The British Transplantation Society
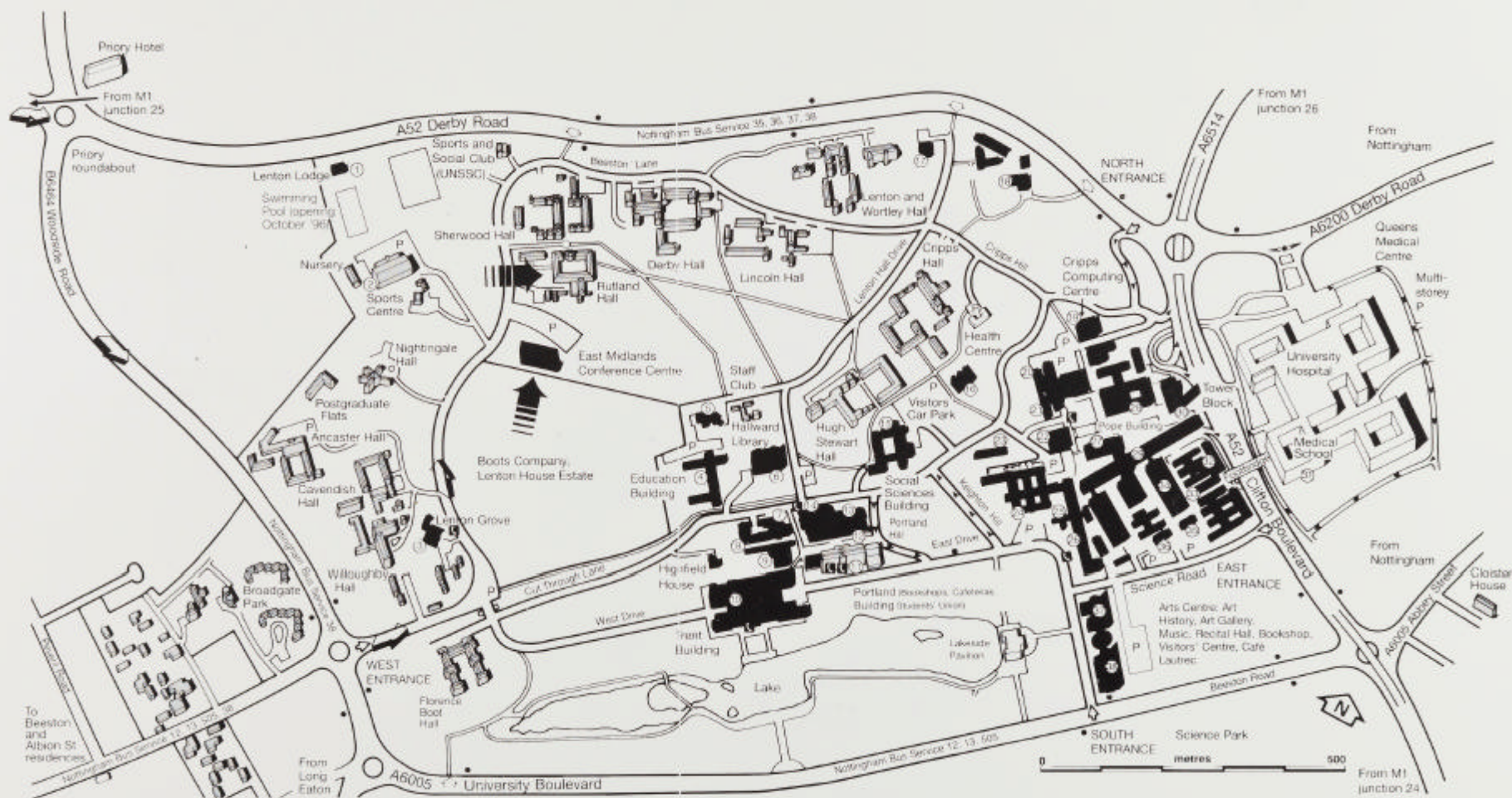
Spring Meeting

Nottingham

15th & 16th April 1997

East Midlands Conference Centre, University Park

Priory Hotel

Priory roundabout

B6464 Woodside Road

A52 Derby Road

Nottingham Bus Service 35, 36, 37, 38

From M1 junction 26

A6514

From Nottingham

A6200 Derby Road

NORTH ENTRANCE

Queens Medical Centre

Lenton Lodge

Sports and Social Club (UNSSC)

Beeston Lane

Swimming Pool (opening October '96)

