARISED CARDIAC ALLOGRAFT SURVIVAL

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M.E. Shapiro, Beth Israel Deaconess Medical Centre, Boston, USA
N. Saxton, A. Nesbitt, R. Foulkes, Celltech Therapeutics, Slough

Antibodies targeting MHC class II molecules have been used extensively to prevent autoimmune and allogeneic responses. Concern about toxicity has limited the use of these antibodies. We have characterised extensively the effects of a non-cytotoxic anti-MHC class II antibody (OX6) in the Biozzi AB/H mouse. In keeping with its mIgG1 isotype, this antibody has been shown to cause only transient depletion of B cells and to have no quantitative effect on priming to foreign antigen or self antigen administered in complete Freund's adjuvant. However, it is still effective at preventing autoimmune disease (1).

In this work we investigated the effect of OX6 in a murine heterotopic cardiac allograft model. BALB/c ($H\text{-}2^d$) hearts were grafted into Biozzi AB/H ($K^aI\text{-}A^{g7}I\text{-}E^bD^g$) recipients and graft survival assessed by palpation. Mice received either OX6 or MOPC21 (isotype matched control antibody) at 40mg/kg ip on days -1, 0 and +1 only. The plasma half life of OX6 following a single dose is <24h. The cell surface half life on MHC class II positive cells is approximately 36h. Median graft survival time was 39 days in OX6 treated animals compared to 11 days in control animals (2).

The gLE1 antibody is a recombinant human anti-DR monoclonal antibody which cross reacts with the cynomolgus mamu MHC class II molecule. Substitutions in the constant regions of gLE1 reduce its ability to bind complement and interact with FcγR1 as compared to the parent molecule. It has a circulating half life of <4h and causes only transient depletion of B cells, similar to the effects of OX6 in rodents. Animals underwent bilateral nephrectomy and then received an intra-abdominal allogeneic kidney transplant. Animals (n=5) were given gLE1 at 10mg/kg on days -1, 0 and +1 or at -2h and then day +1 and +2. No other concommitant therapy was given. Graft function was monitored by measurement of plasma creatinine and animals sacrificed when severely uraemic. gLE1 treated animals survived 14, 24, 24, 27 and 28 days with control animals surviving 6 and 8 days.

Thus a short treatment course of non-cytotoxic anti-MHC class II antibody results in marked prolongation of survival of primarily vascularised solid organ allografts in murine and cynomolgus monkey models. This effect is not dependent on depletion of APC and extends long beyond the survival of antibody in the circulation or on the target cell surface. In vitro studies have shown selective modulation of OKT3 stimulated peripheral blood mononuclear cell cytokine production. Taken together this data suggests active immunomodulation to be the mechanism of action of these antibodies and highlights the antigen presenting cell as an attractive target for immunomodulatory therapies.

1. Smith RM, Morgan A, Wraith DC. Anti-class II MHC antibodies prevent and treat EAE without APC depletion. Immunology 1994; 83 (1): 1-8.

2. Smith RM, Chen ZK, Foulkes R, Metcalfe SM, Wraith DC. Prolongation of murine vascularized heart allograft survival by recipient-specific anti-major histocompatibility complex class II antibody. Transplantation 1997; 64 (3): 525-528.

DETECTION OF HLA-SPECIFIC IgG USING SINGLE RECOMBINANT HLA ALLELES

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Screening for HLA specific antibodies is a complex procedure and it can be difficult to define all the HLA specificities to which antibody has been produced in some patients, particularly those with antibodies to multiple epitopes. Also antibodies binding *in vitro* to lymphocytes may be directed at non-HLA targets. The more recent use of isolated HLA class I and class II antigens from cell lines with ELISA or flow cytometric techniques has removed the confounding factor of non-HLA molecules. However the soluble antigen preparations contain a minimum of 1 A locus, 1 B locus and 1 Cw locus antigen and may have as many as 2 of each of these. Each antigen must therefore be tested at least twice, and usually many more times, in different combinations with other antigens in order to determine which antigen in any preparation is the target. In this study we have investigated the use of soluble HLA molecules of a single allele for antibody screening.

The use of recombinant biotinylated HLA molecules bound onto streptavidin to form tetrameric complexes has been described. Two preparations of biotinylated HLA-A*0201 monomers, each presenting a different peptide, were bound in saturation to streptavidin microspheres. The coated beads were incubated with serum previously shown a) to be specific for HLA-A2 epitopes, b) to have no HLA-specific antibodies or c) to have class I specific antibodies other than anti-A2. Antibody binding to the beads was measured by use of a FITC conjugated anti-human IgG antibody and flow cytometric analysis. All 38 A2 specific sera, and 1 of 15 non-A2 specific sera exhibited binding in the positive range demonstrating very high specificity and sensitivity. The nature of the presented peptide did not appreciably affect the antibody binding.

One A2 monomer/peptide combination was also coupled to streptavidin-coated 96-well plates and an ELISA was employed to detect specific antibody. Preliminary results using 24 anti-A2 positive and negative sera showed 100% concordance with the cytotoxically defined specificities.

These early results demonstrate the potential for a revolutionary anti-HLA screening technique which would facilitate antibody analysis and allow hitherto unattainable specificity definition. We shall be testing more recombinant class I molecules and eventually aim for a comprehensive coverage of all class I and II alleles.

TRANSPLANT ACCOMMODATION IN HIGHLY SENSITISED RENAL ALLOGRAFT RECIPIENTS IS ASSOCIATED WITH UP-REGULATION OF THE ANTI-APOPTOTIC PROTEIN BCL-XL: IN VITRO MODELS IMPLICATE INDUCTION OF ACCOMMODATION BY LOW LEVELS OF ANTI-DONOR ANTIBODY

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Under certain, still ill-defined circumstances, anti-donor antibodies may not cause hyperacute rejection (HAR) but induce endothelial cell accommodation. This process has been reported following transplantation of ABO-incompatible allografts, following the return of anti-HLA antibodies in highly sensitised recipients, and in xenograft models. Active changes in the donor endothelium are thought to be responsible. We have investigated the molecular mechanisms involved in accommodation in vivo, in highly sensitised human allograft recipients, and in vitro using cultured human umbilical vein endothelial cells (HUVEC).
Seven highly sensitised patients were immunoadsorbed, to remove their anti-HLA antibodies prior to renal transplantation. Immunohistochemistry was performed on transplant biopsies from these highly sensitised recipients as well as on control native and transplant biopsies. The sections were stained for Bcl-xL, Bcl-2 and HO-1. 4/7 of the patients transplanted had a return of anti-donor antibodies; This did not lead to HAR. Prominent Bcl-xL expression was observed in the graft microvasculature in 3/4 patients in whom anti-donor antibodies returned and in none in whom they did not, nor in any of the controls. Neither HO-1 nor Bcl2 were specific for the accommodated grafts, endothelial staining having been found in other inflammatory conditions. In vitro we cultured allogeneic HUVEC with anti-HLA antibodies from one of our highly sensitised patients. Prolonged exposure to low doses of anti-donor antibodies led to upregulation of the anti-apoptotic proteins Bcl2 and Bcl-xL in the HUVEC. Following this antibody treatment the cells underwent phenotypic changes consistent with accommodation, including resistance to complement mediated lysis, attenuation of adhesion molecule expression, as judged by ICAM levels, and functional protection from apoptosis. Taken together, these data shed light on the mechanisms responsible for allograft endothelial cell accommodation. Further insights into the mechanisms underlying accommodation may allow the development of specific therapies to promote accommodation in allografts and xenografts prior to transplantation.

THE INDUCTION AND REGULATORY ROLE OF ANERGIC T CELLS IN TRANSPLANT TOLERANCE

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We have previously proposed that alloantigen presentation by costimulation-deficient parenchymal cells of transplanted organs is instrumental in the induction of donor-specific T cell hyporesponsiveness. Consistent with this hypothesis, a substantial fall in frequencies of interleukin-2-secreting T cells with direct anti-donor allospecificity can be observed in renal transplant recipients, and γ-interferon-treated, HLA-DR$^+$ renal tubular epithelial cells induce allospecific anergy in peripheral blood T cells in vitro. Two questions have been addressed in this study: first, is CTLA4 ligation involved in the induction of T cell anergy by costimulation-deficient antigen presentation?, and second, can anergic T cells contribute to the maintenance of T cell tolerance?

It has been suggested that CTLA4 ligation is necessary in the induction of T cell unresponsiveness. At face value this is difficult to reconcile with anergy induction by antigen-presenting cells (APC) that lack B7 expression. Using naive CD8$^+$ H-Y peptide-specific TcR-transgenic T cells as responder cells, and naive CD4$^+$ T cells as APC, we observed low level proliferation in response to high peptide concentrations. This response was dependent upon the low level of CD86 expressed by the T APC, and was abolished by addition of anti-CD86 antibody (mAb). In striking contrast, addition of anti-CD80 mAb led to a substantial (10-fold) increase in proliferation. Flow cytometric analysis of the responding T cells after 3 days stimulation revealed substantial CD80 expression that was absent at the initiation of the culture. Furthermore, addition of anti-CTLA4 mAb led to a comparable increase in the proliferative reponse. Taken together these data suggest that non-cognate T:T interactions involving CD80 and CTLA4, both expressed by the responder cells, impose regulatory effects when APC-derived costimulation is limiting or absent.

To address the second question, we have extended our earlier in vitro observations of regulation by anergic T cells to an in vivo model. Sensitised NOD mice reject NOD^{asp} skin grafts within 15 days. NOD anti-NOD^{asp} alloreactive T cell clones were generated and rendered anergic in vitro. The anergic cells inhibited proliferation by responsive T cells with the same specificity in vitro. This did not appear to be due to cytokine release, in that secretion of IL-2, γ-IFN, IL4, IL-10, and TGFβ was greatly reduced following the induction of anergy, and neutralising mAbs specific for IL-4, IL-10, and TGFβ failed to reverse the inhibition. In vivo, adoptive transfer of the anergic T cells into NOD recipients of NOD^{asp} skin grafts doubled the length of skin graft survival. Consistent with the in vitro data, graft survival was not influenced by the administration of a neutralising anti-IL4 mAb.

These results indicate that anergic T cells can act as antigen-specific suppressor cells both in vitro and in vivo.Thus, alloantigen presentation by graft parenchymal cells may serve to induce T cell unresponsiveness, following which the unresponsive T cells may contribute to the active maintenance of a tolerant state.

THE ALLOANTIBODY RESPONSE IS DEPENDENT UPON THE CLASSICAL PATHWAY OF COMPLEMENT ACTIVATION

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Alloantibodies cause significant morbidity to potential recipients of an allograft. Complement augments the B cell response to threshold amounts of T-dependent antigens, but few studies have investigated the role of complement in antibody production at large doses of antigen, and none have specifically analysed the alloantibody response. This is significant as new specific inhibitors of the complement system are now available for human use. It is important to elucidate basic mechanisms so that potential therapeutic strategies can be developed.

Method To investigate the role of complement in alloantibody production, a skin graft model was utilised in complement knockout mice. Mice received tail skin from fully MHC-disparate donors, and the subsequent alloantibody response in the recipients was assessed by two colour flow cytometry, using donor strain T cells as target cells. The production of allospecific IgM, IgG and IgG isotypes was determined. The responses in mice with different complement deficiencies were directly compared (n=10 in each group).

Results Compared with the wild type controls, mice deficient in the complement component C3 had a marked reduction in allospecific IgG levels ($p<0.0001$). In addition, they demonstrated a defect in IgG subclass production. They were only able to produce the non-complement fixing IgG1 whereas the wild type animals produced the full range of IgG isotypes, including high levels of complement fixing antibody ($p<0.0001$). The IgM response was unaffected. Mice deficient in C3 or C4 had similar responses to eachother indicating the role of the classical pathway of complement activation in this process. These mice also had delayed rejection of the skin grafts ($p<0.0001$). In contrast, mice deficient in C5, or in whom C5 had been inhibited with the monoclonal antibody BB5.1, exhibited normal skin graft rejection, IgM and IgG responses.

Conclusions Even in the presence of a large antigen load, complement plays an important role in the control of alloantibody production. The major defect caused by complement deficiency is in antibody class switching. The classical pathway of activation is critical to this process, but the terminal pathway is unimportant. It is likely that split products of C3 activation facilitate alloantibody production.

Liver

Monday 29th March

SKIN TESTS PREDICT ACUTE CELLULAR REJECTION IN LIVER TRANSPLANTATION

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Aim: Acute cellular rejection following liver transplantation is common but there is no reliable indicator pretransplant which allows tailoring of immunosuppression. Contact sensitisation to a neo-antigen pretransplant was carried in patients with chronic liver disease to assess its ability to predict acute cellular rejection following transplantation.

Method: Patients on the waiting list for transplantation were sensitised to diphenylcyclopropenone (DPC)for 48 hours. An elicitation test was carried out 14 days later and a score given for each of the 5 concentrations tested (bulla = 3, vesicles =2, erythema = 1). Acute cellular rejection was defined as rejection requiring therapy with high dose steroids. The clinicians deciding on therapy were blinded to skin test results as were the pathologists. The histopathological score was also noted in those who had biopsies at day 7. The nutritional status ,aetiology and Child's score was also assessed.

Results: 41 patients (17 PBC,10 ALD,5 PSC,4 HBV,2 cryptogenic,2 AIH,1 HCV) have been tested. Twenty two had no response while the 19 responders had skin test scores from 1-9. Three patients, all non-responders, died on the waiting list. Eighteen of 19 non-responders did not have acute rejection while 14 of 19 responders required treatment for acute rejection ($p<0.001$). The histological grading of rejection according to the Banff criteria in the various skin test scores is shown below (6 non-responders had no biopsy).

Skin test score	No rej.	Mild rej.	Mod. rej.	Severe rej.
0	2	11	0	0
1-3	0	2	7	0
4-6	0	0	8	0
7-9	0	0	0	4

Conclusions: The ability to mount a contact sensitisation response to a neoantigan pretransplant predicts those patients who will have not require treatment for acute cellular rejection. There is a relationship with the magnitude of responsiveness and the severity of acute cellular rejection. This may allow tailoring of immunosuppression on an individual basis.

PRESERVATION INJURY VERSUS ACUTE REJECTION IN DAY 7 LIVER TRANSPLANT BIOPSIES.

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Introduction: Parenchymal changes in day 7 liver transplant biopsies are often attributed to persistent preservation-reperfusion injury. The features include perivenular cholestasis, steatosis and hepatocyte ballooning and necrosis. The precise relationship between preservation injury and early post-transplant liver allograft histology is still not well understood. Furthermore some of these changes could be related to graft rejection, implying different treatment options.

Aim: To determine which parenchymal histological features correlate with preservation-reperfusion injury and/or pre-existing donor change and which with rejection in day 7 biopsies.

Methods: Protocol day 7 liver transplant biopsies from 50 patients who had a day 1 AST<400 IU/L (minimal preservation-reperfusion injury) and 39 patients with a day 1 AST>2000 IU/L (severe preservation-reperfusion injury) were blindly assessed for portal tract inflammation, biliary infiltrate, portal vein endothelialitis, hepatic vein endothelialitis, overall grade of acute rejection (based on the portal tract triad of inflammation, biliary inflammation and portal vein endothelialitis), hepatocyte ballooning, hepatocyte necrosis, perivenular inflammation, steatosis and cholestasis. The severity of the changes was assessed semiquantitatively on a scale of 0 (absent) to 3 (severe). Statistical analysis was performed using the Mann-Whitney U test to compare between the two groups and Spearman's rank correlation coefficient to assess correlation between two different histological features.

Results: There was significantly more hepatocyte ballooning ($p=0.0001$), steatosis ($p<0.0001$) and cholestasis ($p<0.03$) in the higher AST group. There was no significant difference in the other variables between the two groups. There was a correlation between hepatocyte necrosis and perivenular inflammation ($p<0.001$), and hepatic vein endothelialitis ($p=0.003$) but not with the overall grade of rejection or hepatocyte ballooning. The amount of cholestasis correlated with the degree of hepatocyte ballooning ($p<0.04$). There was no correlation between the amount of steatosis or overall grade of rejection and cholestasis.

Discussion: Hepatocyte ballooning, steatosis and cholestasis in day 7 biopsies can still be attributed to preservation-reperfusion injury and/or a pre-existing donor abnormality. Hepatocyte necrosis however appears to be related to rejection, but may be independent of the usual portal tract rejection changes.

ASSOCIATION OF ACUTE REJECTION IN LIVER TRANSPLANTATION WITH TUMOUR NECROSIS FACTOR ALPHA-308 POLYMORPHISMS

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Orthotopic hepatic allograft failure can occur due to chronic rejection and occasionally due to acute rejection. The role of cytokines in both processes is not entirely clear. The aim of this study was to investigate the association between polymorphisms known to affect in-vitro production of certain cytokines and rejection.

Method: Whole blood samples from 138 liver transplant patients (57 PBC,27 ALD, 17 PSC, 7 viral, 8 autoimmune, 13 acute liver failure, 5 cryptogenic and 4 others) were analysed along with 180 controls. DNA was extracted and the presence of polymorphisms in alleles coding for tumour necrosis factor alpha (position -308), transforming growth factor beta (codons 10 and 25) and interleukin 10 (positions -1082,-819 and -592) identified using sequence specific oligonucleotide probes. The diagnosis of acute cellular rejection was made on clinical, biochemical and histological grounds and was said to have occurred if high dose corticosteroid therapy was given. Chronic rejection was a histological diagnosis.

Results: The table shows the occurrence of acute rejection with respect to TNFα genotype.

	308-GG	308-GA	308-AA
Acute rejection	28	24	16
No acute rejection	40	27	3

P= 0.004

There was no significant differences between the rejection and non-rejection group with respect to Il-10 or TGFβ genotype polymorphisms.

Chronic rejection was infrequent in the group as a whole (8/138). None of these patients had a genotype associated with low production of TGFβ.

Conclusion: Acute cellular rejection is more common in patients with a polymorphism associated with high TNFα production in-vitro.

EX-VIVO LIVER PERFUSION SYSTEM FOR HEPATIC FAILURE PENDING LIVER REGENERATION OR LIVER TRANSPLANTATION

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There is a well recognised need for a system capable of providing effective support for patients with hepatic failure pending liver regeneration or liver transplantation. Recent attempts of using bioartificial liver containing capsulated porcine hepatocytes, the development of emergency whole liver or hepatocyte transplantation are complex and not consistently successful. The technique of ex-vivo hepatic perfusion developed and used clinically by Abouna in the 1970s, has now been redesigned in a perfusion circuitry which mimics the physiological conditions of a normal liver. Prior to clinical application of this system, a preclinical trial was carried out in dogs with induced hepatic failure.

Methods: Acute hepatic failure was induced in dogs by an end-to-side portacaval shunt followed 24 hours later, by a two-hour occlusion of the hepatic and gastroduodenal arteries. All animals (n=18) were medically supported and were divided into two groups. In the control group (n=6), only medical support was used. In the experimental group (n=12), the animals were connected to the ex-vivo liver support apparatus during acute hepatic failure via an AV shunt using a dog liver (n=6) or calf liver (n=6). [After a temporary extracorporeal bovine kidney transplant to remove preformed xeno antibody.] Hepatic perfusion was carried out at 37°C through the hepatic artery and portal vein at physiologic pressures and blood flow rate for 6-8 hours.

Results: All control animals died in hepatic failure at 14-19 hours after clamping of the hepatic artery. The animals treated with ex-vivo liver showed remarkable clinical and biochemical improvement. Five animals survived for 36-60 hours. Another seven recovered completely and became long-term survivors with biochemical and histological evidence of regeneration of their own liver.

Conclusions: The observations and results obtained in this trial strongly confirm that extracorporeal perfusion through a whole liver, using the system described, is very successful and cost effective for the treatment of acute, but reversible hepatic failure, as well as serving as a bridge to liver transplantation. The time has come for this form of liver support technology to be reintroduced and widely used.

SHOULD MARGINAL LIVERS BE USED IN TRANSPLANTATION FOR FULMINANT HEPATIC FAILURE (FHF)?

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Purpose: To study the outcome of marginal and "good" donors in liver transplantataion (LT) for FHF.

Methods: 74 consecutive patients received emergency LT for FHF (56 adult, 18 paediatric, median age 28 (3 m – 72 years), 27 males) between January 1994 and September 1997. Marginal donors were defined as those with 1 or more of:- AST >60 U/L, ITU stay > 5 days, sepsis, high inotrope requirement, cardiac arrest, advanced cardiovascular disease, lengthy hypotension, alcoholism.

Results: 47 livers were marginal.(27 - high inotropic support; 16 - AST>60IU/L; 21 - lengthy hypotension; 17 - sepsis; 3 - >5 days in ITU; 1 - uncontrolled hypertension and 1 - excess alcohol). 2 patients required early regrafting -1 primary non-function("good") and 1 acute rejection (marginal). Peak AST (days 1-5) was significantly higher (1367 vs 855 U/L) in the marginal group (p=0.04). There were no differences in the peak bilirubin and lowest INR (days 1-5). Graft survival at 1 and 12 months was 81% and 72% in the marginal group and 81.5% and 74% in the "good" group(p=n.s.). One month and 1 year patient survival was 83% and 75% in the marginal group and 85% and 78% in the "good" group (p= n.s.). The main cause of death in the marginal group was cerebral oedema and in the "good" group was sepsis /multi-organ failure.