Nursery

Sherwood Hall

Derby Hall

Lincoln Hall

Lenton and Wortley Hall

Cripps Hall

Cripps Hill

Cripps Computing Centre

Sports Centre

Rutland Hall

Lenton Hall Drive

Multi-storey P

University Hospital

Nightingale Hall

East Midlands Conference Centre

Staff Club

Health Centre

Medical School

Postgraduate Flats

Ancaster Hall

Boots Company, Lenton House Estate

Hallward Library

Hugh Stewart Hall

Visitors Car Park

Tower Block

Pope Building

A52

Cavendish Hall

Education Building

Social Sciences Building

Portland Hill

Visitor Hill

Clifton Boulevard

Lenton Grove

East Drive

Science Road

EAST ENTRANCE

From Nottingham

A6005 Abbey Street

Cloister House

Broadgate Park

Willoughby Hall

Cut-through Lane

West Drive

Highfield House

Trent Building

Portland (Bookshops, Cafeterias) Building (Students' Union)

Lake

Lakeside Pavilion

Arts Centre: Art History, Art Gallery, Music, Recital Hall, Bookshop, Visitors' Centre, Café Lautrec

Breston Road

To Beeston and Albion St residences

Nottingham Bus Service 36

WEST ENTRANCE

Florence Boot Hall

Nottingham Bus Service 12, 13, 505

From Long Eaton

A6005 University Boulevard

Nottingham Bus Service 12, 13, 505

SOUTH ENTRANCE

Science Park

N

0 metres 500

From M1 junction 24

Any other business

Mr R W G Johnson gave special thanks on behalf of the BTS to Dr Philip Dyer for his hard work and contribution whilst in post as General Secretary

In the absence of any other business the President closed the meeting a 6 p.m.

ABSTRACTS SELECTED FOR PRESENTATION

CLINICAL RESPONSE AND TEMPORAL PATTERNS OF ACUTE CELLULAR REJECTION;
RELATIONSHIP TO CHRONIC TRANSPLANT NEPHROPATHY

Ashraf S, Parrott NR, Dyer P, Roberts I, Johnson RWG.

Renal Transplant Unit, Manchester Royal Infirmary

The association between acute cellular rejection and the development of
chronic rejection has been the subject of much debate. Studies have suggested
that the two phenomena may be linked, or, conversely that there is no
association at all. In order to clarify this relationship, the outcome of 284 renal
allografts were examined. Transplants were performed at a single institution
between January 1989 and December 1991, allowing minimum follow-up of 5
years. In order to classify acute cellular rejection (ACR), this was categorised
into three clinical groups;- I) ACR where the post-rejection serum creatinine
returned to baseline levels (Type 1 ACR), ii) ACR where the creatinine
remained elevated at least 10% above base levels, (Type 2 rejection) and iii)
ACR requiring treatment with mono- or polyclonal antibodies. Where possible
both ACR and chronic transplant nephropathy (CTN) were confirmed by
histology using Banff criteria. Other risk factors possibly associated with CTN
were also examined including transplant number, donor age, gender, infection,
ischaemia times, HLA matching and Cyclosporin dose.

CTN occurred significantly more frequently in those patients with 'late ACR'
(after day 60), than in those who had early rejection (53.5% versus 17.3%
respectively), P< 0.00001.
When ACR was classified by clinical response, there was no association
between ACR Type 1 and CTN (P>0.25), there was a highly significant
association between Type 2 rejection and CTN (P<0.0001), and a similar
association between Type 3 ACR and CTN (P<0.001). There was no correlation
between Banff grading of rejection and the later onset of CTN.

This study suggests that it is the clinical behaviour and response to treatment of
ACR that is paramount in determining the likelihood of chronic rejection. Late
acute rejection, and acute rejection responding poorly to treatment are more
likely to be associated with CTN and late graft loss. Studies that look only at the
phenomenon of ACR without consideration of the whole spectrum of this event
are likely to draw erroneous conclusions about the association of acute and
chronic rejection.

MEASUREMENT OF MATRIX PROTEIN mRNA LEVELS IN STABLE HUMAN RENAL TRANSPLANT BIOPSIES

P. N. Furness, G. R. Bicknell, L. L. Hall, L. Primrose, J. A. Shaw, J. H. Pringle,
M. L. Nicholson[†]

Departments of Pathology and [†]Surgery, University of Leicester
Leicester General Hospital and Leicester Royal Infirmary

The development of improved strategies to prevent chronic allograft nephropathy
depends not only on improved understanding of the process, but also on improved
methods of measuring the rate of progression of damage to the transplant.

Graft survival can be predicted by histo-morphometric assessment of extracellular
matrix at 6 months post-transplant. It is likely that this deleterious accumulation is
stimulated and controlled by cytokine synthesis, especially TGFß1. We therefore
argued that measurement of the rate of synthesis of extracellular matrix, as represented
by tissue mRNA levels, might provide a measure of the rate of graft damage earlier
than 6 months. Changes in matrix mRNA levels could represent a sensitive measure
of the efficacy of different treatments, and may shed light on the nature of the
underlying process.

We therefore measured levels of specific mRNA species in single glomeruli plucked
from the surface of protocol renal transplant biopsies, using semi-competitive reverse
transcriptase-polymerase chain reaction (RT-PCR) with ELISA quantitation of the
products.

Measurements of levels of glomerular GAPDH, a 'housekeeping' gene, showed large
variation between different patients, though much less between glomeruli from one
patient. This is probably due to variation in cellularity between glomeruli.

With or without a correction for GAPDH levels, we found considerable differences in
the amount of mRNA for TGFß1 and several matrix proteins. There is a close
correlation between levels of mRNA for TGFß1 and for several extracellular matrix
proteins, including collagen III, collagen IVα2 and tenascin. Surprisingly, elevated
TGFß1 correlates with elevated matrix metalloproteinase 2; but this may be inactive,
as there is simultaneously an elevation of mRNA for the metalloproteinase inhibitors
TIMP1 and TIMP2.

We do not yet have sufficient follow-up to assess correlations between these data and
graft survival.

TRANSFORMING GROWTH FACTOR BETA (TGFβ) AND LUNG ALLOGRAFT FIBROSIS FOLLOWING TRANSPLANTATION

A El Gamel, P Hasleton, M Awad, E Simm, NA Yonan, J Egan, C Campbell, AN Rahman, AK Deiraniya, P Sinnott and IV Hutchinson

Cardiothoracic Transplant Unit and Department of Pathology, Wythenshawe Hospital, Manchester M23 9LT; School of Biological Sciences, University of Manchester Medical School, Manchester M13 9PT

TGFβ is a potent immunosuppressive cytokine that promotes fibrosis by enhancing the synthesis of extracellular matrix components. The repair process following lung transplantation is initiated by rejection or infection, leading to replacement of lung parenchyma by fibrotic tissue and, ultimately, to pulmonary dysfunction. However, the precise role of TGFβ in this excessive healing process and an increased risk of infection is unknown. We have investigated the relevance of different factors including on allograft fibrosis and its correlation with TGFβ production.

Patients were between 16 and 62 years of age (mean 39.6 years). The procedures were heart and lung (n=32), double lung (n=18) and single lung (n=41) transplantation. Fibrosis was graded in H&E stained sections of endobronchial biopsies while the presence of TGFβ was determined using immunohistochemistry. TGFβ genotypes, corresponding to the ability of individuals to produce greater or lesser amounts of TGFβ in vitro, were determined in all patients.

Forty six patients had fibrosis diagnosed in endobronchial biopsy specimens. Patients who developed interstitial fibrosis suffered significantly (p=0.024) more rejection episodes (3.4±2.8) than those who did not (2.1± 2.2). The presence of eosinophils in the interstitium preceeded and was positively associated with the development of fibrosis regardless of the rejection grade (p=0.0001). TGFβ was significantly (p<0.0001) more heavily expressed in sections with fibrosis (score 6.8± 2.9) compared with sections with no fibrosis (score 2.4±0.6), and TGFβ expression correlated positively with fibrosis score (p<0.0001). Furthermore, the development of graft fibrosis was greatest in patients homozygous for the high TGFβ producer genotype (p=0.01). The use of cardiopulmonary bypass, which causes release of TGFβ into the circulation, was associated with the development of excessive fibrosis (p=0.02). Patient death was correlated with fibrosis score and genotype. The survival of patients with a fibrosis score < 6 was 892±73 days compared with 427±78 days in patients with a fibrosis score >6 (p<0.0001), and seven (17.5%) with severe fibrosis died of septicaemia. The FEV1 was higher in patients without fibrosis (1870±111ml vs 1590±160, p=0.02).

In conclusion, the risk of fibrosis in the transplanted lung increases with recurrent rejection, tissue eosinophilia, homozygous high producer TGFβ genotype and use of the bypass machine. The association between fibrosis, higher mortality and morbity might be accounted for by the immunosuppressive and fibrogenic properties of TGFβ. Immunological strategies to down-regulate TGFβ production might improve survival and function of lung transplants.