Conclusions: There was no difference in the eventual graft and patient outcomes in fulminant recipients who received marginal livers. This should encourage the use of these livers.

Posters

Monday 29th March

THE EXPRESSION OF FIBROSIS ASSOCIATED GENES IN GLOMERULI AFTER RENAL TRANSPLANTATION. A COMPARISON BETWEEN CADAVERIC AND NON HEART BEATING DONORS.

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The main difference between cadaveric and non heart beating donors (NHBD) is in the degree of warm ischaemia the kidney is subjected to. This study was designed to see if this affected the expression of fibrosis associated genes in the early post-transplant period, as these are thought to be involved in the development of chronic allograft nephropathy (CAN).

A series of 20 cadaveric and 17 NHBD renal transplants, performed over the same time period were studied. Mean donor age was higher in the NHBD group (48 vs 38), recipient age and drug therapy were similar between the two groups. Patients underwent ultrasound guided renal transplant biopsies at 1 week, 3 months and 6 months post-transplant. From each biopsy, at least two individual glomeruli were isolated under a microscope and placed in lysis buffer. mRNA was isolated and genes of interest were amplified by RT-PCR, then quantified in an ELISA system, compared to a known housekeeping gene, GAPDH.

Delayed graft function was common in NHBD (82%) but only 15% in cadaveric transplants. Acute rejection rates were 29% and 20% respectively. Mean levels of mRNA expression are shown in the table, presented in arbitrary units. Groups were compared using a Mann-Whitney U test, and in general genes were expressed in similar amounts between the two groups.

		Coll III	Coll IV	MMP2	TIMP1	TIMP2	TGFβ
1 wk	Cad (n=20)	0.74	0.37	0.65	1.31	0.74	0.20
	NHBD (n=17)	0.94	0.42	0.50	2.57	0.91	0.10
	P	*0.50*	*0.60*	*0.26*	*0.02*	*0.12*	*0.53*
3 mth	Cad (n=17)	0.43	0.57	0.34	2.25	0.98	0.35
	NHBD (n=11)	0.57	0.47	0.44	1.76	1.13	0.14
	P	*0.39*	*0.50*	*0.08*	*0.92*	*0.42*	*0.75*
6 mth	Cad (n=16)	0.50	1.05	0.43	1.38	0.71	0.20
	NHBD (n=9)	0.29	0.34	0.18	1.47	0.58	0.12
	P	*0.48*	*0.67*	*0.27*	*0.82*	*0.87*	*0.08*

We conclude that although the increased ischaemic injury in NHBD means a high rate of delayed graft function, it does not seem to affect the level of fibrosis associated gene expression compared with cadaveric donors.

ACTIVE TGFβ EXPRESSION IN KIDNEY TRANSPLANTATION: THE EFFECT OF CYCLOSPORIN A AND TACROLIMUS.

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Chronic rejection remains a major cause of graft dysfunction following kidney transplantation. It has been proposed that this fibroproliferative disease may be promoted by overproduction of the growth factor TGFβ. In this respect it is significant that Cyclosporin A has been associated with upregulation of TGFβ by human renal tubular epithelial and interstitial cells. The current study was designed to measure the active TGFβ expression in renal transplant biopsies.

Diagnostic transplant renal biopsies were divided into two groups on the basis of immunosuppression with cyclosporin A (17) and tacrolimus (26). The sections were first dewaxed and then incubated with primary chicken antihuman active anti-TGFβ antibody. After washing and treating with secondary rabbit anti-chicken antibody conjugated with FITC, the sections were analyzed by semi-quantitative scanning confocal fluorescence microscopy. Data were expressed as the ratio of the mean fluorescence of the experimentally stained tissues (excluding the tubule lumen) to the corresponding value from control sections.

The renal biopsies from patients treated with Cyclosporin A expressed significantly more TGFβ (median ratio of 3) than sections from patients receiving Tacrolimus (median ratio of 1.3; ,$P<0.0001$, Mann-Whitney test).

These results suggest that there is a greater expression of TGFβ in renal tissue from patients receiving cyclosporin A than in biopsies from tacrolimus-treated patients. This may have implications for the development of graft rejection

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THE EFFECT OF ACUTE REJECTION UPON EXPRESSION OF FIBROSIS ASSOCIATED GENES IN RENAL TRANSPLANT RECIPIENTS

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The aim of this study was to determine the role of acute rejection (AR) in the development of chronic allograft nephropathy by determining the expression of fibrosis associated genes (FAG) in renal transplant glomeruli.

A consecutive series of 47 patients (29 male: 18 female) were assessed. Donor categories were cadaveric (n=18), living related (n=9) and asystolic (n=20). Transplant recipients received Neoral or Tacrolimus immunosuppression. Each underwent needle core ®, ultrasound-guided renal transplant biopsies at 1 wk, 3 mths and 6 mths. AR was confirmed histologically and treated with intravenous steroid or anti-T lymphocyte therapy if resistant. Glomeruli were plucked from the biopsy and placed in a lysis binding buffer. Total mRNA extraction was performed with paramagnetic Dynabeads®. Fibrosis associated genes were amplified by reverse transcriptase/PCR and quantified in an ELISA in comparison to a house keeping gene (GAPDH). Both groups were well matched in terms of age, sex, immunosuppressive agents and ischaemic episodes.

There was no statistical difference between either group of patients ($p>0.05$) suggesting that AR does not increase the expression of FAG and may not play a dominant role in chronic allograft nephropathy.

Gene	1 week		3 months		6 months	
	AR (n=14)	No AR (n=21)	AR (n=14)	No AR (n=20)	AR (n=10)	No AR (n=20)
Collagen III	0.22	0.48	0.59	0.48	0.42	0.31
Collagen IV	0.83	0.83	0.55	0.45	0.46	0.42
MMP 2	0.41	0.59	0.54	0.27	0.46	0.23
TIMP 1	1.51	2.32	1.72	2.25	1.56	1.16
TIMP 2	0.83	0.77	0.25	1.30	0.75	0.53
TGF B	0.17	0.13	0.18	0.33	0.25	0.13

All mean values-arbitrary units.

These results suggest that AR does not increase the early expression of fibrosis associated genes and may not therefore play a dominant role in the subsequent development of chronic allograft nephropathy.

THE PRODUCTION OF HLA SPECIFIC ANTIBODIES POST-TRANSPLANT IN TACROLIMUS MONOTHERAPY TREATED RENAL TRANSPLANT RECIPIENTS.

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HLA specific antibodies present in recipient serum prior to transplantation result in hyperacute or accelerated graft failure. Patients with donor reactive antibodies are excluded from transplantion on the basis of a positive result in the pre-transplant crossmatch. Recipients can also develop antibodies post-transplant to mismatched donor HLA antigens in association with transplant failure. The majority of immunosuppressive agents are directed primarily at T cell function but there are limited data to suggest that tacrolimus may also affect B cell function.

Data from this laboratory and others has demonstrated that ELISA and flow cytometry techniques are useful tools for monitoring HLA specific antibody production post-transplant. Therefore, we have investigated antibody production, using ELISA and flow cytometry techniques, in patients treated with tacrolimus monotherapy (Tac, n=28) as compared with a cyclosporin based regimen (CsA, n=28). Both groups had received crossmatch negative primary renal transplants in 1996 and did not differ for gender and pre-transplant antibody status. The mean number of HLA-A,-B and-DR mismatches was 1.86 in each group.

Forty six of the patients were antibody negative both pre-and post-transplantation and were not investigated further. Five patients (2 Tac and 3 CsA) were antibody positive pre-and post-transplant but only 1, from the CsA group and prior to transplant failure, had developed additional antibodies against donor mismatched HLA antigens. There was a decrease in the reaction frequency, as determined by ELISA, for 2 of the Tac monotherapy patients from 65.9% and 48% to 24.4% and 24%, respectively.

Five patients, 1 Tac and 4 CsA, who were antibody negative prior to transplantation, subsequently became positive, although in 2 of the CsA patients this related to a pre-transplant sensitization event. In 3 male patients (1 Tac and 2 CsA) with no history of potential sensitising events, IgM HLA specific antibodies were detectable for the first time following transplantation.

In summary, only 1 patient (3.6%) treated with Tac monotherapy developed de novo HLA specific antibodies following renal transplantation compared with 5 patients (18%) on CsA based immunosuppression.

Post-transplant monitoring of antibody production using a combination of ELISA and flow cytometry techniques suggest that the use of tacrolimus may limit the production of HLA specific antibodies following transplantation. This approach may become a novel method of monitoring the influence of immunosuppressive drugs on the post-transplant course.

THE EFFECT OF ANGIOPLASTY AND STENTING ON RENAL ALLOGRAFT DYSFUNCTION IN PATIENTS WITH RENAL TRANSPLANT ARTERY STENOSIS.

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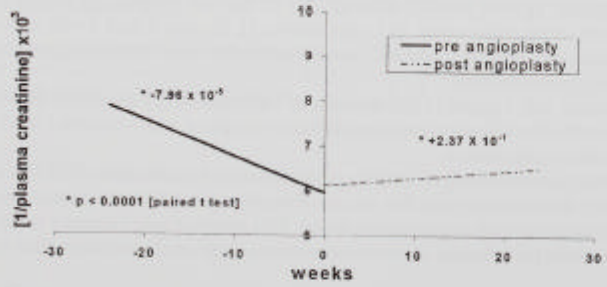
Transplant renal artery stenosis [TRAS] is a significant cause of allograft dysfunction and graft loss. It is technically amenable to treatment by percutaneous transluminal angioplasty [PTA], intra-arterial stenting [IAST] and by-pass surgery. Previous reports have highlighted the beneficial effects of PTA on hypertension but have been circumspect about its value in treating allograft dysfunction.

We have treated 46 renal allograft recipients [30 male 16 female, age range 19-70, mean 44 years] with significant TRAS and a decline in graft function, illustrated by a negative slope of 1/plasma creatinine against time. TRAS was diagnosed by intra-arterial angiography 1 month–30 years post transplant [mean 24 months, median 7 months]. Following intervention with PTA the decline in allograft function over the subsequent 6 months was reversed or slowed in 33 patients [72%]. 13 grafts [28%] were not improved and of these 9 failed [7 from TRAS].

20 patients [43%] underwent 32 further PTA or IAST procedures during the year following the primary procedure [range 1 week–9.5 months, mean 3.3 months]. 8 patients were treated with 10 IAST procedures.

The figure shows the pooled results of the 37 patients with functioning allografts throughout the 6 month follow-up period.

Allograft function pre/post renal artery angioplasty



Although this study clearly demonstrates the beneficial impact of PTA or IAST on allograft function in TRAS, patients may require repeat procedures and careful follow-up.

SUSCEPTIBILITY TO SKIN TUMOURS AFTER RENAL TRANSPLANTATION IS NOT INFLUENCED BY GENOMIC VARIATION IN P53

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An increased risk of skin tumours is a well-recognised complication of renal transplantation. Recently it has been suggested that genomic variation in the tumour suppressor p53 gene may predispose to the development of human papillomavirus-associated tumours, by increasing the susceptibility of the p53 protein to degradation (Storey et al, *Nature* 1998;393:229-34). We investigated the association between p53 genotype and skin tumours in a large cohort renal transplant recipients from a single centre, where the occurrence of skin neoplasms has been well documented for many years.

219 white UK renal transplant recipients were selected for analysis. These individuals had received an initial immunosuppressive regimen consisting of cyclosporin, azathioprine, and prednisolone; and had subsequently received at least five years of immunosuppressive therapy. All were assessed for the presence or absence of skin tumours by interview and review of clinical and pathological records. 51 patients had developed skin tumours, of whom 31 had developed at least one SCC skin tumour. 84 UK cadaveric organ donors were genotyped as a control group.

Allele specific PCR-SSP primers were designed to amplify a 434 bp fragment of the p53 gene. No difference in allele or genotype frequency of the p53 codon 72 polymorphism was detected between any of the groups or subgroups studied, and overall frequencies were comparable to those described in other white populations. Thus, in contrast to earlier suggestions, homozygosity for the p53 codon 72 Arginine allele does not confer susceptibility to the development of skin tumours after renal transplantation.

GENETIC VARIATION IN FREE RADICAL METABOLISM AND SUSCEPTIBILITY TO SKIN TUMOURS AFTER RENAL TRANSPLANTATION

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An increased risk of skin tumours is a well-recognised complication of renal transplantation, and while environmental risk factors are well established, little is known about genetic susceptibility to this condition. Glutathione S-transferase (GST) enzymes detoxify reactive chemical species by catalysing their conjugation to glutathione, and are critical in limiting the toxic effects of reactive oxygen species on lipid membranes and DNA. Polymorphisms associated with impaired free radical detoxication, such as those of GST isoenzymes, may predispose to the development of skin cancers. These genetic susceptibilities may be particularly important in immunosuppressed individuals, who experience the additional insults of long term immunosuppression and human papillomavirus infection.

We investigated the association between polymorphisms in three GST isoenzymes and the development of skin tumours in a large cohort renal transplant recipients from a single centre, where the occurrence of skin neoplasms has been well documented for many years. 219 white UK renal transplant recipients were selected for analysis. These individuals had received an initial immunosuppressive regimen consisting of cyclosporin, azathioprine, and prednisolone, and had subsequently received at least five years of immunosuppressive therapy. All were assessed for the presence or absence of skin tumours by interview and review of clinical pathological records. 51 patients had developed skin tumours, of whom 31 had developed at least one SCC skin tumour. A control group of 84 UK cadaveric organ donors were also studied. All individuals were genotyped for polymorphisms in GSTM1, GSTT1 and GSTP1 using allele-specific PCR-SSP assays that use identical amplification and detection conditions.

Significant differences were found in the distribution of GSTP1 alleles, with the less common GSTP1*C being significantly associated with the development of SCC skin tumours after transplantation ($p<0.006$). This was most marked in individuals who developed skin tumours within the longevity of their first graft ($p<00003$). No associations with non-SCC skin tumours, or with GSTM1 or GSTT1 alleles were noted.

These results demonstrate that genetically determined functional differences in GSTP1 activity contribute to the development of skin tumours in renal transplant recipients. If confirmed in additional cohorts, this will permit the identification of individuals at particularly high risk for the development of skin tumours.

SKIN CANCER RISK FOLLOWING RENAL TRANSPLANTATION

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Renal transplantation is associated with a variable increase in the risk of malignancy, particularly non-melanoma skin cancer (NMSC). Exposure to ultra-violet (uv) light is a major contributory factor; polymorphisms of the glutathione-s-transferase (GST) gene family are associated with variability in the ability to handle uv-induced oxidative stress. We performed a study examining clinical and genetic markers of NMSC risk.

All renal allograft recipients under continuing follow-up at this centre were systematically interviewed and examined by a single dermatologist (HMR). Data was gathered on patient characteristics including skin type, sun exposure, hair and eye colour; case notes were carefully reviewed for details of previous skin cancer. Informed consent was given for the extraction of DNA from peripheral blood and genotype analysis; performed by standard PCR techniques. 171 patients (67% male) with a mean (SD) age at transplantation of 38 (17) years and mean (SD) follow-up interval of 6.5 (5.7) years were studied. 13% had NMSC, 15% solar keratoses, 57% viral warts and 1% lentigo maligna melanoma (n=2). Duration of immunosuppression influenced skin cancer risk; NMSC occurred in 5%, 14%, and 32% of those with allograft survival of 0-5 (n=78), 5-10 (n=59) and >10 (n=34) years respectively. Older age at transplantation [odds ratio (OR) 1.04; p=0.02], male gender (OR 4.02; p=0.03), green eyes (OR 7.4; p=0.01), outdoor occupation (OR 1.04; p=0.003) and actinic keratoses (OR 47.6, p<0.001) were significantly associated with increased risk of NMSC. Squamous cell carcinoma (SCC) but not basal cell carcinoma (BCC) was associated with a history of having ever smoked. GSTM3 BB genotype was significantly associated with increased risk of NMSC (OR 12.7, p=0.04) and reduced time to first tumour (hazards ratio 7.5; p=0.01).

Skin cancer poses a significant problem post-transplantation, even in temperate climates. No standard practice exists in the UK with regard to follow-up of such patients with respect to skin cancer. The early identification of those at highest risk using clinical and/or genetic markers may allow the development of more structured and effective surveillance strategies than currently exist.

UK TRANSPLANT PREGNANCY REGISTRY - FIRST REPORT

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Data collection commenced in March 1997 and by December 1998 a total of 132 pregnancies had been notified to the Registry, with participation from 86% of UK transplant units. Initial identification was by means of a quarterly mailing from UKTSSA which asked transplant units to report any pregnancies in recipients aged between 14 and 50 years. Thereafter questionnaires were issued requesting additional transplant related and obstetric information, including maternal and neonatal complications. Of the 132 pregnancies in 123 women, 15 have been in liver or cardiothoracic transplant recipients with the remainder in kidney recipients. Of these 117 kidney recipients, 93 proceeded to delivery while there were 9 therapeutic terminations, 14 miscarriages and 1 ectopic pregnancy.

Of the pregnancies attaining delivery all infants were born alive although 2 subsequently died. The incidence of premature delivery was 49% and 54% of the infants were of low birth weight (<10centile). The caesarean section rate was 63%, a figure comparable to the 53% reported by the US National Transplantation Pregnancy Registry[1].

Logistic regression analysis demonstrated a relationship between premature delivery and the use of anti-hypertensive drugs prior to pregnancy ($p=0.01$) and renal dysfunction during pregnancy ($p=0.02$). Renal dysfunction during pregnancy was also associated with low birth weight ($p=0.05$) and pre-pregnancy serum creatinine was related to successful pregnancy outcome ($p=0.05$). Hypertension and pre-eclampsia were reported in 75% and 5% of pregnancies respectively with deteriorating renal function evident in 16%.

This is the first analysis of this Registry and accumulation of pregnancy data at the current rate over the next 5 years will result in a database containing information on approximately 500 pregnancies, including more than 50 pregnancies in liver and cardiothoracic transplant recipients. Future analyses will include a case control study to examine the relationship between pregnancy and renal allograft dysfunction.

Reference

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HYPERTENSION AND SURVIVAL FOLLOWING RENAL TRANSPLANTATION

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The influence of recipient blood pressure on graft and patient survival and targets for blood pressure control post-transplantation is not clear. We performed a prospective study in 637 renal transplant recipients, looking at the influence of clinic blood pressure on graft and patient survival after forty months. Of the 634 patients with follow-up data 163,(25.7%), had a blood pressure less than or equal to 140/90 on no therapy. 267,(42.1%) achieved this target with anti-hypertensive therapy and 204,(32.2%) had a blood pressure greater than 140/90,despite therapy with between one and four agents.64 grafts failed and 64 patients died during follow-up, (Death with a functioning graft was censored as a cause of graft loss). Increased levels of blood pressure were associated with a graded increase of subsequent graft failures (see table) and in a Cox regression analysis blood pressure was independently associated with graft failure. While increased levels of systolic and pulse pressure were associated with increasing numbers of subsequent deaths, (see table), in a multivariate analysis blood pressure did not significantly predict death independently of age, creatinine, and diabetes. We were able to demonstrate an independent relationship for systolic and pulse pressure and death when we performed subgroup analysis on diabetic patients.

SBP	graft	mort.	DBP	graft	mort.	PP	graft	mort.
96-119	3.4	4.3	59-74	6.1	7	27-41	4	4.8
120-129	11	7.9	75-79	7	12.6	42-47	12.3	5.3
130-136	9	7.7	80-82	11	13.6	48-53	8.1	12.5
137-146	13.2	11.6	83-86	9.6	11	54-61	11.5	8.4
147-193	17.6	16.8	87-105	16.2	6.2	62-115	15	21.3

(graft = % graft loss and mort. = % mortality)

This study suggests graft survival benefits at levels of systolic blood pressure below that currently recommended, stressing the need for interventional blood pressure trials in this population to lower blood pressure below current guidelines. Clinic blood pressure did not adequately predict mortality in this population and other risk factors amenable to therapy need to be identified in this population at high risk of cardiovascular death.

SCREENING FOR URINARY INFECTIONS IN RENAL TRANSPLANT RECIPIENTS: DIPSTICK URINALYSIS VERSUS URINE CULTURE

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Introduction. Urinary tract infections are common and often asymptomatic following renal transplantation. If not promptly treated they may lead to graft dysfunction. Hence, screening with urine culture is common practice in most renal transplant outpatient departments. This is relatively expensive and time consuming. The new generation of multiple reagent strips detect (in addition to the standard blood and protein) nitrites and leucocyte elastase, which, if positive, are claimed to be better indicators of a urinary infection. The multiple reagent strips have the potential benefit of a rapid diagnosis and substantial cost saving.

Methods and Materials. Three hundred and five mid-stream urine specimens were collected from forty-three renal transplant patients over a sixteen month period from October 1996 to February 1998. Each sample was sent for culture and analysed simultaneously using Bayer's Multistix 8 SG reagent strips and read by an automated Bayer Clinitek 50 machine.

Results. Twenty-one of the 305 samples were positive on culture for a single organism using standard laboratory techniques. For all modalities on the Multistix a 'trace' result was considered as negative. The following table compares the results obtained from the standard dipstix parameters of blood and protein, with those of nitrite and leucocyte elastase.

	Blood and protein	Nitrite and leucocyte elastase
Positive predictive value	10.3%	70.0%
Negative predictive value	93.3%	98.7%
Sensitivity	24%	82%
Specificity	84%	97%

Conclusion. The nitrite and leucocyte elastase parameters on Bayer's Multistix 8 SG reagent strips provide a sensitive and reliable screening tool that can substantially reduce the number of urine culture requests. With a high positive predictive value the strips provide the opportunity to initiate prompt empirical treatment while awaiting the formal culture results.

APPLICABILITY OF THE UPDATED NATURAL HISTORY MAYO MODEL FOR PRIMARY BILIARY CIRRHOSIS (PBC) IN PATIENT SELECTION FOR LIVER TRANSPLANTATION.

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Background: The Updated Natural History Mayo model (Murtaugh PA et al, Hepatology,1994) is a useful mathematical model for prediction of survival in PBC and may assist clinicians in patient selection and timing of orthotopic liver transplantation (OLT).

Aims and Methods: We retrospectively assessed the applicability of this model in a cohort of PBC patients referred to our Unit for OLT, by comparing the Mayo estimated probability of survival (EPS) with our clinical judgement regarding patient selection and timing of orthotopic liver transplantation (OLT). 44 out of 58 patients who underwent OLT between Nov.'92 and Jun'98 for PBC were randomly selected to calculate the Mayo EPS. For comparison, randomly selected records of 22 / 28 patients, who were assessed for OLT but not transplanted for various reasons were also being analysed using the Mayo model.

Results OLT Patients: 21/ 44 patients (47.7%) were transplanted for quality of life (EPS >64% at 2y) and 2/44 (4.5%) for recurrent variceal bleeding (EPS >59% & >76% at 2y, respectively). From the remaining patients, 11/44 (25%) had EPS<50% at 1 y, 4/44 (9%) had EPS >50% at 1y but <50% at 2 y; 6/44 (13.6%) were transplanted despite Mayo EPS >50% at 2y, because of deteriorating liver function. Non-OLT Patients: 16/22 of patients (72.7%) not transplanted, had Mayo EPS >50% at 1y. 14/16 of these patients (87.5%) did survive beyond 1y post-assessment. 2/16 (12.5%) patients died within 1y of assessment despite a Mayo EPS >50%in 1 y.
5/6 patients (83.3%), who were either considered too ill to transplant or a donor liver did not become available in time, had a Mayo EPS <50% at 1y and died shortly after being assessed. 1/6 (16.7%) survived for >2y despite a Mayo EPS <50% at 1y and 16% at 2y.

Conclusion: 1. In our patient cohort, the Mayo model had over 80% accuracy in predicting survival in PBC patients and can be used to enhance clinician's decision making with regards to timing of OLT. 2. Clinician's judgement regarding patient selection and timing of OLT was in concordance with the Mayo model estimated survival probability.

OUTCOME OF HEART TRANSPLANTATION FOR CARDIOMYOPATHY AND ISCHAEMIC HEART DISEASES

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Background: Cardiomyopathy (CM) and ischaemic heart disease (IHD) are the commonest indications for heart transplantation. The aim of this study was to investigate the difference in clinical outcome in these two groups.
Methods: At our institution between 1987 and 1998 transplantation was performed in 145 patients with IHD and 98 with CM. Follow up was complete for all patients (mean 83 months)
Results: Mean age at time of surgery was 39±9 years for CM versus 51±5 years for IHD recipients (p=0.02). There was no difference in donor age, donor sex, pre-operative haemodynamics, renal function, or ischaemic time between the two groups. NYHA status was 1.1±0.2 and 2.0±0.3 for CM and IHD recipients respectively (p=0.013). The onset of post operative rejection episodes was 18±9 days for CM versus 29±6 for IHD recipients (P <0.001). The frequency of rejection episodes ≥grade 2 was lower in CM recipients (4.1±0.9 vs 6.9± 1.0, p<0.01). No difference was observed in the intra-cardiac pressures or incidence of renal dysfunction, infection or malignancy between the two groups following transplantation. At 2 years after transplantation coronary artery disease was present in 6 % of CM recipients and 17 % of IHD recipients (p<0.02). At 5 years the incidence of severe coronary artery disease was 2% and 8% respectively (pNS). The operative mortality was 5.6 % in CM recipients and 10.5% for IHD recipients. (p NS). The actuarial survival at 1, 5, and 10 years was 85%, 82% and 80% for CM recipients compared to 77%, 62% and 39% for IHD recipients (p=NS, <0.0001, <0.0001 respectively).
Conclusions: After heart transplantation the medium and long term outcome is significantly better for CM than IHD recipients. In view of limited donor availability, it may be appropriate to vigorously explore alternative treatments for terminal IHD patients.

LIVER DONATION IN SCOTLAND. NO INCREASE OVER THE LAST SIX YEARS

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Introduction: Liver donation continues to fall behind organ demand. Furthermore, the number of organ donors in the U.K. have fallen by 15% over the current decade. Prior to the establishment of the Scottish Liver Transplant Unit (SLTU) in November, 1992, liver donation in Scotland had risen from 4.9 livers per million population to 13.7 between 1989 and 1992. The aim of this audit was to examine organ donation in Scotland following the establishment of the SLTU with the associated increased medical, public and media interest in organ donation.

Methods: The date and hospital of donation along with the age, sex and cause of death of the donor was obtained from the UKTSSA database for all Scottish liver donors between November 1992 and October 1998. The date and hospital of donation for all solid organ donors in Scotland was also obtained over the same six year time period.

Results: Liver donation and solid organ donation (including liver) rates per million population in Scotland by transplant region are shown in the table.

LIVER DONATION	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**
Glasgow (2.75 million)	8.4	11.3	8.0	7.3	6.6	9.1
Edinburgh (1.22 million)	12.3	12.3	9.0	10.7	9.8	9.0
Dundee (0.39 million)	10.3	10.3	10.3	17.9	10.3	7.7
Aberdeen (0.79 million)	10.1	12.7	24.1	20.3	10.1	19.0
Scotland (5.15 million)	9.7	11.7	10.9	10.9	8.2	10.5
SOLID ORGAN DONATION	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**
Glasgow	16.7	18.5	14.9	16.4	12.7	16.0
Edinburgh	18.0	16.4	14.8	13.9	20.5	18.9
Dundee	15.4	17.9	15.4	28.2	10.3	12.8
Aberdeen	17.7	16.5	29.1	21.5	13.9	19.0
Scotland	17.1	17.7	17.1	17.5	14.6	16.9

Liver donation and solid organ donation rates in Scotland have not changed significantly over the past six years. However, variation between region is noted with the liver donation rates for Aberdeen generally higher in recent years than for the other three regions despite the overall solid organ donation rate similar between the four regions. The mean age, sex and cause of death of the liver donors was not significantly different between the four regions.

Conclusion: Despite the establishment of the SLTU with its concomitant increased publicity in Scotland, overall liver and solid organ donation rates in Scotland have not changed significantly. However, variation between region is evident and is worthy of further study to maximise potential multiorgan donation.

SEQUENTIAL ASSESSMENT OF NON-IMMUNOLOGICAL RISK FACTORS FOR THE DEVELOPMENT OF CHRONIC GRAFT NEPHROPATHY: COMPARISON OF PROGRAF AND NEORAL REGIMENS.

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The importance of non-immunological risk factors in the development of chronic graft nephropathy is increasingly being recognised however temporal changes in these parameters have not been well defined. The aim of this study was to document sequential changes in the plasma levels of recognised biochemical and haematological risk factors and to investigate the relationship between these parameters and the primary immunosuppressive agents prescribed.

As part of an ongoing study, patients undergoing cadaveric renal transplantation were randomised to receive either Prograf (PRO, n=44) or Neoral (NEO, n=43) as primary immunosuppression in a triple therapy regimen. Sequential samples were obtained for the assessment of cholesterol, LDL, triglycerides, lipoprotein a, urate, fibrinogen, insulin and PTH at baseline (n=87) and at follow-up of 3 months (n=62), 6 months (n=44) and 1 year (n=25).

The results are summarised below and are expressed as medians:

Timepoint	0	0	3	3	6	6	12	12
Agent	PRO	NEO	PRO	NEO	PRO	NEO	PRO	NEO
Cholesterol	5.7	5.7	5 # *	5.8	5.1 #	5.5	4.4	6.1
TAG	1.9	2	1.45 *	2.15 #	1.65 #	2	1.5	2.5
Lip a	220	181	95 #	59.5 #	101 #	59 #	98.5 #	98.5 #
Urate	0.31	0.3	0.35 *	0.39 #	0.38 #	0.42 #	0.36	0.46 #
Fibrinogen	3.9	3.7	3.15 # *	3.55	3.3 #	3.6	3.25 #	3.5
Insulin	13 *	20	17 #	12	19 #	17	22 #	16
PTH	21.35	22.35	8.9 #	7.4 #	7.8 #	4.3 #	12.6	10.6

#p<0.05 versus Baseline (Wilcoxon Rank test), *p<0.05 Prograf versus Neoral (Mann Whitney U test)

Reductions in lipoprotein a, fibrinogen and PTH were universal as was an increase in plasma urate. Prograf patients exhibited increased levels of insulin compared with the Neoral group whilst cholesterol and triglyceride levels were elevated in Neoral-treated patients.

CHRONIC REJECTION IN A LIVER TRANSPLANTATION POPULATION

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Chronic rejection in orthotopic liver transplantation results in around 5-10% of primary allografts.The aetiology of chronic rejection is unclear. We have investigated the occurrence of chronic rejection in our transplant population.

Method: The notes of all patients with a histological diagnosis of chronic rejection were retrieved and the following information obtained- aetiology,sex, age at transplantation, HLA mismatch, T-cell lymphocytotoxicity, cyclosporin levels at day 7, 1 month and 3 months, number of acute rejection episodes and severity of acute rejection. Controls for age,sex and time of tranplantation were also investigated. Cytokine genotype details for 6 patient and 6 controls for TNFalpha,TGF beta and interleukin 10 were available.

Results: Nine patients out of 189 patients had biopsy proven chronic rejection of their primary hepatic allograft. The time to diagnosis varied from 6 weeks to 13 months. Six patients had primary biliary cirrhosis, 2 patients had paracetamol induced acute liver failure and one had primary sclerosing cholangitis. In comparison to the whole transplant population patients transplanted for PBC were more likely to develop chronic rejection (p=0.03). Eight of the nine patients were female, which again is significantly different to the transplant population as a whole ($p< 0.01$). The mean age of those with chronic rejection was 44.2 (SEM 4.78) compared with 47.1 (SEM 1.1) of the whole transplant population.

In comparing cases with age sex matched controls there was no difference in number of HLA mismatches and no patient in either group had a positive lymphocytotoxic crossmatch. The cyclosporin levels and rejection episodes are shown in the table.

	Day 7	1 month	3 months	Mean no. of rej episodes
Case	200	160	146	1.9
Control	146	166	148	.44

p = 0.004

The cytokine genotypes were only available in 6 out of 9 patients and they showed no significant difference from controls.

Conclusions:Chronic rejection in our population is not common but is more likley to occur in female patients and patients with primary biliary cirrhosis. There is no significant difference in cyclosporin levels, HLA mismatch or cytokine genotypes between cases and controls. There is however a highly significant increase in the number of acute rejection episodes in patients who go on to develop chronic rejection.

RENAL TRANSPLANTATION IMPROVES LONG-TERM PATIENT SURVIVAL.

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Although renal transplantation improves quality of life, there are still few reports that transplantation improves long-term patient survival compared with maintenance dialysis. The aim of the present study was to assess the survival of all patients commencing renal replacement therapy (RRT) between Jan 1984 and Dec 1995, and to compare the outcome of those who were transplanted with those who remained on the transplant waiting list.

891 consecutive patients were included in the analysis, 678 were ultimately transplanted. 316 patients died during follow-up. The median (range) follow-up from initiation of RRT was 6.6 (0.1,14.5) years. Median age at RRT was 42.4(16.1,70.9), and was significantly higher in those who were not transplanted [49.9(18.5,69.7) vs. 38.8(16.1,70.9) years, P<0.001]

The median survival in transplant recipients was 14.5(11.3,17.0) years compared with 4.6(4.0,5.2) years in those who remained on dialysis (P=0.0001, Log Rank test). The observation that older patients are less likely to be transplanted introduces bias into the analysis. We therefore compared the influence of transplantation on survival in older patients. In those aged 50 or over transplantation increased survival from 4.2(3.5,4.9) to 9.6(7.2,12.0) years (P<0.0001); a similar improvement was seen in those aged 60 or over at initiation of RRT [5.0(4.0,6.1) to 10.9(6.0,15.7) years; P<0.001]. Transplant function was also a major predictor of long-term survival, median survival was reduced to 9.2 years in those patient whose first graft failed.

Studies to compare survival on dialysis vs. transplantation have an unavoidable bias, since patients who die on dialysis cannot be transplanted. However, the median survival on dialysis in this study was 4.6 years suggesting that this was not the sole reason that patients were not transplanted. Nor was age the only explanation, since transplantation improved survival in older age groups. Furthermore, successful renal transplantation was associated with improved survival compared with those whose graft failed, and who returned to dialysis. Overall, these findings suggest that successful renal transplantation prolongs life expectancy in patients entering RRT programmes. They emphasise the need to increase the numbers and survival of renal allografts and also the need to address the risk factors for premature (principally cardiovascular) death in those patients who remain on long-term dialysis.

TACROLIMUS / CYCLOSPORIN CONVERSION FOR BRONCHIOLITIS OBLITERANS SYNDROME. EFFECTS ON LUNG FUNCTION.

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Background
Empirical augmentation of immunosuppression has been advocated to stabilise the decline in lung function associated with bronchiolitis obliterans syndrome (BOS) following lung transplantation.

Method
We studied serial lung function in 11 patients (HLTx 5, BSLTx 4, SLTx 2) with BOS (8) and/or obliterative bronchiolitis (5) receiving triple immunosuppression. BOS was defined as a sustained 20% or greater decline in FEV_1 from established post-operative values without infection or acute rejection. The mean time to tacrolimus conversion was 709 days (range 137-1406). Mean FEV_1 decline was 22±103ml/month ($p<0.05$) and the mean FEF_{25-75} decline was 160±97ml/sec/month ($p<0.01$). The rate of decline of FEV_1 and FEF_{25-75} in the year pre-conversion were 53±120 ml/month and 114±117 $mlsec^{-1}$/month respectively. Following conversion, the rate of decline in FEV_1 slowed in the first six months (mean decrease 24±44ml/month, $p=0.35$) and an increase in FEV_1 was seen in the following six months (mean increase 12.5±50ml/month, $p=0.31$). The decline in FEF_{25-75} was reversed in the first six months (mean 0.5±77mlsec-1/month, $p<0.001$). Tacrolimus conversion was associated with a stabilisation of FEV1. The rate of decline of FEF25-75 improved significantly in the six months post conversion. This benefit was sustained over the first year ($p<0.05$).

Conclusion
These results indicate that tacrolimus conversion slows the decline of lung function in BOS. The attenuation of decline in lung function is sustained for at least a year following conversion.

BLOOD PRESSURE AND LEFT VENTRICULAR MASS (LVM) CHANGES FOLLOWING RENAL TRANSPLANTATION IN PATIENTS RECEIVING CALCINEURIN INHIBITORS.

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Hypertension, abnormal diurnal rhythm and left ventricular hypertrophy (LVH) are strong predictors of risk of death in renal transplant recipients. Several studies have suggested that these risk factors are not modified following successful renal transplantation due to the vasoconstrictive effects of calcineurin inhibitors. The aim of this study was to prospectively compare cardiovascular parameters in a longitudinal study of patients randomly allocated to treatment with the calcineurin inhibitors Neoral and Prograf.

Patients had baseline 24-hr blood pressure measurements and LVM estimation by echocardiography in the first week following transplant and at 3, 6 and 12 months follow-up. An antihypertensive drugs index (AHDI) was recorded. As paret of our policy to reduce nephrotoxicity, all patients received Nifedipine SR 10 mg b.d. The results are summarised in Table 1. Data shown as median and maximum values. AHDI as mean +/- (S.D.).

	Neoral (n=43)				Prograf (n=44)			
Assessment	0	3	6	12	0	3	6	12
Day Mean BP	106(133)	100(116)	97(122)	100(135)	105(135)	97(109)	94(111)	98(117)
Night Mean BP	108(140	100(111)	96(115)	92(118)	108(151)	92(109)	91(110)	93(105)
Nocturnal "dip"	+0.5	0	-5	-5	+2	-1	-4	-5
Day Sys. BP	148(179)	134(174)	133(166)	135(192)	143(188)	131(142)	127(147)	131(150)
Night Sys. BP	151(197)	136(156)	133(164)	129(172)	151(203)	125(142)	125(145)	128(140)
Nocturnal "dip"	+1	-1.5	-3.5	-6	+2.5	-4	-4	-6
Day Dia. BP	82(112)	82(97)	80(96)	82(99)	86(108)	82(93)	76(92)	83(97)
Night Dia. BP	82(115)	81(94)	80(95)	75(96)	87(116)	77(92)	73(98)	76(89)
Nocturnal "dip"	0	0	-4	-6	+3	-2	-5	-4
LVMI (<120)	201(305)	182(330)	182(330)	164(205)	187(295)	141(242)	182(299)	157(220)
AHDI	1.77(1.1)	1.54(1.2)	1.6(1.2)	1.14(1)	1.58 (1)	1.23(1)	1.4(1.1)	1.08(1.2)

Patients entering the transplantation program have grossly abnormal BP patterns and marked LV hypertrophy. Ttransplantation significantly improves BP profiles, reverses diurnal abnormalities and improves LVM though the changes take several months to manifest. Improvement in diastolic BP was more significant in the Prograf group ($p<0.028$-0.001). Patients receiving tacrolimus, but not Neoral, showed a significant drop in antihypertensive drugs requirement ($p<0.02$).

URINARY PI AND ALPHA GLUTATHIONE-S-TRANSFERASE EXCRETION IN THE EARLY PERIOD POST-RENAL TRANSPLANTATION

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The enzyme glutathione-s-transferase (GST) forms approximately 3% of the cytosolic protein in human kidneys. The alpha form (alpha GST) is confined to the proximal and the pi (pi GST) to the distal tubule in human kidneys. Acute cellular rejection typically causes tubular destruction of the distal tubule and may be predicted to proportionately increase the excretion rate of pi compared to alpha GST.

Timed urine collections were made in recipients of cadaveric renal transplants during the initial inpatient and early intensive out patient follow up period post-transplantation. The excretion rate of pi and alpha GST was measured as was the ratio of pi divided by alpha GST. The medical records and investigations for patients were retrospectively reviewed. The clinical course of the patients is summarised in the next four paragraphs.

For seventeen patients there was no evidence of rejection during this early period. Pi and alpha GST excretion rates are always high in the first few days post surgery presumably principally due to ischaemic injury. In these seventeen patients they fell rapidly and remained low, (Pi GST excretion rate mean 3.97 ng/min (s.d. 2.86), alpha GST excretion rate mean 4.06 ng/min (s.d. 2.17), after day 5).

Five patients had biopsy proven rejection preceded by a rise in the excretion rate of pi GST and an increase in the pi over alpha GST ratio, mean increase in pi GST 19.24 to 39.02, coincident alpha GST measurements 6.29 to 5.23 ng/min. The mean ratio of pi over alpha GST increasing from 3.06 to 7.46.

Two patients had biopsy proven acute cellular rejection preceded by low pi and alpha GST excretion rate (pi <4.87, alpha <3.14 ng/min). One patient had a late rise in alpha GST unaccompanied by any obvious clinical event (alpha GST increased from 3.71 to 99.28 ng/min).

Therefore the clinical course and urinary enzymes were concordant in 22, but contradictory in 3 patients. There was wide intra-individual variation in the excretion rate of the enzymes.

This pilot project suggests that serial measurements of urinary enzymes can help predict early rejection and may contribute to achieving the diagnosis of the cause of graft dysfunction.

The project was supported by Biotrin International, The Rise, Mount Merrion, Co. Dublin, Ireland.

FAS LIGAND TRANSFECTED MYOBLASTS AND ISLET CELL TRANSPLANTATION- AN ANALYSIS OF THE IMMUNE RESPONSE

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Considerable controversy exists as to whether the expression of Fas ligand (FasL) within the local environment of an islet allograft will confer protection against rejection.