THE OUTCOME OF A TACROLIMUS MONOTHERAPY SAFETY STUDY IN PRIMARY RENAL ALLOGRAFT TRANSPLANTATION

Campbell B A; Johnson R W G; Parrott N R; Pearson R C; Riad H N; Short C D; Roberts I S D; Turnbull C; Bendle J, Asderakis A; Nagy K K.

Renal Transplant Unit, Manchester Royal Infirmary.

Tacrolimus was used for the first time as the sole immunosuppressive agent in a prospective, open, single centre study to assess the efficacy and safety in first renal transplant recipients.

Exclusion criteria included second and subsequent grafts, patients aged < 18, Pregnant and nursing mothers or women of child bearing age unwilling to use adequate contraception during the study, failure of immediate function, PRA score > 50% and patients with clinically significant cardiovascular, pulmonary or liver disease.

Forty-five patients were entered into the trial between January and September 1996 of which 17 patients were withdrawn because they did not meet the criteria. The following data refer to the remaining 28 patients.

There were 23 males (82%) and 5 females. Ages ranged from 20 - 70 years. With a mean of 43.5yrs (SD = 11).
Tacrolimus was given orally at an initial dose of 0.15mg / kg body weight twice daily commencing within the first 24hrs of transplantation. The target whole blood trough level within the first month was 10 - 20ng / ml. Subsequently a lower maintenance trough level of 5 -10 ng / ml was sought.

No patient had more than 3 HLA mismatches, no patient had 2 DR mismatches. Mean number of mismatches (A,B,DR) was 1.82.
Patient and graft survival has been excellent. Six episodes of suspected rejection (in 6 patients) were confirmed histologically. The allografts were biopsied on each occasion. In 5 other biopsies, 2 exhibited changes compatible with toxicity and 3 were non-specific. No patient required ATG therapy or maintenance steroids and no grafts were lost. 50% of the rejections occurred in the first week. One patient had his first and only rejection at 5 weeks. Currently, the mean follow up is 6.25months and all 28 patients remain on Tacrolimus. The mean serum creatinine concentration at 6 months after transplantation is 174.4 mmol / L. Eleven patients were treated for infections such as UTI (7), wound (2), CMV (1) candida (1). Five patients exhibited raised blood sugar post operatively, only one required an oral hypoglycaemic agent. The mean period for hospitalisation was 10.4days.

Tacrolimus has proved to be a remarkably safe and effective agent when used as the sole immunosuppressive agent for renal transplantation. An acute rejection rate of 21.4% (6/28) compares favourably with rates for all previously reported immunosuppressive protocols. In addition the very low rate of opportunistic infection is a significant advantage over triple and quadruple therapy. These results indicate the need for a larger, multicentre, prospective trial.

MEDICATION INFLUENCING THE DEVELOPMENT AND SEVERITY OF GINGIVAL OVERGROWTH IN RENAL TRANSPLANT PATIENTS.

Varga E., Mair L.H. (Bakran A).

Department of Clinical Dental Sciences, The University of Liverpool, UK.

Gingival overgrowth is a well recognised complication in renal transplant patients which has been associated with the use of cyclosporin. Other researchers have implicated nifedipine as an important co-factor. This longitudinal study was initiated to determine the effect of peritransplant medication on gingival overgrowth in patients awaiting renal transplants and then to follow them over a five month period post-transplant.

Ninety-four patients on the renal transplant waiting list were seen on two occasions and their pre-transplant medication was recorded. Of these 94 patients, 35 received renal transplants during the study period. These patients were recalled at 4-6, 10-12, 16 and 20 weeks post-transplant. All the post-transplant medication was recorded together with the serum cyclosporin level (SCL) corresponding to each recall. In addition the mean of all the SCLs recorded during the 1st month post-transplant (SCL1M) was ascertained. At 1 pre-transplant and 3 post-transplant visits dental impressions were taken and used to make models from which the Gingival Overgrowth Index (GOI) was measured as described by Semour *et al.* (J. Clin Periodont 1985, 12: 413-419).

The 35 patients were divided into three distinct groups by the severity of their gingival overgrowth: (1) Severe overgrowth (n=13), (2) Mild overgrowth (n=16) and (3) No overgrowth (n=6). There was no significant difference between the mean SCL's of the three groups at any visit or between the SCL1M's (Kruskal-Wallace $Chi^2 < 2.28$, $p > 0.32$). The number of patients on nifedipine in the severe group was 9 (69%) compared with 12 (75%) in the mild group. These values were not significantly different. Most importantly, it was noted that all the patients in the severe group had evidence of gingival hyperplasia prior to transplant. Of these only 4 patients had taken nifedipine prior to their transplant.