We explored this potentially valuable therapeutic strategy by engineering C3H ($H2^K$) myoblast lines to express Fas ligand at high ($FasL^{hi}$) and low ($FasL^{lo}$) levels. Mice rendered diabetic by a single dose of streptozotocin (250µg/ml), received either a syngeneic graft consisting of islets alone, or a composite syngeneic graft consisting of islets mixed with $2x10^6$ FasL (lo/hi) or non-transfected myoblasts (C2C12).

Examination at day 30 post-transplantation revealed that the animals which had received islets alone were normoglycaemic (3/3), however mixing syngeneic islets with FasL-expressing myoblasts resulted in failure of all the islet grafts ($FasL^{lo}$=0/7; $FasL^{hi}$=0/5). The majority of animals which had received syngeneic islets mixed with non-transfected myoblasts (C2C12) had functioning grafts at day 30 (5/8), although an explanation for the failure of some of the grafts was initially unclear.

Macroscopic examination of the grafts revealed that FasL expressing myoblasts ($FasL^{lo}$ + $FasL^{hi}$) had generated formation of massive intra-abdominal adhesions, with only sparse amounts of tissue remaining at the site of transplantation. Animals that had received islets mixed with non-transfected syngeneic myoblasts (C2C12), developed a 3-4mm well demarcated tumour at the site of transplantation which encased the islets, thus explaining why some grafts failed to maintain normoglycaemia. Immunohistochemical analysis highlighted massive infiltration by neutrophils into FasL expressing grafts which peaked between day 6 and day 13, and which had resolved by day 30 post-transplantation.

Our results provide further support for the opinion that unless FasL-dependent neutrophil infiltration can be prevented, then it is unlikely that this method of protection against allograft rejection will be beneficial.

Supported by The Wellcome Trust and The Allison Foundation

Eligible for Medawar Medal

Medawar Medal

Tuesday 30th March

Delegates are reminded that the eligibility criteria for the Medawar Medal are as follows:

Candidates must be a member of the Society and aged 35 years or under on the first day of the Annual Congress

The work must be original and innovative and have been performed largely or entirely in the UK

WHAT IS THE TRUE INCIDENCE OF ACUTE REJECTION (AR) IN RENAL TRANSPLANT RECIPIENTS RECEIVING CALCINEURIN INHIBITORS?

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The incidence of AR has fallen by 50% since the introduction of calcineurin inhibitors, but the incidence of chronic graft nephropathy has remained unaltered. One explanation could be a significant incidence of unsuspected acute rejection injury (USAR) that escapes current anti-rejection therapy. The aim of this study was to investigate, in a longitudinal manner, the histological incidence of acute rejection on protocol biopsies.

Patients were randomised to receive either Neoral (NTT) or Prograf (PTT) based triple therapy. Each AR was confirmed histologically. Patients also underwent protocol biopsies at 3, 6, and 12 months post-transplant regardless of renal function. No patient received anti-rejection therapy on the basis of scheduled biopsy alone. This interim analysis of 87 patients consists of 122 clinically-indicated and 123 scheduled biopsies. The incidence of AR + USAR was 63% in the NTT group and 41% in PTT group ($p<0.05$). The difference between the two groups was more significant for the incidence of AR (42%vs.23%; $p<0.0001$).

SCHEDULED BIOPSIES	NTT			PTT		
Biopsy time	3	6	12	3	6	12
No. Biopsies	24	17	9	32	26	15
No Rejection %	42	41	44	56	58	47
Banff 3 (Borderline) %	29	29	11	28	23	0
Banff 4 Grade 1 %	21	12	0	13	0	0
Banff 4 Grade 2 %	4	6	0	0	0	0
Banff 5 (CGN) %	4	12	45	3	19	53
Creatinine in non-rejectors (μmol/l)	143	150	149	139	138	130
Creatinine in Rejectors (μmol/l)	156	157	167	137	148	127
Creatinine in No CGN group (μmol/l)	147	156	153	136	137	122
Creatinine in CGN group (μmol/l)	170	157	173	148	161	144

Features of rejection were seen in up 59% of protocol biopsies performed in the first post-transplant year. Our data demonstrates that the incidence of USAR falls with time but is "replaced" by chronic graft nephropathy. Continuation of this study will determine if USAR has a significant detrimental effect on long-term graft function. Continuation of this study will determine if USAR has a detrimental effect on long-term graft function.

THE INFLUENCE OF DELAYED GRAFT FUNCTION IN RECIPIENTS OF CONVENTIONAL HEART BEATING AND NON-HEART-HEART BEATING DONOR KIDNEYS

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Delayed graft function (DGF) has a strong association with reduced graft survival and is common in recipients of kidneys from non heart beating donors (NHBD). This study was performed to compare outcome of renal transplants from conventional heart beating donors who developed DGF and recipients of kidneys from NHBD with or without DGF.

All renal transplant recipients of cadveric or LRD with a period of DGF (defined as the need for dialysis within the first 7 days after transplantation) were retrospectively identified during the period 1987-1997. A uniform immunosuppression protocol was used (cyclosporin, azathioprine and steroid). Similarly a prospective analysis of all recipients of NHBD performed during 1992-1998 were also analysed. In this cohort patients received either cyclosporin, or Tacrolimus with additional steroids. All patients with DGF underwent weekly needle core biopsies using ultrasound guidance to exclude acute rejection. Histologically proven acute rejection was treated with intravenous steroid and those resistant received anti-T lymphocyte therapy (e.g OKT3 or ATG). All patients with primary non function were excluded from analysis.

During the study period a total of 464 transplants were performed. The overall incidence of graft survival at 1 year was 85%. The incidence of DGF in recipients or cadaveric or LRD was 17% and 93% for recipients of kidneys from NHBD. Both groups receiving conventional heart beating donors with DGF (n=69) or NHBD (n=59) were well matched for donor age (median 43 years versus 48) and recipient age (median 45 versus 46). Similarly, there was no difference in the rates of acute rejection between either group (median 23% versus 24%). The duration of cold ischaemia was 21 hours in the conventional heart-beating donors with DGF compared to 17 hours in NHBD. Finally graft survival in the NHBD recipients was significantly better at 3 years (84% versus 73%) compared to the conventional heart-beating donor recipients with DGF ($p<0.05$).

In conclusion these results suggest that despite the high incidence of DGF in recipients of kidneys from NHBD the overall graft survival is significantly better when compared to recipients of kidneys from conventional heart-beating donors. This may be due to an absence of the complex effects of brain stem death in NHB donors.

IL13 AND IL15 INDUCE A20 GENE EXPRESSION IN ENDOTHELIAL CELLS

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Introduction

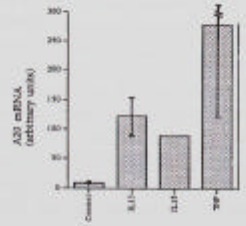
The zinc finger protein A20 inhibits TNF-mediated activation of endothelial cells and expression of NF-κB dependent inflammatory molecules such as E-selectin, VCAM-1 and tissue factor. A20 also protects endothelial cells from TNF-mediated apoptosis. Expression of this 'protective' gene may be important in the context of graft rejection; Bach et al (Nature Medicine 1997; 3: 196-204) recently demonstrated that A20 expression was associated with accommodation of rat-hamster heart xenografts. In this study we examine the effects of IL13 and IL15 on A20 expression in human umbilical vein endothelial cells (HUVEC).

Methods

HUVEC were grown to confluence in gelatin-coated 24-well plates before exposure to IL13 or IL15; both were used at 20ng/ml for 6 hours. TNF, a known inducer of A20, was used as a control. Cells were harvested and total RNA was extracted. Competitive quantitative RT-PCR was performed for A20 and a 'housekeeping gene' (β-actin); mRNA transcripts containing primer sequences for A20 and β-actin but of differing length compared to natural sequence were constructed, titrated and combined with extracted cellular RNA to control for efficiency of reverse transcription and PCR and to act as a competitor.

Results

IL13 and IL15 upregulated transcription of A20. The results shown are based on duplicate wells and are representative of three independent experiments.



Conclusion

The Th2 cytokines IL13 and IL15 induce A20 gene expression in HUVEC and may protect endothelial cells from TNF-mediated activation and apoptosis.

THE EFFECT OF LINK NURSES AND A NURSE EDUCATION PROGRAMME ON ORGAN AND TISSUE DONATION

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In late 1992, two separate innovative programmes were instituted within the region served by our Unit: i) A Link Nurse Programme in which 1-2 nurses are identified on each ITU whose remit is to link with the Regional Transplant Co-ordinator and assist in identifying the educational needs of ITU staff. The Link Nurses enable effective communication, increased motivation and a team approach to organ and tissue donation. A training programme and follow-up study days are provided;
ii) A Nurse Education Programme which enables health care professionals working within the ITU's, Ward areas, Accident and Emergency Departments and Operating Theatres to learn about organ and tissue donation and transplantation.

We have analysed our experience of organ and tissue donation comparing, where possible, a period of 5 years prior to institution of the programmes with a period of 5 years following. The following outcomes were noted:-

i) An increase in brain-stem dead potential organ donors from an average of 32 per year to an average of 40 per year, which has been sustained.
ii) A reduction in refusal rates from 23% (37 of 161) to 14% (28 of 200) – $\chi^2 = 4.87$ $p < 0.05$.
iii) A progressive annual increase in skin donation.
iv) An increase in bone donation.
v) An initial increase in corneal donation which has not been sustained.

We believe that the Link Nurse and Nurse Education Programme have contributed to improved awareness of the need for organ and tissue donation and have thus improved their provision within our region. The training and education days, and follow-up study days are provided free to participants and form part of the overall education programmes provided by the transplant co-ordinators.

TISSUE BINDING PROPERTIES OF A SYNTHETIC PEPTIDE DNA VECTOR DERIVED FROM SNAKE VENOM AND TARGETED TO CELL MEMBRANE INTEGRINS

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Our group has previously demonstrated that a synthetic 31 amino acid peptide (polylysine-molossin) comprising a 15 amino acid moiety for targeting cellular integrins (derived from the snake venom, molossin) and a 16 amino acid polylysine moiety for DNA binding, is an effective DNA vector for cell lines and for corneas *in vitro*. In this study, we have studied the binding properties of this DNA vector system, both to identify the appropriate target tissues in the commonly transplanted organs, and to define the specificity of the binding. The 31 amino acid vector, as well as its separate 15 amino acid molossin and $(lys)_{16}$ components, were individually synthesised and a monoclonal antibody was raised to the molossin peptide. Flow cytometry studies with the ECV304 cell line demonstrated that the binding of polylysine-molossin and polylysine-molossin/DNA complexes involved both electrostatic and integrin-mediated interactions with the cells, with the electrostatic binding being sufficient for maximal binding. However, binding to cellular integrins was essential for successful gene transfection. Thus non-specific (and from the point of view of gene transfer) ineffective binding of this vector is likely to occur by electrostatic interactions to many cell types and to components of the tissue matrix. Binding studies on frozen tissue sections of the rat and pig demonstrated that the molossin peptide bound to many cell types of interest in transplantation, but not to all. Among the negative tissues were vascular endothelium, pancreatic islets, skeletal muscle and intestinal epithelium. However, most other cell types were positive. Small species differences in tissue binding were noted between rat and pig. This study defines the cooperative nature of the binding of this vector system, and the cell types in transplanted organs most likely to be effectively targeted for DNA transfer.

Size reduction of adult cadaveric small bowel for pediatric recipients of less than 10 kg.: a way forward ?

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Small bowel transplantation in children is limited by the relative lack of size matched donors. Children of less than 10 kg are particularly susceptible to liver damage by TPN and other problems related to short bowel syndrome and have a high mortality whilst waiting for small bowel transplantation. Patient survival for isolated small bowel transplantation of 85-90 % at one year is currently being reported by experienced Surgical teams. The concept of reduced size small bowel procured from adult cadaveric donors to transplant children of less than 10 kg could offer a way forward and a study has been performed to evaluate the anatomical and technical feasibility.

15 small bowels were retrieved from adult cadaveric donors following the consents of the Hospital Ethical Committee and the donor family . A graft consisting of 1 metre of ileum was produced whilst keeping the whole length of the superior mesenteric artery and vein. The volume of the graft created was 271 ± 42 ml in 7 thin patients with Body Mass Index (BMI) < 25 (weight from 60 to 82 kg), 390 ± 120 ml in 5 mildly obese patients with BMI >25 and < 30 (weight from 85 to 100 kg) and 490 ± 118 ml in 3 obese patients with BMI >30 (weight from 95 to 103 kg). The size reduction was safer and easier in thin patients where transillumination permitted in situ dissection of the mesentery. The diameter of the adult ileum was 2 cm and no diameter reduction was required. CT scans from children of less than 5 kg (N=5) and from 5 to 10 kg (N=5) were used to calculate the abdominal cavity volume available for the small bowel graft in these weight groups which were 261 ± 45 ml and 460 ± 117 ml respectively. The use of an abdominal silastic patch may be needed in children with contracted abdominal cavities. The space would allow the implantation of up to 2 metres of small bowel from a thin adult weighing up to 80kg into a children of 5 to 10 kg. Angiography confirmed the preservation of a suitable vascular supply to the graft. Modification of this technique will also allow the creation of 2 grafts, one ileal and the other jejunal from a single donor. However, the jejunal graft occupies 1.5 to twice the volume of the corresponding ileal graft.

In conclusion, size reduction of adult cadaveric small bowel can provide suitable grafts for children of less than 10 kg and could alleviate the shortage of donors for small children. Thin adult donors weighing up to 80 kg are the most suitable donors for providing ileal grafts of which up to 2 metres could be implanted into children of 5 to 10 kg.

MANIPULATION OF CD4-DEPENDENT ALLOANTIBODY RESPONSES TO CLASS I MHC BY ALLOPEPTIDES

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Alloantibody plays an important role in both acute and chronic allograft rejection. In this study, the ability of a synthetic allopeptide to influence the generation of a T cell-dependent alloantibody response to allogeneic class I MHC was studied in an experimental rat model. Congenic PVG-RT1^u rats mount a strong alloantibody response to RT1.A^a class I MHC, with the response being critically dependent upon CD4$^+$ T cells which recognise A^a alloantigen by the indirect pathway.

Previous studies have identified a 15-mer allopeptide, designated peptide 7, which corresponds to the hypervariable region of the α1 domain of the RT1.A^a class I MHC molecule (aa 61-75), and encompasses an immunodominant T cell epitope. When injected subcutaneously with adjuvant, peptide 7 resulted in an accelerated cytotoxic alloantibody response to A^a-disparate PVG-R8 blood transfusions, and accelerated rejection of R8 cardiac allografts.

The ability of peptide 7 to modulate the alloantibody response to A^a alloantigen was examined by pre-immunising PVG-RT1^u rats with 300μg of the allopeptide intravenously 12 days before challenge with an A^a blood transfusion. Treatment with peptide 7 resulted in a diminished alloantibody response to intact donor class I following R8 blood transfusion; IgM alloantibody levels remained low while the IgG2b alloantibody response was delayed by several days. Cytotoxic alloantibody-mediated lysis of donor target cells was markedly reduced compared to that in control animals.

Conclusion: These results show that *in vivo* treatment with a class I MHC allopeptide encompassing a dominant T cell epitope may be of use for impairing CD4$^+$ T cell-dependent alloantibody responses.

STAT 6 UPREGULATION BY FK506 IN THE PRESENCE OF IL4

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Background.
FK506 perturbs normal phosphorylation in lymphocytes by inhibition of the PP2B protein phosphatase, *calcineurin*. Calcineurin activity is required for intracellular signal transduction and lymphocyte activation which in turn leads to either TH1, or TH2, -type responses to antigen. This choice of response involves differential phosphorylation of STATS (Signal Transducers and Activators of Transcription) for induction of STAT activity. Interferon γ (γINF) activates STAT1, a TH1-type mediator, and IL4 activates STAT6, a TH2-type mediator.

Aim.
To determine if FK506 predisposes cells towards a STAT 6 response.

Experimental.
The RAW 2647 mouse macrophage cell line expresses both the IL4 receptor and the γINF receptor. Raw 2647 cells treated with IL4, plus or minus 2nM FK506, were lysed and separated into nuclear and cytoplasmic fractions. Gel shift and supershift assays measured the induction of STAT6 transcriptional activity by STAT protein binding to the P^{32}-labelled DNA consensus sequence for STAT6. Total STAT6 protein in nuclear and cytoplasmic fractions was assayed by Western blot.

Results.
(i) FK506 per se did not induce STAT 6 in the RAW cells, whereas
(ii) IL4 induced activation of STAT 6.
(iii) Cultures which had been preincubated with FK506 showed an enhanced STAT6 activity in response to IL4.
(iv) Pretreatment with FK506 was associated with increased levels of STAT 6 protein following stimulation by IL4 when compared to cells without FK506.

Conclusion.
Exposure to therapeutic levels of FK506 may enhance immunosuppression for allografts indirectly by enhancing STAT6 activity. This effect would be additional to the known inhibitory effect of FK506 on IL2 synthesis.

Concurrent Sessions

Tuesday 30th March

Clinical

Tuesday 30th March

IMPACT OF LOCAL EXCHANGE OF CADAVERIC RENAL TRANSPLANTS ON HLA MATCHING AND COLD ISCHAEMIA TIME

Newstead C.G. on behalf of the Leeds / Liverpool / Manchester Renal Transplant Alliance. Renal Unit, St.James's University Teaching Hospital, Leeds.

In September 1997 the renal transplant units in Leeds, Liverpool and Manchester initiated an exchange protocol designed to improve the degree of HLA matching in recipients of cadaveric renal transplants. Organs retrieved within the region are allocated to a recipient pool, which includes all the patients (approximately 1000) on the transplant waiting list of the three units, after allocating kidneys according to the national matching rules.
We have examined the impact of the new system on HLA matching and cold ischaemia time. The results are tabulated in the next two tables comparing the first year with the twelve months prior to the start of the scheme.
For all cadaveric transplants:

		Mismatches as percentage			Cold ischaemia time Hr : min	
Era	Number	000	0 DR	100,010,110	Mean	Median
1.9.96 – 31.8.97	252	8	69	43	22 : 33	20 : 10
1.9.97 – 31.8.98	260	12	80	65	20 : 58	19 : 23

For locally retrieved and exchanged transplants only:

		Mismatches as percentage			Cold ischaemia time Hr : min	
Era	Number	000	0 DR	100,010,110	Mean	Median
1.9.96 – 31.8.97	169	6	63	38	21 : 55	19 : 52
1.9.97 – 31.8.98	191	8	78	59	19 : 49	18 : 30

We have also examined the cold ischaemia time between 1.9.97 and 31.8.98 comparing kidneys which were imported from UKTSSA with those retrieved and implanted by the same centre and those retrieved by one of the other two alliance centres and implanted by the third centre.

	Cold ischaemia time: Hr : :min	
	Mean	Median
UKTSSA imports	25 : 10	20 : 33
Locally retrieved and implanted	18 : 41	16 :55
Other Alliance retrieved	19 : 53	19 :10

These results show that within this alliance the exchange of cadaveric renal transplants achieved improved HLA matching with no increase in the cold ischaemia time.

PRE-EMPTIVE THERAPY AGAINST CYTOMEGALOVIRUS DISEASE IN RENAL TRANSPLANTATION - A REALISTIC OPTION?

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Prophylaxis against CMV disease is now a realistic option with the availability of both oral and intravenous preparation of ganciclovir. However, a significant proportion of patients with the highest risk of CMV, donor CMV seropositive recipient CMV seronegative (D+R-), do not even contract CMV infection, and would receive therapy needlessly. The concept of pre-emptive therapy against CMV is more attractive since it only targets patients who are likely to get CMV disease but requires an early indication of CMV activity. This marker should be sensitive and specific and enable treatment before onset of disease. Eight laboratory markers were prospectively evaluated for their ability to guide pre-emptive therapy - pp65 antigenaemia (DAT), CMV IgG level, CMV IgM, Hybrid Capture assay (HCS), plasma PCR (in house -PPCR, Roche Amplicor -PAMP), leucocyte PCR (in house -LPCR, Roche Amplicor -LAMP).

Patients were classified according to donor (D) and recipient (R) CMV serostatus (+ or -). 77 renal transplant recipients (D+R- 21, D-R+ 13, D+R+ 43) were followed for 12 weeks with weekly blood samples for the above tests. CMV disease was defined as confirmed organ specific illness or the presence of unexplained fever, malaise, leucopenia or thrombocytopenia in association with laboratory confirmed CMV activity. 62% (13) D+R- and 7% (4) or R+ recipients developed CMV disease ($p<0.000001$). In 29% D+R- recipients CMV transmission did not occur. Of the high risk category of patients (D+R-), LPCR and LAMP identified 54% and 69% of patients requiring pre-emptive therapy at a mean time of 2.3 and 8.4 days before disease onset, respectively. Plasma PCR was less sensitive (31-46%) and the non-PCR methods were generally positive after the onset of illness. In the low risk group (R+ patients) these markers were either not sensitive enough (DAT, IgM, IgG) or would have led to overtreatment (LPCR 35%, LAMP 50%). Viral load analysis is ongoing.

Whilst none of the markers were absolutely predictive using our weekly sampling strategy, leucocyte PCRs appear to be the best and most realistic options to guide therapy in the high risk D+R- recipient. A reliable marker has yet to be found to guide therapy in the low risk recipient, although clinically this is a relatively less important issue since only 7% of such patients contracted CMV disease.

"OLD AND COLD" CADAVER KIDNEYS ARE A HIGH RISK TRANSPLANT

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We review the experience of a single UK centre between 1990-97 for the interaction of cold ischaemia time (CIT), organ exchange, HLA-DR mismatching and donor age on short and long-term survivals. CIT data was available in 66.4% of 991 first cadaver donor transplants; 36.4% of these were imported from other centres. CIT was significantly increased for imported kidneys (mean 28.9h) over local kidneys (mean 23.9h), p<0.0001. Kidneys stored >=20h had significantly reduced survivals. CIT was <20h in 27% of local and in 14% of imported kidneys.

	Number of cases	% Graft Survival		
		1 year	5 years	Log rank p
First, cadaver donor, 1990-97	991	86.0	76.7	
First, cadaver donor with CIT data	658	86.6	76.7	
Stored >= 20 hours	510	84.7	73.4	
Stored < 20 hours	148	93.2	89.4	<0.0001
Stored < 20 hours + 0 HLA-DR mm	54	97.8	92.8	
Stored >= 20 hours + donor > 55y	86	83.9	61.7	
Stored >= 20 hours + donor > 55y + HLA-DR mm	26	80.4	52.0	

0 HLA-DR mismatching was achieved in 62.8% of <20h and 60.9% of >=20h stored kidneys. The incidence of acute rejections (26%) did not differ between CIT groups. Kidney donor age > 55 years was found to significantly reduce transplant outcome. Where the donor was aged >55y and CIT was >=20h poor survivals resulted. In 26 of these transplants there was HLA-DR mismatching giving the poorest outcomes.

We conclude that CIT >=20h adversely affects kidney graft survival and efforts should be made to control this variable. Kidneys should only be exchanged if there is no HLA-DR mismatch or transport times are short. High five year survivals of over 90% may be achieved by avoiding HLA-DR mismatches with CIT of <20h. In such instances the attrition rate of graft loss is less than 1% per year after the first year suggesting ischaemia damage and HLA-DR mismatching may contribute to the mechanisms of chronic transplant loss.

IS THE USE OF ELDERLY DONORS JUSTIFIED IN OUR QUEST TO EXPAND THE DONOR POOL?

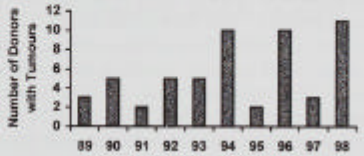
G. Morris-Stiff, S. Benson, J. Casey.

The Carrel Club.

The recent expansion of the donor pool by the use of elderly donors has lead to commendable clinical results. However, one issue not touched upon is the potential risk of renal cell carcinoma, a tumour which has increased its incidence by 38% during the era of renal transplantation. The aim of this study was to analyse the UKTSSA database to determine whether the increasing use of elderly donors has lead to an increased identification of renal neoplasia.

Data from the UKTSSA were examined over a 10 year period to determine the change in donor demographic characteristics and the reported annual incidence of renal neoplasia. The time point of identification of the tumour was also noted together with the fate of both kidneys.

During the period 1989-1998, the mean donor age increased from 34 to 38 years. More importantly, the proportion of donors over 40 years increased from 39% to 52%. Fifty-eight donors were reported as having 1 or more renal tumours (41 bilateral, 17 unilateral). The mean age of donors with tumours was 48.8 years. The temporal prevalence is as shown.



The fate of the 116 kidneys from the 58 donors is summarised below:

Bilateral (n=82)					Unilateral (n=34)				
NT	NO	TNA	TAN	U	NT	NO	TNA	TAN	U
8	10	22	42	0	2*	0	5	12*	15

NT=not taken; NO=not offered; TNA=taken, not accepted, TAN=taken, accepted, not used, U=used. * 15/17 normal kidneys were transplanted and 2 were not (1 NT, 1 TAN)

Of the donors with unilateral tumours, no tumours have developed in the recipients of the normal kidneys. Disappointingly, in approximately 47% of cases the tumours are identified by the recipient hospital at the time of preparation for implantation.

With increasing use of the older donor population, more renal tumours are being identified but this does not appear to have had a detrimental effect. In the light of the Standards Document, the "late" diagnosis of these tumours warrants further investigation and the development of a pre-harvest investigation protocol.

THE SIGNIFICANCE OF POSITIVE FLOW CYTOMETRIC CROSSMATCHES IN LIVE DONOR TRANSPLANTATION

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The flow cytometric crossmatch (FCXM) is the most sensitive pre-transplant test for identification of donor specific antibodies. A positive FCXM in sensitised recipients is widely considered to be a contra-indication to transplantation. The significance of positive FCXMs in unsensitised recipients of primary grafts is less clear. In this laboratory the FCXM is used as the principle crossmatch for all potential live donor transplant pairs irrespective of the sensitisation status of the recipient. In this study we have reviewed the results of FCXMs performed for all potential live donor/recipient pairs from 3 centres. A total of 126 donor/recipient pairs were tested over a period of 2 years, 57 of which have been transplanted to date. Thirty of these pairs had 1 or more positive FCXMs.

In 14 of the 30 cases the positive FCXM was not classed as a contra-indication to transplantation. In 5 cases the recipients were shown to have a positive auto FCXM. In 7 cases an immediate repeat FCXM was shown to be negative, including 2 patients with previous failed transplants and high levels of panel reactive antibodies. The false positive FCXMs in these cases were primarily due to the low B cell numbers obtained from the donor peripheral blood, with insufficient cells for a reliable result to be obtained. A repeat FCXM allowed greater numbers of cells to be prepared for analysis. In 2 cases positive FCXMs were obtained on more than 1 occasion but testing over a period of time showed that the FCXM became negative. These cases were associated with donor &/or recipient infections concurrent with the crossmatch samples and additional antibody screening did not show any evidence of HLA specific antibodies in these samples. Overall 11 of the 14 recipients had no evidence of sensitisation on antibody screening. Nine of the 14 donor/recipient pairs have been transplanted to date. There was one technical graft failure in this group compared with 1 failure in the 48 negative FCXM pairs.

In the remaining 16 positive FCXM pairs the result was regarded as a contra-indication in 14 pairs and of unknown significance in two. In 14 cases the positive result was consistent with defined HLA specific antibodies (mean panel reactivity 60%) and included repeat mismatched antigens in 5 cases where the recipient had a previous failed graft. In the 2 unknown cases one patient has been shown to have antibodies specific for HLA-DP, the clinical significance of which is unclear. One patient has a consistently positive FCXM and a negative auto FCXM but no evidence of HLA specific antibodies on screening. The primary disease in this patient is SLE and it is probable that the positive FCXM is due to non-HLA antibodies which are associated with the disease.

The results of this review show that a positive FCXM need not be a contra-indication to live-donor transplantation where the possibility of donor HLA-specific antibodies can be discounted and indicates that full investigation of positive results is worthwhile in these cases for both sensitised and unsensitised recipients.

Intra-thoracic Transplantation

Tuesday 30th March

DEVELOPMENT OF A SIMPLE RISK MODEL FOR PREDICTING SURVIVAL AFTER ADULT ORTHOTOPIC HEART TRANSPLANTATION

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Purpose: Knowledge of key donor and recipient factors and their relative importance can assist in advising patients of the likely outcome of their transplant and in selecting an appropriate recipient for a donor heart. We have previously presented a simple model for classifying adult orthotopic heart transplants based on the number of risk factors per transplant (JHLT 1998;17:82). That model was however limited by the equal weighting given to risk factors. We present a refinement of our original model that allows for the differential weighting of risk factors.

Methods: 682 transplants performed over a three year period, from all 8 adult transplant units in the UK, were studied. Fourteen potential risk factors for 30-day survival were identified from the literature and a Cox proportional hazards model was used to assess the combined influence of each. A risk score was calculated from the model. On the basis of this score grafts were classified as low, moderate, high or very high risk. The model was further assessed by testing it on twelve random half samples taken from the full dataset.

Results: 6 factors were found to be associated with 30 day survival in a multivariate model: recipient age (regression weight 0.06 per yr>50 yrs), ventilatory support (1.23), elevated PVR>2.5 Wood units (0.56), re-transplantation (2.62), female donor (0.45), total ischaemia time (0.22 per hr>1 hr). Circulatory support, >1 previous open heart operation, donor-recipient size mis-match, donor inotropes, age, drug abuse and diabetic donor were considered but were not significant. Transplant risk scores ranged from 0-3.84 and were grouped low risk (score 0-0.5), moderate risk (0.51-1.0), high risk (1.1-1.5) and very high risk (1.6+). 30 day survival estimates were: low risk 95% (95% CI 92-99); moderate risk 89% (85-93); high risk 86% (81-91); very high risk 79% (69-89). In 10/12 random samples the relative risk (RR) of failure increased with increasing risk group (RR for the moderate risk transplants ranged from 1.31-3.28, for high risk from 1.59-5.67 and for very high risk from 2.80-7.35 across the 12 samples).

Conclusion: This preliminary model represents a simple way of classifying heart transplants according to risk and could have several potential applications. As further data become available the model will be further refined and validated on an independent data set.

PROTECTION FROM CARDIAC TRANSPLANT VASCULOPATHY; TGF BETA POLYMORPHISM

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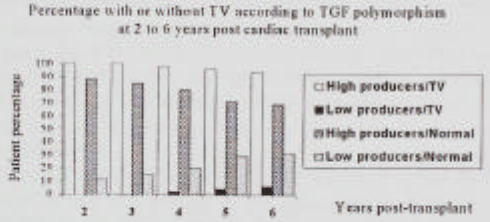
The development of cardiac transplant vasculopathy (TV), characterised by myointimal proliferation, is the most important determinant of long term survival following heart transplantation. Transforming growth factor-β1 (TGF) influences vascular remodelling by regulating smooth muscle cell growth, matrix production and growth factors. Polymorphism of the TGF gene affects TGF production, individuals being high or low producers. We assessed this allelic variation on the development of TV.

Methods. TV was diagnosed by coronary angiography. Following DNA extraction sequence specific primers amplified the polymorphic TGF gene region by PCR. Electrophoresis allowed genotype identification. Fishers test was used for comparison of patients.

Results: 137 patients and 242 angiograms were studied. 89.5% were high producers of TGF-β1. High producers were more likely to have TV (p=0.023, 0.022, and 0.036 at 4, 5, and 6 years). Low producers were protected from TV, representing an increased proportion of normal patients with time (p=0.013). Very few low producers developed TV. The results were independent of pretransplant diagnosis and acute rejection



episodes.

Conclusions: TGF polymorphism may be important in the aetiology of TV as high producers are more likely to get earlier disease. This discovery offers the prospect that TV might be controlled by the pharmacological inhibition of TGF.

CLINICAL RELEVANCE OF ANTIMYOSIN ANTIBODIES IN PATIENTS TRANSPLANTED FOR IDIOPATHIC DILATED CARDIOMYOPATHY.

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Cardiac rejection remains an important clinical problem after heart transplantation. Factors determining rejection remain uncertain. Myosin heavy chain (mhc) is an important antigen in murine models of myocarditis and dilated cardiomyopathy (DCM). Results from a previous study demonstrated autoantibodies against mhc with proinflammatory characteristics in a select group of patients with DCM. Our objective was to determine whether these preformed antibodies (Abs) influenced cardiac rejection and the clinical course after transplantation. Frequency and reactivity of Abs (IgM, IgG and IgG subclass) was evaluated by ELISA prior to transplantation in 64 patients with DCM and 53 with IHD

RESULTS: Anti α- and β-mhc antibody (IgG and IgM) levels were similar in patients with DCM and IHD but statistically higher than in controls. Prevalence of raised antibodies and reactivity to β-mhc in patients with and without rejection differed in the DCM group. In 50 DCM patients with rejection (ISHLT grade 1 and above), frequency and reactivity of IgM antibodies was 26% (13/50), 0.618± 0.07 and 7% (1/14), 0.393± 0.05, in the remaining 14 patients with no rejection $p < 0.05$. Antibody positive patients had a significantly greater frequency and severity of rejection episodes and required more immunosuppression. Anti-mhc antibody positive patients also had an earlier incidence of rejection than antibody negative patients, $p<0.009$. Levels of preformed antimyosin Abs in patients with IHD did not affect the rejection status after transplantation. The distribution of IgG subclass levels in the two diseases differed. Patients with DCM had statistically higher IgG3 reactivity. 70% of IgG3 antibodies were restricted to patients with moderate rejection. IgG3 antibody positive patients had a greater incidence of grade 3 as the initial rejection.

CONCLUSION: Preoperative antimyosin antibody status correlated with rejection in patients with DCM. Antibodies at class and subclass level were differentially associated to post transplant clinical course in these patients. Levels of preformed antimyosin antibodies in patients with IHD did not affect the rejection status after transplantation

ZONAL ALLOCATION FOR THORACIC ORGANS IN THE UK; HAS IT BEEN SUCCESSFUL IN A SINGLE CENTRE VIEW ?

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Background Zonal allocation for thoracic organs has been introduced in the UK since 1993. The primary aim of this scheme was to avoid coupled retrieval to allocation and to minimise much unnecessary movement of retrieval teams.

Objectives The purpose of this study was to study the impact of the zonal allocation in the activity of our transplantation practice and operative outcome

Methods We analysed the results of 451 consecutive heart, lung, and heart lung transplantation performed at our institution between April 1987, and December 1998. The transplants were divided into three groups: local donors retrieved by our team (DL group; = 171 hearts, 61 lungs, and 19 heart lungs); distant donors retrieved by out team (DD group; = 58 hearts, 35 lungs, and 14 heart lungs); and distant donors retrieved by other teams (DX group; = 51 hearts, 41 lungs and, 1 heart lung).

Results

Heart transplants;

No significant differences were noted between the three groups in terms of operative events or requirement for early mechanical or ventilatory support. Mean Cardiac index at 24 hours post-operatively was (DL group, 2.6± 0.4 L/M^2; DD group, 2.7±0.6 L/M^2; DX group, 2.5±0.7 L/M^2). The duration of post operative ITU and hospital stay were similar in all groups. The 30 days mortality was 7.1%, 8.6 %, and 7.8% for DL, DD, and DX groups respectively. The actuarial 1- year survival (DL group 89%, DD group 88% and DX group 88%).

Lung transplants;

No significance difference was noted between the three groups in operative events. Alveolar-arterial oxygen gradient was similar between different groups both immediately (DL group, 358 ± 19 mmHg,; DD ggroup, 345± 17 mmHg; DX group,329 ±21 mmHg) and up to 3 post-operative days. The post-operative ITU and hospital stays were similar in all groups. The 30 days mortality was 11%, 10.5%, and 11.9% for DL, DD, and DX groups respectively. The actuarial 1- year survival (DL group, 79%; DD group, 78%; DX group, 78%).

Conclusion The application of zonal organ allocation for thoracic organs in the UK has been safe and successfully applied to our transplantation programme. We have used donor organs retrieved by other teams and have achieved equivalently satisfactory outcomes for both heart and lung transplantation.

ASSESSMENT OF HEALTH RELATED QUALITY OF LIFE IN PULMONARY TRANSPLANTATION: APPLICATION OF A SIMPLE GENERIC TOOL

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Purpose: Health-related quality of life (HRQoL) is a complex outcome, the measurement of which traditionally requires use of detailed and complex tools. The EuroQol EQ5D is a generic questionnaire, requiring only 1 minute to complete, developed to provide a simple method for assigning utility values to health. EQ5D asks 5 questions from which an aggregate utility score for HRQoL is derived (on a scale where 0 represents death and 1 represents the best possible health state). We have used the EQ5D to measure HRQoL before and after lung transplantation, comparing HRQoL in recipients of single (SLT), bilateral/double (BSLT) and heart-lung (HLT) grafts.

Methods: Cross-sectional study of 86 patients awaiting lung transplantation and 255 post-transplant patients attending follow-up clinics in four transplant units.

Results: The mean (SD) utility value of patients on the waiting list was 0.31 (0.31). Utility values after transplant are shown in the table. Health utility values correlated well with patients' own assessment of their health with a 0-100 visual analogue scale (VAS). In the waiting list group, 61% reported extreme problems in at least one of the five EQ5D domains; in comparison, at 3 or more years after transplant only 20% SLT, 4% BLST and 2% HLT reported extreme problems. After 6 months the health utility scores and health-profile of the bilateral (BSLT) and heart-lung (HLT) were consistently superior to that of single lung (SLT) recipients ($P<0.05$) with problems in all 5 domains more frequent in the SLT group.

Mean utility values (VAS scores) after lung transplantation				
months	0-6	6-18	18-36	>36
SLT	0.69 (67)	0.66 (65)	0.65 (65)	0.61 (60)
BSLT	0.75 (79)	0.83 (78)	0.81 (79)	0.82 (77)
HLT	0.67 (76)	0.85 (79)	0.86 (79)	0.87 (79)

Conclusions: 1) The EQ5D is simple method of deriving a single utility value for HRQoL and is responsive to changes after lung transplantation. It is worth considering as a means for monitoring of HRQoL post-transplant and as an index of quality of survival in research studies in solid organ transplantation. 2) These data suggest that HRQoL after transplantation of one lung (SLT) is inferior to that after transplantation of two lungs (HLT,BSLT).

Posters

Tuesday 30th March

PROLONGATION OF CONCORDANT CARDIAC XENOGRAFT SURVIVAL BY NON-CYTOTOXIC ANTI-MHC CLASS II ANTIBODIES: SYNERGISM WITH CYCLOSPORIN A

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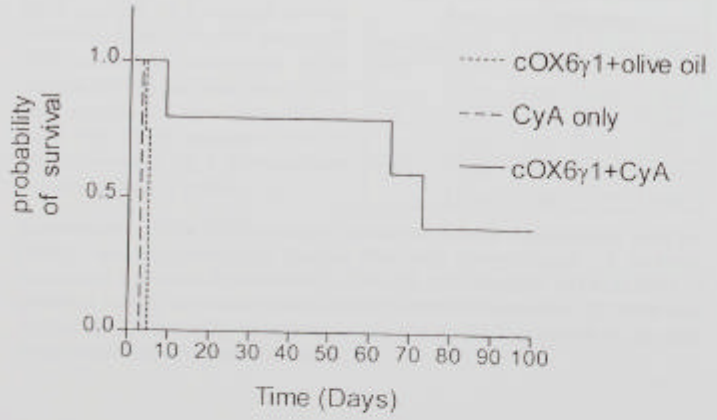
OX6 is a murine IgG1 antibody specific for a non-polymorphic determinant on rat MHC class II. It cross reacts with a polymorphic determinant (Ia17) on murine I-A molecules of H-$2^{k,f,s,u,f,g7}$ haplotype.

We have shown cOX6 to be able to modulate T cell function resulting in prolonged survival of fully allogeneic murine cardiac allografts. A three day course of antibody treatment also: 1) causes transient depletion of splenic and peripheral blood B cells 2) induces changes in morphology of splenic B cell follicles 3) induces changes in B cell surface phenotype 4) transiently inhibits T cell independent anti-TNP antibody production.

This dual action on T and B cells suggested that cOX6 may be effective also in prolonging heterotopic cardiac xenograft survival. This has been investigated in the concordant syrian hamster to rat (DA, $RT1^{av1}$) xenograft model, which has been shown to be predictive of effects seen in primate models.

OX6 was administered at 40mg/kg on days -1, 0 and +1 only, either alone or in combination with cyclosporin A (CsA, 30 mg/kg for days 1-7 then 20 mg/kg every 2 days). CsA alone had no effect on graft survival. OX6 alone prolonged graft survival from 3 days in untreated animals to 5 days (n=6). When given in combination with CsA (n=6), graft survival could be extended to >100 days. There were no obvious side effects of antibody administration. In untreated animals anti-hamster antibodies were detectable on day 2 post transplantation. OX6 treatment delayed appearance of anti-hamster antibodies to day 5.



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THE ROLE OF COMPLEMENT IN RENAL ISCHAEMIA/REPERFUSION INJURY

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Ischaemia/reperfusion (I/R) injury has a direct impact on graft outcome and allograft rejection. The mechanism of I/R injury is complex. Complement is thought to be one of major factors playing a role in the I/R injury. Understanding of pathogenesis of I/R injury, and its contributory factors might lead to therapeutic interventions.

To investigate the role of complement in renal I/R injury, complement C3, C5 and C6-deficient and wild type mice were studied. (C3: n=16; C5: n=4; C6: n=16 in each group). Renal arteries and veins were bilaterally occluded for 40 min (C5 mice) and 58 min (C3, C6 mice). Following reperfusion, the mice were tail bled at different time-points and sacrificed at the end time-point. Renal function was assessed by meseauring serum urea and creatinine. Morphological changes were examined by PAS and H+E staining. Infiltration of neutrophils was examined by immunochemical staining.

In this study, we showed that complement-deficient mice were protected from renal I/R injury. Compared with complement-sufficient controls, complement-def mice had reduced serum urea nitrogen levels, after 24 h reperfusion (C3: 35.94%; C5: 31.6%; C6: 22.0%), 48 h reperfusion (C3: 55.22%; C5: 30.26%; C6 44.0%) and 72h reperfusion (C3:69.49%; C6: 44.78%). Serum creatinine correlated with serum urea and was reduced in complement–def mice after 72 h reperfusion (C3: 69.49%; C6: 47.32%). Histological and immunochemical analysis demonstrated that infarction and cell necrosis were minimised in complement-def mice, and that the number of neutrophils were decreased in complement-def mice.

These data suggest that complement plays a role in renal I/R injury. Participation of complement in pathogenesis of renal I/R injury not only involves the early (C3) and intermediate (C5) components, but also the late components (C6). This provides evidence of a role for the terminal attack complex of complement, in addition to a neutrophil mediated mechanism.

MODIFIED REPERFUSION AMELIORATES ISCHAEMIA REPERFUSION INDUCED ENDOTHELIAL DAMAGE ASSOCIATED WITH COLD ISCHAEMIC INJURY

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Background Primary graft failure after lung transplantation has a high mortality and is related to ischaemia-reperfusion injury. Although reperfusion injury is multifactorial in aetiology recent work [1] has shown that controlling the pressure of the initial reperfusate may ameliorate it. So far investigations into ischaemia reperfusion injury in the lung have used functional markers as end points. Recent work has shown that endothelial markers may well better correlate with the experience of clinical lung transplantation. The capillary filtration coefficient is one such marker of endothelial function in that it correlates with fluid permeability. Thus the aim of this work was to assess the effect of modified reperfusion on the capillary filtration coefficient of rodent lungs undergoing a cold ischaemic insult followed by reperfusion.

Method Groups of five rat lungs were flushed with University of Wisconsin solution (UW) then stored at 4°C for 4 hours. After this period they underwent reperfusion for a half hour period using a parabiotic animal [2] during which time functional measures (gas exchange, pulmonary artery pressure and airway pressure) of lung performance were made. After reperfusion for 30 minutes the lungs underwent estimation of their capillary filtration coefficient (units g/ cm water/min/g wet lung tissue) using a gravimetric technique and lung samples were taken for estimation of wet/dry ratio. Three groups were studied; Group I underwent no reperfusion, Group II underwent 30 minutes of reperfusion at 25 cm H_2O pressure and Group III underwent 15 minutes reperfusion at 12 cm H_2O pressure and then 15 minutes at 25 cm H_2O pressure.

Results There was no difference in change in airway pressure, pulmonary artery pressure or gas exchange between Group II and Group III . Reperfusion injury was demonstrated between Group I and Group II animals by an increase in capillary filtration coefficient ((mean±SD) 1.05±0.77 to 3.07±1.23 ($p<0.01$)). There was no increase in wet-dry ratio between group I and II. Diminution in reperfusion injury was demonstrated by a decrease in filtration coefficient (3.07±1.23 to 1.32±0.51 ($p<0.01$)) between Group III and II. This was also associated with a decrease in the wet – dry ratio (5.65±0.33 to 4.31±0.82 ($p<0.05$))

Conclusion Changes in endothelial properties are not associated with changes in functional measures of pulmonary performance. Modifying the pressure of the initial reperfusate is associated with an amelioration of reperfusion injury as evidenced by a change in fluid permeability but no change in gas exchange or haemodynamics.

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A NOVEL APPROACH TO BINDING SOLUBLE MOLECULES TO CELL SURFACE MEMBRANES: APPLICATION TO TRANSPLANTATION

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We have applied a novel method for cell targeting of soluble pharmaceutical proteins to the problem of complement-dependent injury associated with ischaemia and reperfusion during transplantation. Our approach utilises a linear combinatorial array of discrete membrane-interactive units which we term Sequential Membrane Addresses [SMAs]. These are designed to bind to cells and display a functional molecule of choice. The first of these units interacts with the hydrophobic interior of a membrane bilayer and the second with the headgroups of the component phospholipids. A third address is designed to act as a ligand to bind marker proteins relatively specific for particular cell types, such as those of the vascular endothelium. As a functional molecule, derivatives of the first three short consensus repeats of human complement receptor type 1 (a naturally occurring complement inhibitor) were expressed in E.coli, refolded and linked chemically to synthetic membrane binding peptides containing either 2 or 3 SMAs.

By this means we have prepared a set of fully water-soluble complement regulatory molecules which can bind with high affinity to outer cell membranes *in vitro*. Assay of these constructs as inhibitors of antibody-mediated haemolysis showed significant increases in potency associated with combinations of two or more SMA tags, compared to constructs displaying no or only one SMA. In pharmacokinetic analyses in rats, using an ELISA developed to quantitate these complement inhibitors in rat plasma, systemic administration of a construct with 2 SMAs exhibited prolonged pharmacokinetic clearance compared with that of a construct with no SMA which was rapidly excreted into urine, suggesting retention in the body. We are assessing the use of these molecules in models of transplantation. Rat kidneys prior to transplantation were perfused with a bolus of one construct (APT070) in a bid to load the endothelium of such organs with a cell-bound complement regulator. By immunohistochemistry we stained transplanted organs pretreated in this way and could localise APT070 to glomerular and peritubular capillaries during the post-transplant period. The effectiveness of these constructs to modulate complement-mediated damage and reperfusion injury is under current study.

THE TRANSFUSION EFFECT: TGF-β1 AND CLASS II MHC EXPRESSION IN A CLASS II AND CLASS I-LIKE DISPARATE RAT MODEL OF CHRONIC HEART REJECTION.

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Chronic rejection is the main cause of late graft failure. It is characterised histologically by concentric myointimal thickening with mononuclear cellular infiltration into the media and intima of arteries. This disease accounts for 4-6% of annual heart graft loss in clinical situations.

The PVG-R23 to PVG-RT1[u] rat model of chronic rejection share the same Class I MHC but differ at the MHC Class II and Class I-like region. Previous studies using this animal model have shown that these heart allografts show the histological changes associated with chronic rejection, from as early as 30 days and at 60 days the arteries show severe signs of chronic rejection, especially luminal obstruction of the arteries by smooth muscle cells.

The administration of donor specific transfusion (DST) 14 days prior to grafting abolishes these histological changes. This effect is thought to be cell mediated and not antibody mediated as both DST and non- DST rats produce alloantibody, although a slight reduction in alloantibody production by the DST group has been shown.

The aim of this experiment was to investigate the expression of TGF- β1 and Class II MHC in the heart grafts at various time points corresponding to acute and chronic rejection episodes in transfused and non-transfused rats.

We found no significant difference in TGF- β1 expression in the matrix of the grafts, however, in the arteries of the DST group there was a significant reduction in the TGF-β1 expression compared to the non- transfused group. Furthermore, there was no significant difference in Class II MHC expression and regulation in both animal groups.

These results suggest that in a cell mediated model of chronic rejection reduced myointimal thickening is accompanied by lack of TGF-β1 expression and that TGF-β1 plays a role in the intimal thickening.

PROLONGATION OF PORCINE ISLET XENOGRAFT SURVIVAL BY NON-CYTOTOXIC ANTI-MHC CLASS II ANTIBODIES

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M. Koulmanda and T.E. Mandel, Walter and Eliza Hall Institute, Melbourne, Australia
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Transplantation of pancreatic endocrine tissue offers the prospect of a cure for diabetes. However both allogeneic whole pancreas and purified pancreatic islet transplantation are severely restricted by organ availability. Xenotransplantation is one possible solution to this problem.

Transplantation of solid organ xenografts (eg kidney or whole pancreas) remains problematic with the hurdle of hyperacute xenograft rejection still incompletely resolved. In contrast islet grafts derive their vasculature from the recipient and are thus not a target for antibody mediated hyperacute rejection. In keeping with this conventional T cell targeted immunosuppressive therapies are effective in preventing islet xenograft rejection in murine and primate models.

We have studied the efficacy of a non-cytotoxic anti-MHC class II antibody (OX6) in delaying porcine islet xenograft rejection in NOD mice. In this model the grafted tissue is devoid of donor vascular endothelium and antigen presenting cells and rejection is therefore dependent on indirect T cell mediated mechanisms of graft rejection. Furthermore, the donor MHC molecules are not recognised by the antibody used and indirect recognition of graft antigens alone is therefore targeted by the immunotherapeutic regimen.

Cultured fetal porcine grafts were placed under the kidney capsule of NOD mice. One mg of OX6 was given ip the day before, day of and day after grafting. Grafts were scored according to an accepted scheme at days 7, 14 and 21 after grafting. Data is representative of two similar experiments.

	Number of grafts surviving		
Treatment	Day 7	Day 14	Day 21
OX6	5/5	6/8	1/7
PBS	3/5	0/8	1/6

At day seven control grafts showed an eosinophil infiltrate with graft necrosis. OX6 treated grafts showed eosinophilic infiltrates but no necrosis. At day 14 both control and OX6 treated grafts showed heavy infiltrates but only OX6 treated grafts showed intact endocrine tissue. By day 21 OX6 treated and control grafts showed heavy infiltrates with loss of endocrine tissue.

This work demonstrates the unique potential for the use immunomodulatory agents targeting indirect antigen recognition for prevention of rejection of xenogeneic islets.

PRESERVATION WITH PHOSPHATE BUFFERED SUCROSE: COMPARISON WITH UW IN AN ISOLATED PERFUSED LIVER MODEL

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Phosphate buffered sucrose (PBS140) is a clinically proven and inexpensive kidney preservation solution but it has not been fully evaluated in liver preservation. We report successful 24 hour liver preservation with PBS140 in a model of isolated perfused rat liver (IPRL) with results comparable to University of Wisconsin (UW) Solution.

The experiment was carried out under a Home Office license and according to standard guidelines for animal care. Male Wistar rats (200-250g) were anaesthetised with pentobarbitone (50 mg kg^{-1}). After midline laparotomy, the liver was flushed with 20 ml of ringer lactate (control), PBS140 or UW through the aorta and 5ml of the same solution via the portal vein. The liver was removed and stored in the flush solution at 4°C. After 24 hours of cold storage, the liver was set up on a recirculating circuit system at 37 °C for two hours with physiological saline containing 35% washed bovine red blood cells and 2% bovine albumin maintained at pH 7.4. Taurocholic acid (4.84%) was added to the inflow system to support bile production. Bile was collected during the reperfusion period. Liver enzymes (ALT and LDH) were determined after preservation and reperfusion.

The results are summaraised below as mean ± standard error. ALT and LDH results are for the first flush* after 24 hour preservation and the last flush# after reperfusion for 2 hours.

Test solution	n	Bile flow (µl/2h/g)	Bile acid extraction (%)	Bilirubin in bile (mg/dl)	ALT (U/L)	LDH (U/L)
Control	6	45±7	8.0±2.7	1.55±0.36	171±29* 118±19#	1640±132* 1107±182#
PBS140	8	147±16	51.0±4.5	6.65±0.74	33±3* 44±3#	161±15* 181±16#
UW	6	145±7	55.0±3.4	4.41±0.49	17±0* 24±1#	180±20* 84±8#

Both PBS140 and UW achieved significantly improved results for all parameters when compared to the control group ($p<0.05$ by t-test and ANOVA), and the results for PBS140 and UW were similar. These results suggest that PBS140, which was developed for kidney preservation, is worthy of further investigation for preservation of organs such a liver.

IMPROVED LIVER PRESERVATION WITH PHOSPHATE BUFFERED SUCROSE

SB Song, MS Attia, DJ Potts, JPA Lodge

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Phosphate buffered sucrose (PBS140) is a clinically proven and inexpensive kidney preservation solution but it has not been fully evaluated in liver preservation. In this experiment we used a model of isolated perfused rat liver (IPRL) to compare the relative effects of PBS140 and University of Wisconsin (UW) Solution in cold and warm ischaemia in order to mimic clinical liver transplantation.

The experiment was carried out under a Home Office license and according to standard guidelines for animal care. Male Wistar rats (200-250g) were anaesthetised with Nembutal (60mg/kg). After midline laparotomy, the liver was exposed and catheters were introduced into the abdominal aorta and portal vein. The liver was flushed with ice-cold PBS140 or UW and stored at 4°C for 24 hours. After 24 hours of cold storage, the liver was set up on a recirculating circuit for perfusion for two hours at 37 °C with physiological saline containing 35% washed bovine red blood cells and 2% bovine albumin maintained at pH 7.4. Taurocholic acid (4.84%) was added to the inflow system to support bile production.

Two experimental groups (n=6) were compared. In Group 1, the livers were flushed and stored immediately but were subjected to 30 minutes warm ischaemia after storage and before reperfusion. In Group 2 (Control), there was no warm ischaemia. During the reperfusion period, measurements were made of liver enzymes, O_2 consumption and bile flow with the following results (mean ± standard error, *$p<0.05$ PBS140 vs UW, t-test).

Group	Bile flow (µl/2h/g)	ALT (U/L)	AST (U/L)	LDH (U/L)	O2 consumption ((µmol/min/g)
1-PBS140	55.7±16.0	95±6*	101±9.5*	448±65	0.334±0.025*
1-UW	43.8±6.0	179±11*	241±38*	583±58	0.237±0.010*
2-PBS140	64.4±13.0	108±15*	146±15*	428±47	0.352±0.030
2-UW	93.4±14.0	56±8*	62±9*	344±41	0.358±0.020

Although, UW was more effective than PBS140 in the absence of warm ischaemia, with pre-reperfusion warm ischaemia PBS140 was more effective than UW. This latter situation more closely mimics clinical liver transplantation and suggests that a pre-reperfusion warm ischaemic period should be included in experimental protocols for testing organ preservation solutions.

COMPARISON OF THE PROTECTIVE EFFECTS OF PHOSPHATE BUFFERED SUCROSE AND UW IN A NON-HEART BEATING LIVER DONOR EXPERIMENT

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Phosphate buffered sucrose (PBS140) is a clinically proven and inexpensive kidney preservation solution and our laboratory work has demonstrated efficacy in liver preservation. In this experiment we used a model of isolated perfused rat liver (IPRL) to compare the relative effects of PBS140 and University of Wisconsin (UW) Solution in cold and warm ischaemia in order to mimic the non-heart beating donor scenario.

The experiment was carried out under a Home Office license and according to standard guidelines for animal care. Male Wistar rats (200-250g) were anaesthetised with Nembutal (60mg/kg). After midline laparotomy, the liver was exposed and catheters were introduced into the abdominal aorta and portal vein. The liver was flushed with ice-cold PBS140 or UW and stored at 4°C for 24 hours. After 24 hours of cold storage, the liver was set up on a recirculating circuit for perfusion for two hours at 37°C with physiological saline containing 35% washed bovine red blood cells and 2% bovine albumin maintained at pH 7.4. Taurocholic acid (4.84%) was added to the inflow system to support bile production.

Two experimental groups (n=6) were compared. In Group 1, the liver was subjected to 25min warm ischaemia (37°C) prior to being flushed and stored. In Group 2, (Control), there was no warm ischaemia. During the reperfusion period, measurements were made of liver enzymes, O_2 consumption and bile flow with the following results (mean ± standard error, $^*p<0.05$ PBS140 vs UW, t-test).

Group	Bile flow (µl/2h/g)	ALT (U/L)	AST (U/L)	LDH (U/L)	O2 consumption ((µmol/min/g)
1-PBS140	30.7±4.0	283±31	772±108*	566±103	0.234±0.010
1-UW	47.2±12.0	220±22	383±40*	306±55	0.269±0.020
2-PBS140	64.4±13.0	108±15*	146±15*	428±47	0.352±0.030
2-UW	93.4±14.0	56±8*	62±9*	344±41	0.358±0.020

In both experimental groups UW was more effective than PBS140, suggesting that UW should be more effective than UW as a preservation solution if non-heart beating liver donors are to considered in the future.

ANERGIC T CELLS ACT AS SUPPRESSOR CELLS THROUGH ANTIGEN PRESENTING CELLS

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The phenomena of infectious tolerance and linked-suppression are well established, but the mechanisms involved in these forms of immunoregulation remain controversial. We, and others, have shown that anergic T cells can cause immunosuppression using both *in vitro* and *in vivo* systems. In this study we tested the hypothesis that anergic T cells exert regulatory effects by inhibiting antigen-presenting cell (APCs) function.

Allospecific mouse T cell clones were rendered unresponsive *in vitro* using an immobilised anti-CD3 mAb. These anergic T cells inhibited proliferation of non-anergic T cells in response to allogeneic dendritic cells. The inhibition did not appear to be due to IL-4, IL-10 or TGFβ, because secretion of these cytokines by the T cells was reduced upon anergy induction. In addition, neutralising mAbs specific for IL-4, IL-10 or TGFβ failed to reverse the inhibition.

The inhibition imposed by the anergic T cells was not observed if responder T cells were activated by plastic-immobilised anti-CD3 and anti-CD28 mAbs, thereby eliminating APCs. This suggested that the suppressive effects of anergic T cells required APCs. Furthermore, when dendritic cells were cocultured for 48 hours with anergic T cells and subsequently isolated by negative selection using anti-CD4 mAb and magnetic beads, they were unable to activate antigen-specific CD4 or CD8 T cells. Coculture with non-anergic T cells had no inhibiting effect. Transwell experiments indicated that suppression of dendritic cells by anergic T cells can not be accounted for by secretion of soluble factors and required cell-cell contact between APCs and the anergic T cells. In addition the phenotypic analysis of dendritic cells after interaction with anergic T cells revealed that the expression of MHC class II, CD80 and CD86 was downregulated .

Taken together, these experiments suggest that one mechanism by which anergic T cells can function as suppressor cells involves the inhibition of dendritic cells in their antigen presenting capacity.

DIFFERENTIAL INHIBITION OF THE FAS CELL SURFACE RECEPTOR EXPRESSION BY CsA AND FK506: IMPLICATIONS FOR THE INDUCTION OF ALLOSPECIFIC T CELL APOPTOSIS.

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Introduction Cyclosporin A (CsA) and FK506 are powerful immunosuppressive drugs that inhibit *de novo* synthesis of cytokines such as IL-2, thereby preventing the proliferation of antigen-specific T cells. In addition to this mechanism, it has been shown recently that, unlike CsA, FK506 does not inhibit activation-induced apoptosis of T lymphocytes. As this process is triggered by the Fas receptor (CD95), a series of experiments was designed to assess the effects of CsA and FK506 on the expression of Fas during *in vitro* activation of allospecific T cells.

Methods Allospecific T lymphocytes were activated by mixture with an allogeneic, EBV-transformed B cell line. Cells were cultured either in the presence of CsA (100ng/ml), FK506 (1ng/ml) or control medium. The expression of Fas on the surface of T cells was measured daily for 7 days by semi-quantitative flow cytometric analysis.

Results Freshly isolated T lymphocytes expressed low levels of Fas on their cell surface; this was unaffected by treatment with CsA or FK506. Following activation normal T cells quickly upregulated expression of Fas. Maximal levels were observed after culture for 5 days; this period coincided with that for acquisition of sensitivity to Fas-mediated apoptosis. Significantly, cells stimulated in the presence of CsA showed reduced Fas expression compared to cells treated with FK506.

Conclusion It is possible that apoptotic deletion of activated, allospecific T cells can contribute to the development of graft tolerance. In this series of experiments it was shown that CsA and FK506 have differential effects on the expression of Fas by alloantigen-stimulated T lymphocytes. This may have implications for the development of specific graft tolerance by immunosuppressed transplant recipients.

COMPARISON OF FOUR HEART PRESERVATION SOLUTIONS USING AN EXPERIMENTAL MODEL OF TWO HOURS ISCHAEMIA AT ROOM TEMPERATURE

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The objective of this study was to assess the efficacy of cardiac preservation under moderately hypothermic conditions similar to those present during intraoperative myocardial surgery. Many different cardioplegia solutions are used in routine cardiac surgery but St.Thomas's Hospital solution No 2 (STH2) is currently favoured for heart preservation for transplantation. We have investigated the efficacy of STH2 in cardiac preservation for 2 hours at room temperature (22°C) and compared it with three other solutions with demonstrated efficacy in kidney and liver preservation: University of Wisconsin solution (UW), Celsior Solution (CS) and Phosphate Buffered Sucrose (PBS140).

The experiment was carried out under a Home Office license and according to standard guidelines for animal care. It involved using an isolated rat heart model (male Wistar rats, 200-250g). We carried out initial control studies on each heart by assessing five haemodynamic parameters: heart rate (HR), systolic pressure (SP), aortic flow (AF), coronary flow (CF) and cardiac output (CO). The hearts were rapidly excised and perfused with oxygenated Krebs-Henseleit buffer (KHB) in a Langendorff whole heart model for approximately 5 minutes, and then switched to working mode for 20 minutes after cannulation of the left atrium. This was followed by cardiac preservation by infusion of 10 ml of the preservation solution followed by storage in the same solution for 2 hours at controlled room temperature (21-22 °C). The same parameters were measured after reperfusion.

Results (mean ± standard error) for the four experimental groups are shown in the table, expressed as the % recovery of pre-ischaemic function for each of the measured haemodynamic parameters.

Group	n	HR	SP	AF	CF	CO
STH2	8	97.2±1.4	89.5±1.4	70.1±6.7	88.0±3.4	76.0±4.6
UW	9	93.6±2.3	77.3±4.0	39.3±3.9	68.1±2.8	47.7±2.2
CS	7	95.2±1.4	89.7±2.1	57.6±4.5	89.9±3.7	67.1±4.3
PBS140	9	90.5±2.8	82.8±1.5	53.0±4.8	68.1±7.7	58.3±5.8

Although STH2 achieved the best results in this study, the other solutions were also effective. Manipulation of these solutions may increase the efficacy of heart preservation and allow the development of a single multi-organ flush solution for transplantation.

THE PATHWAY FOR FRUCTOLYSIS DURING ATP REGENERATION IN THE COLD HYPOXIC PIG LIVER.

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The aim of the current study was to promote glycolysis during periods of cold ischaemia in order to protect metabolic pathways important to cell survival. Pig livers (n=5 / group) were retrieved, stored on ice for 3h and hypothermically reperfused (HtR) with no substrate and then with either 10mM glucose (group G) or fructose (group F). Changes in hepatic ATP and phosphomonester (PME) levels were monitored every 2min using ^{31}P NMR. HtR with glucose/fructose produced similar quantities of ATP but rates of regeneration were dissimilar. Group G had an increased rate of 36% but group F had a 60% ($p<0.001$) reduction from 'no substrate' HtR values. Following cessation of HtR, ATP hydrolysis was 85% ($p<0.001$) slower with fructose. From the PME differences there was a clear alteration in the metabolic response of glycolysis to fructose as the substrate. During the first half of the HtR period a significant decrease was observed, but thereafter the PME increased to 37% higher than Group G ($p<0.01$). Following cessation of HtR with glucose, the PME level equilibriated back to its cold storage value within 30min. However, in Group F the PME increased steadily during ischaemia to 19% higher than its cold storage levels ($p<0.02$), thereafter it returned to pre-HtR levels. Analysis of *in vitro* extracts of these livers indicated that in group G, there were fewer components within the PME region ie. phosphoethanolamine & phosphocholine, glucose 6-phosphate (G6P) & 3-phosphoglycerate, the latter two resonances indicating glucose flux. However in group F, G6P was absent and in its place was fructose 1-phosphate (F1P) and other glycolytic intermediates ie. dihydroxyacetone phosphate, glyceraldehyde 3-phosphate (G3P) & α-glycerophosphate. These findings supported the changes observed in the *ex vivo* liver experiments where PME levels remained elevated over a longer period of time during ischaemia. Glucose is first phosphorylated by hexokinase followed by phosphofructokinase (PFK), a key regulatory enzyme for glycolysis. Fructose can either be phosphorylated by hexokinase (non-specific) or by the very specific ketohexokinase – fructokinase. Fructokinase has a very low K_m for fructose and hence production of F1P occurs rapidly, after which aldolase B splits the six carbon ring into dihydroxacetone phosphate and free glyceraldehyde (which is subsequently phosphorylated to G3P). By producing the triose phosphates in this manner, regulation of glycolysis due to the allosteric inhibition of PFK can be eliminated. During subsequent ischaemia, Group F contained greater quantities of substrates such as F1P, hydroxyacetone phosphate and G3P which maintained ATP levels through anaerobic glycolysis, therefore buffering its hydrolysis. Thus, in cold preserved livers, energy supply *via* glycolysis is not inherently blocked; if substrate can be supplied by the correct route, significant ATP generation during hypoxia can be promoted.

EXPRESSION AND CELLULAR COLOCALISATION OF FRACTALKINE IN ACUTE RENAL ALLOGRAFT REJECTION

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The CX_3C chemokine Fractalkine is a unique transmembrane molecule for which initial studies have indicated endothelial cell restricted expression and cytokine-inducibility. Fractalkine mediates the capture, integrin-independent adhesion and activation of NK cells, CD8+ T-cells and monocytes. Trafficking of these cells to interstitial sites is central to cellular immune processes in acute renal allograft rejection (AAR). The aim of this study was to identify the expression and cellular colocalisation of Fractalkine in AAR.

Using *in situ* hybridisation with a radiolabelled Fractalkine riboprobe (generated from a RT-PCR synthesised cDNA product ligated into a pGEM-7Zf(+) vector between SP6 and T7 promoter regions) and indirect immunohistochemistry with a polyclonal goat anti-human Fractalkine antibody (R & D systems) we studied snap frozen, cryostat sectioned renal biopsy material from 15 patients with AAR. Fractalkine was expressed at mRNA and protein level by endothelium at interstitial sites local to inflammatory cell infiltrates. Serial section immunohistochemistry identified presence of NK cells (CD56 +ve), monocytes/macrophages (CD68 +ve) and CD8+ T-cells within these infiltrates. In moderate and severe AAR there was heavy expression of Fractalkine mRNA by tubular epithelial cells, however there was low level protein expression at these sites. There was no expression of Fractalkine mRNA or protein by infiltrating leukocytes. There was sparse and occasional endothelial cell expression in normal kidney. Semi-quantitative RT-PCR showed IL-1β and TNF-α inducible upregulation of Fractalkine mRNA expression (maximal at 6 hours) by cultured human vein endothelial cells and proximal tubular epithelial cells (PTEC). Expression was heaviest by endothelial cells. Immunohistochemistry on cultured PTEC confirmed cytokine-inducible expression of Fractalkine by this cell type.

In conclusion: (i) Fractalkine is expressed by interstitial endothelium and PTEC in AAR, (ii) colocalisation to infiltrating leukocyte subsets indicates a central role for this chemokine in directing their recruitment in AAR and (iii) cytokine-inducible patterns of expression *in vitro* are consistent with *in vivo* expression. Fractalkine may represent a novel therapeutic target in AAR.

INVESTIGATION OF FACTORS IMPORTANT FOR THE *IN VIVO* DELIVERY OF AN INTEGRIN-TARGETED NON-VIRAL DNA VECTOR

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Polylysine-molossin is a 31 amino acid synthetic peptide that has previously been demonstrated to function as a DNA vector *in vitro*. It incorporates the 15 amino acid integrin-binding domain of the venom of the American Pit Viper, *Crotalus molossus molossus* as the targeting moiety and a chain of 16 lysines as the DNA-binding moiety. Several parameters of importance for *in vivo* applications were evaluated.

- *Optimal DNA/vector concentration*. Saturation of vector/DNA binding sites on the ECV304 cell line occurred at 6 μg/ml of DNA, using vector/DNA complexes at the optimal transfection ratio of 3:1 weight to weight. The concentration of vector/DNA complexes required for optimal gene transfection was found to be 2-8 μg/ml of DNA, corresponding to the concentration needed for saturation binding.
- *Optimal target cell exposure times*. Vector/DNA complexes saturated target cell binding sites within 5 minutes of incubation. However, lengthy exposure times (>5 hours) to the transfection medium were essential for substantial gene transfer. This was a consequence of two complementary factors. Firstly, it was essential that target cells be exposed to vector/DNA complexes for 2-3 hours at 37 °C. Saturation of target sites and then removal of the transfection medium was not effective. Secondly, exposure to chloroquine for 8-10 hours *following* uptake of vector/DNA complexes was essential for optimal gene transfer.
- *Inhibitory effects of serum*. Target cells previously saturated with vector/DNA complexes and then exposed to 10 % serum showed excellent gene transfer. However, exposure of complexes to even 1 % serum *prior* to transfection, markedly inhibited gene transfer.
- *Extravasation and binding stability in vivo*. Cold *ex vivo* perfusion of rat hearts with vector/DNA complexes demonstrated that little, if any, complex moved out of the vascular system. Following transplantation of the heart, most of the complex bound to the vasculature was lost within 30 minutes of re-establishing the blood circulation.

HLA-G INHIBITS THE TRANSENDOTHELIAL CELL MIGRATION OF HUMAN NK CELLS; A STRATEGY FOR INHIBITING XENOGRAFT REJECTION

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HLA-G is a non-classical MHC class I molecule expressed mainly within the placenta during pregnancy. Recently it has been shown to interact with multiple NK cell receptors including p58 killer inhibitory receptors (KIR), p70 KIR and CD94. In vivo, it may function predominantly to protect foetal cells against lysis by maternal uterine NK cells, a hypothesis in accordance with several in vitro studies which have demonstrated that HLA-G-transfected cells are protected from lysis by NK cell clones. However, the expression of HLA-G is restricted to several specific areas within the placenta; it is found on foetal endothelial cells within placental vessels and also on invading extravillous cytotrophoblast cells, which form the interface between maternal and foetal tissue. During placentation, some of these cells develop an endothelial cell-like phenotype and invade uterine arteries, displacing and replacing maternal endothelial cells. HLA-G therefore comes to be expressed on the vascular endothelial cells of vessels on both sides of the maternal / foetal interface, in effect marking the boundaries between placenta and foetus and between placenta and mother.

We have examined the hypothesis that HLA-G may modify the transmigration of NK cells across the endothelium. Immortalised porcine endothelial cells were transfected with cDNA encoding HLA-G or, as a control, HLA-A2. Expression of neither molecule protected the endothelial cells against lysis by human NK cells freshly isolated from peripheral blood. However, in 4 out of 5 transmigration assays across endothelial cell monolayers, HLA-G inhibited the early passage of highly purified NK cells, but not that of a control T cell line, J6. The influence of HLA-G was specifically inhibited by prior incubation of the transfected endothelial cells with a monoclonal antibody W6/32 which binds MHC class I.

Our data support the conclusion that HLA-G specifically inhibits NK cell migration. We postulate that in vivo, HLA-G may inhibit the migration of NK cells into the placenta from both maternal and foetal aspects, with the obvious advantages to both mother and foetus of limiting NK cell contact with each others tissue. The original impetus behind these experiments was to develop strategies for prolonging the survival of transplanted xenografts; NK cells are known to play a role in xenograft rejection, specifically in the process of 'delayed xenograft rejection'. Our results imply that HLA-G expression on the endothelium of transplanted xenografts may have a beneficial effect on graft survival by limiting the intragraft migration of NK cells. However, for complete effectiveness, such a strategy would need to be combined with others designed to prevent NK cell-mediated activation or lysis of xenograft endothelium.

CYTOKINE mRNA EXPRESSION LEVELS FOLLOWING EXPERIMENTAL CORNEAL TRANSPLANTATION IN THE RAT: ANALYSIS USING QUANTITATIVE COMPETITIVE RT-PCR.

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The aim of this work was to compare the cytokine mRNA levels between allogeneic corneal transplants (BN→Lewis rats) and syngeneic controls (Lewis→Lewis).

Corneal transplants were carried out and scored for vascularization, oedema, and clarity as described previously (*Clin Exp Immunol 1997;107:381*). Donor and recipient corneal tissue was excised and stored at -70°C from ungrafted corneas and from grafted corneas at days 3,5,7,9,11 and 13 post transplant. Cornea samples were then homogenized, the RNA isolated using RNAzol B and reverse transcribed into cDNA. Quantitative competitive RT-PCR for HPRT (used as a housekeeping gene), CD3, CD25, IL-1β, IL-2, IL-6, IL-10, IFN-γ and MIP-II was carried out.

All allografts were rejected, observed onset being day 9-10 whereas no syngeneic grafts were rejected. Expression of CD3, CD25, IL-1β, IL-2 and IL-6 remained elevated but low from day 3-13 in syngeneic grafts. Similar low levels were detected in allogeneic grafts from day 3, however, these levels increased significantly upon rejection at day 9. Interferon-γ expression was too low to be detected in syngeneic grafts at all time points examined and was only detected in allogeneic grafts from day 9. Elevated levels of IL-10 expression was seen in both allogeneic and syngeneic grafts from day 3. A peak of MIP-II expression was seen at day 5 in both allogeneic and syngeneic grafts, a second peak of expression was also detected at day 9 in allogeneic grafts.

There is elevated expression of both Th1 and Th2 cytokines in allogenic and syngeneic corneal grafts post transplantation. Increased expression of Th1 cytokines (coincident with expression of the T-cell markers - CD3 and CD25) were detected upon rejection.

A NOVEL ENDOTHELIAL-SPECIFIC FREE RADICAL SCAVENGER FOR ORGAN PRESERVATION

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Organs used for transplantation undergo varying lengths of cold ischaemia and consequently oxygen free radical mediated damage following reperfusion. In clinical renal transplantation, prolonged cold ischaemia times are strongly associated with delayed graft function and poor graft survival. We have performed an in vitro study using a human microvascular endothelial cell line HMEC-1, to investigate the potential benefits of incorporating a novel form of superoxide dismutase conjugated with lecithin (lec-SOD), into organ preservation solutions to enable specific targeting of lec-SOD to the endothelium.

Confluent monolayers of HMEC-1 were incubated with lec-SOD in Marshall's solution for 1, 3, 12 and 24 hours at 4°C. Increased levels of SOD were detectable by FACS analysis when HMEC-1 were incubated with 5-800μg/ml lec-SOD at all time points analysed. In marked contrast, incubation with unmodified SOD under identical conditions showed no increase in the level of SOD. In addition, following cell permeabilisation, lec-SOD was detectable within endothelial cells suggesting that it may be internalised during cold incubation. Furthermore, lec-SOD was found to have a protective role against cold hypoxia/ reoxygenation-induced cell death on HMEC-1.

The results from this study suggest that, unlike previous forms of SOD, lec-SOD may be a more potent inhibitor of free radical mediated damage following organ preservation and reperfusion as a result of its high affinity for endothelium and intracellular uptake.

THE INDUCTION OF MHC CLASS II EXPRESSION IS SUFFICIENT FOR THE DIRECT T CELL ACTIVATION OF HUMAN CD4+ T CELLS BY PORCINE VASCULAR ENDOTHELIAL CELLS

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The role played by donor MHC class II positive vascular endothelial cells (VECs) in allograft and xenograft rejection is not well defined. *In vitro* T cell activation studies always involve interferon gamma treated VECs, as VECs rapidly lose MHC class II expression on culture. Such studies demonstrate potent direct T cell activation of human CD4+ T cells by both allogeneic human VECs and xenogeneic porcine VECs. However, interferon gamma is a pleiotropic cytokine and induces the expression of many immunologically active proteins (e.g. costimulatory ligands) in addition to MHC class II antigens. In these circumstances, the relevance of the *in vitro* data to the role of donor MHC class II positive VECs is *in vivo* uncertain.

For these reasons, we have taken advantage of the recent discovery of an MHC class II transcriptional activator (CIITA), the function of which is purely to induce MHC class II expression. A porcine vascular endothelial cell line (PIEC) was transfected with the full length human CIITA gene under the control of the CMV promoter, and stable transfectants were selected. Normal PIECs do not express MHC class II antigens, but the CIITA cell lines express large amounts of SLA-DR and SLA-DQ The amounts expressed are very similar to those seen in interferon gamma treated PIEC.

Highly purified human CD4+ T cells were co-cultured with normal PIEC, interferon gamma treated PIEC, and CIITA transfected PIEC lines. Whereas the normal PIEC failed to stimulate the human T cells, both interferon gamma treated and CIITA transfected PIEC gave potent stimulation. The magnitude of the human CD4+ T cell proliferation was the same for both interferon gamma treated and CIITA transfected PIEC, but it was interesting that the peak responses occurred at different times, at day 5 and day 3 respectively. The human CD4+ T cells response to the CIITA transfected PIEC could be inhibited by >50% with antibodies to SLD-DR, but could not be inhibited by CTLA4Ig. These data demonstrated that the PIEC line normally expresses potent costimulatory ligands, and that the induction solely of MHC class II antigens results in strong T cell activation. This supports the hypothesis that VECs are potent APCs *in vivo*.

BIOLOGICAL PROPERTIES OF SYNTHETIC ANTISENSE OLIGODEOXYNUCLEOTIDES WHICH SUPPRESS IL-2 BIOSYNTHESIS IN THE RAT

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Because of the central role played by interleukin-2 (IL-2) in allograft rejection and tolerance, a series of antisense oligodeoxynucleotides was developed with the objective of inhibiting IL-2 expression in rat lymphocytes. The expression level was measured in CON A stimulated lymph node lymphocytes using both genetic (RT-PCR) and protein (CTLL-2 cell line) indicator methods *in vitro*. Antisense oligo 1 (AS1) covered the rat IL-2 initiation codon (ATG) and its upstream (5') sequence; antisense oligo 2 (AS2) covered the IL-2 initiation codon and its downstream (3') sequence; antisense oligo 3 (AS3) covered a region immediately downstream of the initiation codon; the nonsense oligo (control oligo) was a scrambled version of the AS1 sequence. When administered without any delivery vehicle *in vitro*, all antisense oligos (up to 10μM) had no effect on inhibition of IL-2 expression. However, when administered with 5-10μg/ml of Lipofectamine, 1μM AS1 strongly inhibited IL-2 mRNA expression and AS2 had weak inhibition. AS3 and the control oligo gave no suppression. Time course experiments indicated that, after a 4-hour treatment, IL-2 mRNA inhibition appeared immediately and persisted more than 3 days. At a protein level, IL-2 activity was below 5U/ml in all CON A supernatants from day 1 to day 4 after treatment with Lipofectamine plus AS1. The activity of IL-2 in the supernatants of cultures treated with Lipofectamine plus control the oligo was more than 15U/ml. 10U/ml of exogenous IL-2 reversed the proliferation of inhibition induced by AS1. These data indicate that AS1 suppression of cell proliferation was due to IL-2 specific inhibition. *In vivo* studies showed that high doses of AS1 oligo in the absence of Lipofectamine could reduce IL-2 expression level in some transplanted rat kidney allografts, but could not prolong allograft survival.

GENE DELIVERY TO THE LIVER USING A NON-VIRAL DNA VECTOR

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Genetic modification of the liver is an attractive option for controlling rejection of liver allografts (by genetic modification of the graft) and for the correction of enzyme defects which require liver transplantation (by genetic modification of the patient's own liver). The development of optimal DNA vectors for these applications is a major area of research. Our group has been concentrating on non-viral DNA vectors because of their many advantages over viral vectors. We have studied a synthetic peptide vector directed at target cell integrins in 3 critical areas for *in vivo* gene delivery.

- Using a novel technique for *in situ* perfusion via the portal vein of isolated lobes of the rat liver, we have examined the localisation of the vector/DNA complexes. We report that the complexes show excellent penetration into the hepatic lobule within 15 minutes of infusion into the isolated lobes, and that the complexes are still detectable 15 minutes after re-establishing the portal and hepatic circulations. We also report good penetration into the hepatic lobule in whole livers perfused at 4 °C *ex vivo*.
- Once the vector/DNA complex is internalised by endocytosis by the target cell, the complexes must be released from the endocytic vessels for translocation to the nucleus of the target cell. We report *in vitro* studies with several agents known to assist endocytic exit. Chloroquine, lipofectamine, and the fusogenic peptide of the haemagglutinin of the influenza virus have been studied, in isolation and in combination, to determine optimal conditions for gene delivery to the HepG2 human hepatoma cell line.
- The optimal combinations are being tested for *in vivo gene* delivery, using *in situ* perfusion of isolated lobes. In an early result, we report 5 %-8 % transduction rate of hepatocytes using a combination of chloroquine and fusogenic peptide.

REGULATED ENDOTHELIAL CELL EXPRESSION OF NOVEL ANTICOAGULANTS: A STRATEGY FOR THE PREVENTION AND THERAPY OF INTRAVASCULAR THROMBOSIS

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Intravascular thrombosis resulting in organ infarction occurs during hyperacute rejection (HAR) of allografts transplanted into sensitised patients and remains a major problem in hyperacute and delayed xenograft rejection.

We have constructed two membrane-tethered anticoagulant fusion proteins based on human tissue factor pathway inhibitor (hTFPI) and the leech anticoagulant hirudin. When expressed constitutively on the surface of porcine endothelial cells (EC), anchored by the transmembrane / cytoplasmic tail of human CD4, hTFPI bound to and inhibited factors Xa, VIIa and porcine tissue factor (pTF) and hirudin retained potent thrombin binding activity. Using a human plasma recalcification clotting assay and interleukin-1 (IL-1) α-activated, immortalised porcine EC, it was possible to distinguish between pTF–dependent (clotting time (CT) ~ 90 sec) and pTF–independent (CT – 140 sec) fibrin generation (compared to CT ~ 350 sec in the absence of any EC). Expression of hTFPI-CD4 on immortalised EC effectively prevented pTF–dependent clotting, whereas hirudin-CD4 inhibited both pTF-dependent and -independent fibrin generation. When both constructs were co-expressed in the same cell, the potent procoagulant properties of in vitro cultured, IL-1α-activated porcine EC were almost completely abolished (CT ~ 330 sec).

Constitutive expression of these fusion proteins on the endothelium of transplanted allo- or xenografts might be associated with significant clinical problems. One obvious risk might be bleeding from the vascular anastomosis at the time of revascularisation. Another might be severe bleeding after transcutaneous biopsy of a transplanted organ such as the kidney. Because of these risks, the constructs have been modified by the addition of a P-selectin sequence to the cytoplasmic tail in order to localise them in Weibel-Palade (WP) bodies. They have been transfected into WP body-positive primary porcine EC isolated from the inferior vena cava of normal pigs. In resting EC, fusion protein expression co-localised with P-selectin and was restricted to WP bodies. These cells had a potent procoagulant phenotype in recalcified human plasma just as control untransfected cells. However, after activation with the phorbol ester PMA, to mimic 'type 1 EC activation', the anticoagulant proteins were rapidly relocated to the cell surface where they specifically inhibited the clotting of human plasma.

These novel anticoagulant molecules may prove useful therapeutic agents for gene therapy in clinical situations where thrombotic complications are envisaged, including organ transplantation and vascular surgery. They may also be useful for transgenic expression in animals whose organs could be used for clinical xenotransplantation. In these settings, the risks associated with constitutive expression in vascular EC may be minimised by inclusion within the fusion proteins of P-selectin cytoplasmic sequence, to restrict cell surface expression to activated endothelium.

Plenary Session 3

Wednesday 31st March

CADAVERIC RENAL TRANSPLANTATION FACILITATES THE INDUCTION OF DONOR-SPECIFIC HYPORESPONSIVENESS

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Chronic allograft failure represents a major impediment to successful renal transplantation. We are interested in uncovering the nature of the ongoing immunological stimulation that contributes to chronic allograft rejection. Previous work from our group has shown that the prolonged residence of organ allografts can induce donor-specific tolerance in experimental rodent models and that renal tubular epithelial cells induce allospecific T cell tolerance due to a lack of costimulatory molecule expression.

We have isolated $CD4^+$ T cells from recipients of renal transplants and quantified the IL2-secreting frequencies against donor cells both before and then again four months after transplantation. We have also estimated the frequencies of cells secreting IFNγ and IL5.

We have shown that 36% (8/22) of patients develop hyporesponsiveness to directly presented donor alloantigens. Of the remaining patients, 89% (8/9) showed a marked decrease in anti-donor frequencies after the addition of monoclonal αCD4 antibodies to the assay, implying that the high frequencies were maintained by low avidity clones. Based on previous work we postulated that memory/primed T cells ($CD45RO^+$) would be preferentially downregulated, due to their ability to traffic through the graft.

Studies in a further ten patients have been carried out, quantifying $CD4^+$ T cell frequencies to donor cells according to secretion of IL2, IL5 and IFNγ. This has been accomplished for both the $CD45RA^+$ (naïve) and $CD45RO^+$ compartments. Prior to transplantation we found that the frequencies were similar in both compartments for IL2. However frequencies of IL5 and IFNγ secreting cells were notably lower in the $CD45RA^+$ compartment. Four months after transplantation the IL2-secreting frequencies have dropped in the majority of patients studied. There is no significant difference between $CD45RA^+$ and $CD45RO^+$ compartments.

Conclusions: in renal transplant recipients, $CD4^+$ T cells usually become hyporesponsive to direct presentation of donor alloantigens and this phenomenon occurs in both the naïve and primed/memory compartments. Further efforts to elucidate the immunological contributions to chronic rejection should focus upon defining the role of indirect presentation of donor alloantigens

QUANTITATION OF CYCLOSPORIN SENSITIVE AND RESISTANT ALLO-SPECIFIC CYTOTOXIC CELL PRECURSORS AT BIRTH.

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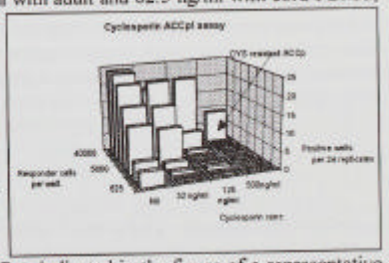
Acute rejection due to cyclosporin (CyA) resistant allo-specific T-cells is a major obstacle to successful transplantation. Naive T-cell precursors are negative for the Interleukin 2 receptor ($CD25^-$), but sensitised T-cells are positive ($CD25^+$). CyA inhibits the *de-novo* transcription of Interleukin 2 (IL-2) and CD25. But, sensitised T-cell precursors express CD25 and are able to respond to exogenous IL-2, even in the presence of therapeutic concentrations of CyA. The frequency of CyA resistant allo-specific T-cell precursors in normal individuals and in patients awaiting transplantation is completely unknown. Therefore we have attempted to measure these drug resistant cells in the peripheral blood mononuclear cells (PBMC) of healthy adults (15), dialysis patients (15), transplant recipients (9) and in cord blood (15). Responder PBMC were incubated for one hour in concentrations of CyA spanning the therapeutic range (32-1,000 ng/ml). Control cultures contained no CyA. CyA levels were confirmed by radio-immunoassay. HLA mismatched stimulator PBMC were irradiated (30 Gy) and added to responders and cultured for seven days with 25 Cetus-units/ml recombinant IL-2. Target cells (PHA-blasts) were prepared in parallel cultures of stimulator, responder and third party PBMC with phytohaemagglutinin. PHA-blasts were labelled with Europium (Eu) and added to the cultures. The amount of Eu-release by lysed PHA-blasts gave % allo-specific lysis and % inhibition of allo-specific lysis. Both single dilution analysis (SDA) and limiting dilution analysis (LDA) were performed to investigate the relationship between allo-specific cytotoxic cells (ACC) and CyA concentration. SDA indicated a plateau of maximum inhibition at 125 ng/ml with adult and 62.5 ng/ml with cord PBMC, suggesting that cord ACC were more sensitive to CyA. LDA analysis measured the ACC precursor frequency (ACCpf). These ranged from 10 to 1,500 /10^6 adult PBMC (n=10) and 12 to 800 /10^6 cord PBMC (n=6) in absence of CyA (NS). LDA also indicated a linear relationship between CyA concentration and ACCpf such that the higher the concentration, the lower the ACCpf. Nevertheless, CyA resistant ACCpf were found in cord PBMC as indicated in the figure of a representative experiment. At 500 ng/ml CyA these ranged from 7 to 20 ACCpf per 10^6 cord PBMC. A possible cause of sensitisation of cord PBMC is non-inherited maternal alloantigens in-utero. In-vitro this may have lead to cross-reactivity with allo-peptides on HLA mismatched PHA-blasts. We conclude that CyA resistant ACCpf are present at birth and may influence the outcome of transplantation.



IN VITRO ACCOMMODATION OF PORCINE ENDOTHELIAL CELLS; LOW DOSE ANTI-PIG ANTIBODIES INDUCE 'SURVIVAL GENE' EXPRESSION BY NITRIC OXIDE DEPENDENT AND INDEPENDENT MECHANISMS.

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Transplanted xenografts, protected from hyperacute rejection and delayed xenograft rejection by xenoreactive natural antibody (XNA) depletion or inhibition of complement can sometimes continue to function despite the return of anti-donor antibody and complement back to pre-transplant levels. This phenomenon, termed graft 'accommodation' was first described in allografts transplanted across ABO barriers and later in those transplanted into allosensitised recipients. Although the precise mechanisms underlying accommodation are still not clear, it is thought to involve changes in xenograft endothelial cell (EC) physiology, rendering the cells resistant to activation. In some models of xenotransplantation, evidence suggests that anti-xenograft antibody may promote the EC changes that characterise accommodation, but this data has been difficult to reconcile with the evidence from other models, which indicates that XNA depletion is crucial for accommodation to occur.

An attractive hypothesis is that accommodation might only arise if endothelium is initially exposed to a low concentration of antibody. We have established an in vitro model of porcine EC accommodation to investigate this possibility. In previous studies, we have reported that low concentrations of human IgG induce a change in the phenotype of porcine EC consistent with the development of accommodation. After incubation for at least 72 hours with human IgG XNA, immortalised and primary porcine EC populations developed resistance to lysis by human complement and downregulated the expression of VCAM and MHC class I. These accommodated EC bound fewer human T lymphocytes than control cells. Another prominent feature was the neo-expression of inducible nitric oxide synthase (iNOS) and a consequent increase in the basal production of nitric oxide (NO) by accommodated EC.

We now report that these accommodated EC upregulate the expression of the 'survival genes' bcl-2 and bcl-xl in a time dependent manner after incubation with human IgG XNA. Only EC that were initially exposed to low concentrations of XNA developed a sustained expression of bcl-2.

'Survival gene' expression was also induced in these cells by incubation with the NO donor detanonoate, suggesting that iNOS expression and NO release during incubation with XNA may enhance the development of an accommodated phenotype. However, addition of the iNOS inhibitor L-NMMA into the EC cultures along with XNA failed to consistently suppress the induction of either bcl-2 or bcl-xl expression. These results are most consistent with the hypothesis that XNA mediate accommodation by at least two different mechanisms, one of which may be dependent on NO production.

This model is expected to continue to provide valuable insights into the molecular basis of accommodation, which may allow the development of novel strategies to promote xeno- and allograft survival.

HUMAN IgG_2 ANTI-GALα1-3GAL IS NON-CYTOTOXIC BUT DOES NOT INDUCE ACCOMMODATION IN PORCINE AORTIC ENDOTHELIAL CELLS

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The biological significance of IgG in pig to primate xenotransplantation is not clearly defined, whereas IgM anti-Galα1-3Gal is known to cause hyperacute rejection [HAR]. It is claimed that 1% of human IgG is anti-Galα1-3Gal and that it may therefore block IgM binding. There is evidence that IgG anti-pig can cause endothelial cell activation and complement-dependent lysis. Conversely, pooled polyclonal human IgG preparations have been used to ameliorate HAR in animal models and to induce a protective phenotype, termed accommodation, in immortalised porcine aortic endothelial cells [PAEC].

To clarify the role of IgG, we have produced highly purified IgG anti-Galα1-3Gal from pooled human normal immunoglobulin [HNIg, BPL] and three individual sera, by means of elution from protein G-agarose followed by elution from Galα1-3Gal- and Galα1-3Galβ1-4Glc-agarose. The eluates were IgM free and comprised IgG_2 [>95%] with small amounts of IgG_1 [<5%]. Primary cultures of PAEC [LDL receptor and von Willebrand Factor positive], rather than immortalised cells, were used in binding and cytotoxicity studies.

We found that only 0.25% of HNIg was anti-Galα1-3Gal. All IgG eluates bound to Galα1-3Gal- neoglycoproteins and PAEC [ELISA], and to porcine lymphocytes [flow cytometry]. Binding sites were not saturated in any of these assays, even at supra-physiological concentrations [200μg/ml]. All eluates bound to proximal convoluted tubules, collecting ducts and non-glomerular endothelial cells in formalin fixed pig kidney sections. It was not possible to block purified IgM anti-Galα1-3Gal [34μg/ml] binding to PAEC or lymphocytes using IgG anti-Galα1-3Gal at 200μg/ml. The eluates [up to 50μg/ml] were all non-cytotoxic in complement-dependent micro-cytotoxicity assays using PAEC and porcine lymphocytes. To investigate accommodation, PAEC were successfully cultured with sub-saturating doses [0.25, 1, 2.5 and 10μg/ml] of the four IgG anti-Galα1-3Gal eluates for 24, 72, 120 and 144 hours. Sensitivity of the PAEC monolayers to complement-mediated lysis by fresh human serum [0.2–100%] was then measured in a colorimetric cell viability assay. PAEC were <u>not</u> rendered resistant to complement-mediated lysis in any of the experiments, relative to control cells passaged and plated at the same time.

Our data indicate that much less than 1% of human IgG is anti-Galα1-3Gal but confirm that IgG_2 is the predominant subclass. The eluates were not cytotoxic, presumably because IgG_2 does not fix complement. It was not possible to block IgM binding, which reflects the huge number of available epitopes per cell. IgG_2 anti-Galα1-3Gal did not induce accommodation in primary PAEC. It is possible that the accommodation previously described using HNIg is due to IgG of different specificity or subclass, or that immortalised, trypsinised PAEC behave differently in cytotoxicity assays.

Plenary Session 4

Wednesday 31st March

SCREENING AND PROGRESSION OF CORONARY ARTERY DISEASE IN RENAL TRANSPLANT RECIPIENTS.

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Coronary artery disease [CAD] is the most common cause of death following renal transplantation, despite increasingly proactive treatment of important risk factors such as hypertension and hyperlipidaemia. In view of this we have a rigorous policy of screening patients in our unit for coronary artery disease, prior to renal transplantation. Since 1991 we have performed coronary angiography on all patients awaiting renal transplantation with symptoms of ischaemic heart disease, all diabetics and most patients with other vascular disease or over 50 years of age. A total of 136 patients [95 male 41 female, age range 26-72, mean 53 years, 58 diabetics] have had coronary angiograms. The table below shows the results:

	Symptomatic No. of patients[%]	Asymptomatic No. of patients[%]
ALL PATIENTS	60	76
Insignificant CAD	21 [35]	66 [87]
Significant CAD	39 [65]	10 [13]
DIABETICS	19	39
Insignificant CAD	7 [37]	33 [84]
Significant CAD	12 [63]	6 [16]

22 patients with significant CAD have undergone revascularisation with coronary artery bypass grafting [CABG] or percutaneous transluminal coronary angioplasty [PTCA] and have then actively awaited [13 patients] or received [9 patients] a renal allograft. 3 [13.6%] of these patients have suffered a cardiac death [0.2, 4.2 and 5.9 years post cardiac intervention]. 73 patients with insignificant CAD have undergone [38 patients] or awaited [35 patients] renal transplantation. 3 [4.1%] of these patients have had subsequent cardiac deaths [1.2, 2.4 and 3.2 years post coronary angiography].
In this cohort of 136 patients there is a significant incidence of silent CAD [13%], which has proved to be almost as high as the diabetic subgroup [16%]. This shows the importance of screening selected, asymptomatic non-diabetics, as well as diabetics, pre-transplantation. Despite revascularisation and attention to associated risk factors there is considerable continued cardiac mortality in the medium term, not sparing those with initially insignificant CAD. These patients require ongoing cardiac surveillance, potentially with follow-up coronary angiography, to detect progressing CAD and provide the opportunity for intervention prior to serious clinical events.

ADRENERGIC RECEPTOR AND ENDOTHELIAL DYSFUNCTION IN RENAL TRANSPLANT RECIPIENTS MAINTAINED ON CYCLOSPORIN

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Hypertension is almost universal following renal transplantation, and may contribute to the already poor cardiovascular prognosis of this group. Cyclosporin-induced hypertension is a particular problem and has variously been attributed to increased sympathetic nerve activity, salt and water retention and increased circulating endothelin levels. However, the effects of cyclosporin (CsA) on the L-arginine/nitric oxide (NO) system *in vivo* in man are unknown. In this present study we examined basal and stimulated NO production from the vascular endothelium of CsA-treated renal transplant recipients using forearm strain-gauge plethysmography.

This technique allows measurement of forearm blood flow (FBF) and the changes induced by drugs infused into the brachial artery through a sterile 27gauge cannula. FBF was measured in both arms and expressed as a ratio of infused:non-infused arm to correct for background variations in FBF. Responses to drugs were measured as the % change in FBF ratio from baseline.

Two separate studies were performed. In study 1, stimulated NO production was assessed using carbachol (endothelium-dependent vasodilator) and sodium nitroprusside (SNP; endothelium-independent vasodilator). Nine CsA patients were studied, with 7 azathioprine-treated controls and 12 controls without renal disease. In study 2, basal NO production was assessed using L-NMMA (inhibits NOsynthase) and noradrenaline in 9 CsA patients and 11 controls. Groups were comparable for age, sex, cholesterol and smoking habits, although blood pressure was higher in the CsA patients. Statistical comparison between the three groups in study 1 was by Kruskal-Wallis analysis; in study 2 comparison between CsA patients and controls was by Mann-Whitney test.

In study 1, the three groups had similar vasodilatation to SNP. However, the response to 3μg/min carbachol was attenuated in the CsA group (188.8 (72.5,385.1); median (range)%change FBFratio) compared to the azathioprine (378.1 (124.0,548.9)) and normal controls (303.8 (124.8,813.3); $p=0.045$). In study 2, CsA patients had impaired vasoconstriction to 4μmol/min L-NMMA (-19.5 (-4.7,-63.1)) compared to controls (-39.5 (-15.7,-52.8); $p=0.056$). While controls vasoconstricted to noradrenaline as expected (-26.9 (-1.4,-38.6)), CsA patients as a group tended to vasodilate (7.9 (-36.8,92.6); $p=0.020$).

These studies demonstrate reduced basal and stimulated NO production from the vascular endothelium of CsA-treated renal transplant recipients. As NO is anti-atherogenic, these defects may predispose the CsA group to the development of premature atherosclerosis. A surprising result was reduced vasoconstriction or paradoxical vasodilatation to noradrenaline in the CsA group. This may reflect chronic activation of α_1-adrenoceptors such that infusion of noradrenaline vasodilates through the β_2-adrenoceptor. Post-transplant hypertension may therefore involve endothelial dysfunction and/or chronic activation of α_1-adrenoceptors.

PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY OF RENAL TRANSPLANT ARTERY STENOSES: LONG TERM RESULTS IN ADULTS AND CHILDREN

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Method: Percutaneous transluminal angioplasty (PTA) of transplant renal artery stenosis (TRAS) has become an established technique. Good initial results are generally reported, but the long term outcome is less well documented in the literature. Between 1980 and 1997, 1139 renal transplants were performed in adults and 526 in children. During this period, PTA was attempted on 86 occasions in 38 adults (M:F 27:11 age 25-73, mean 39.2) and 24 children (M:F 13:11 age 1.5-15, mean 8.8) for hypertension (21 cases), decreasing renal function (15 cases), both (24 cases) or primary non-function of a new transplant (2 cases). The medical notes and radiology of these cases were retrospectively reviewed and complications and outcome recorded.

Results: Initial PTA was clinically successful in 12 children (50%) and 20 adults (53%). Symptomatic recurrent stenoses occurred in 2 children (8.3%) at 3 months and 4 years and 4 adults (10.5%) at 3 months -12 years. Two had successful treatment with PTA, one had successful surgical revascularisation, one graft failed and two grafts are still functioning on medical treatment (follow up 7 months and 88 months). Twelve children had clinically unsuccessful PTA (50%). Three children died shortly after (5-20 days), two of renal failure. Two remained on medical therapy, one of whom lost function 5 months later. Seven children had attempted surgical revascularisation.

Seventeen adults failed to benefit from PTA (47%). Two with primary non-functioning transplants never gained function. One transplant infarcted due to arterial occlusion following PTA with loss of function. Six hypertensive patients were treated medically and continue to have function or died with function (mean follow up 33 months). Two patients died with function (at 6 months and 12 months) Four patients had continuing decreasing renal function, with gradual loss of function (6 months to 4 years). Two patients underwent surgery.

Of the nine patients who had surgery, successful revascularisation was achieved in five of seven children (71%) and both adults, resulting in good long term function (mean 82.9 months) with one restenosis at 120 months successfully treated with PTA. Major complications of PTA were transplant artery occlusion in 3 cases (3.5%), one resulting in graft loss, one successfully revascularised surgically and one recanalising with heparin.

Conclusion: Despite a moderate clinical success rate, PTA remains the first line treatment of TRAS at our institution, since complication rates are low and subsequent surgery is not precluded.

CRISIS IN TRANSPLANTATION IN THE UK - WHERE ARE THE FUTURE TRANSPLANT SURGEONS?

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The biggest challenge in renal transplantation in the UK today is not related to immunosuppression, immunology of transplantation, tissue typing or even the number of transplants performed but the need to find, train and keep adequate numbers of surgeons willing to consider Transplant Surgery as a career. Already there have been several surveys of surgical trainees who have voiced their concerns regarding transplantation training and their ideal Consultant post but none has approached Consultant Surgeons who have trained in transplantation but then elected to choose another general surgical speciality. With this in mind, Consultant General Surgeons with varying degrees of training in transplantation, were approached for their views including:-

1. Their current post.
2. How many years training were spent in transplantation and where?
3. What attracted them to transplantation initially?
4. Why did they leave transplantation?
5. Did they perform research in a transplant topic and did this lead to a PhD/MD/Ch.M?
6. Would they have accepted a transplant post if it had been appropriately advertised/organised and if so what would have been their ideal post?
7. What would be their ideal training for a career in transplantation?
8. Did lack of private practice play a role in their choice of job?
9. Did the amount of out of hours on-call/call-out affect your choice of career?
10. Would extra remuneration for on-call have made a difference?
11. Did the lack of overall post-transplantation control of patients affect their outlook to transplantation?
12. Did the controversial nature of transplantation affect their final choice?

Responses indicate that a career as a full-time Transplant Surgeon was not attractive to these Consultant Surgeons equipped to perform General Surgery and highlights the problems and fears of these surgeons regarding Transplant Surgery.

This and other data points to the need to nurture and support trainees keen to pursue a career transplantation and to arrange posts around the trainee rather than speculatively advertise posts without the certainty of availability of trainees to fill them, as is done currently.