It is concluded that the factor most likely to be associated with the development of gingival overgrowth is a pre-existing hyperplastic response in the gingivae. There was no evidence to show that the development and severity of the overgrowth was related to the cyclosporin serum level or to the combined medication with nifedipine.

THE UK ASSESSMENT OF THE BANFF CLASSIFICATION OF TRANSPLANT PATHOLOGY AND A NEURAL NETWORK APPROACH TO IMPROVED DIAGNOSIS OF ACUTE REJECTION

P. N. Furness, J. I. Kazi*, M. L. Nicholson[†], U. Kirkpatrick[†], N Taub[#], D. Davies[¥], K. Solez[§]

Departments of Pathology & [†]Surgery, Leicester General Hospital; [#]Epidemiology, Leciester University; [¥]Pathology, Oxford; [§]Pathology, Edmonton, Canada; *Sindh Institute of Urology & Transplantation, Karachi, Pakistan.

To test the validity of the Banff classification of renal transplant pathology in routine practice, we selected 21 transplant biopsies where it had been difficult to make or exclude a diagnosis of early acute rejection. All biopsies had diagnoses validated by the subsequent clinical course. We circulated sections to pathologists in all but one of the UK's renal transplant centres. Diagnoses made using 'conventional' criteria and by applying the Banff classification were returned for central analysis.

In diagnosing early acute rejection, the Banff classification produced greater reproducibility than a conventional approach, but it did not produce more correct diagnoses.

We argued that this was because the Banff classification concentrates on tubulitis and arteritis, and ignores other features of possible benefit. To re-introduce such features in a systematic way, we developed a 'Bayesian Belief' network, a simple type of neural network, in the hope that this would improve the accuracy of diagnosis.

The network was 'primed' by grading each of 10 features in each of 100 transplant biopsies where review had showed that clear retrospective diagnoses of rejection or not rejection could be made. The data produced was used to calculate a conditional probability matrix, (CPM) which was entered into the program 'Bayes for Win'.

The network was then tested on the 21 selected difficult biopsies which were used in the UK trial of the Banff Classification. When seen by the transplant pathologists of the U.K., the average number of correct diagnoses had been 62%, and the maximum achieved by any one pathologist was 18/21.

Applying the network, the trainee pathologist whose observations produced the CPM got 19/21 diagnoses right. A more experienced pathologist also got 19/21 right, but his observations were shifted towards over-diagnosis of acute rejection due to inter-observer variation.

The results demonstrate that consideration of features not currently included in the Banff classification of renal transplant pathology can improve the accuracy of diagnosis of early acute rejection.

The approach is applicable to other problems in clinical medicine and pathology.

PREEMPTIVE KIDNEY TRANSPLANTATION THE ATTRACTIVE ALTERNATIVE

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T AUGUSTINE MD, NR PARROT FRCS, RWG JOHNSON FRCS

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Haemodialysis and peritoneal dialysis while life saving for patients with End Stage Renal Failure are associated with severe complications and a significant health care expenditure. Preemptive kidney transplantation is now an established form of transplantation.

Between January 1980 and December 1995 we performed 1715 kidney transplants out of which 1463 were first kidney transplants. In this group of first transplants, there were 161 patients transplanted without prior dialysis. These patients had no difference to those dialysed as far as donor age, donor and recipient sex, and cold ischaemic time was concerned. The preemptive group contained more patients with delayed graft function ($p=0.001$) which might be expected to compromise survival in the preemptive group.

There was no difference in HLA-DR mismatches between the two groups (50.6% zero DR mismatches in the preemptive group compared to 47.9% in the dialysis group). There were significantly more live donor transplants in the preemptive group (14.2% v 7.3%) [$p=0.0002$].

Results

More patients in the preemptive group had rejection (30% with one rejection and 37% with greater than one) than in the dialysis group (25% and 31%) [$p=0.02$]. The actuarial graft survival in the preemptive group at 1, 5, and 10 years (84, 76.4, 69 percent) was significantly better than in the dialysis group (83, 69 and 56.7percent) [$p=0.033$].

If the live donor transplants were excluded from the analysis, there was still a survival advantage in the preemptive group but the difference was not significant until 5 years of follow up.

In conclusion, preemptive kidney transplantation not only avoids the risks, cost and inconvenience of pretransplant dialysis, but is also associated with better graft survival than post dialysis transplantation.

PATIENT ASSESSED OUTCOMES IN RENAL TRANSPLANTATION

Rigg KM[1], Turnbull N[2], Rose M[1], Davis P[2], Burden RP[1]

[1]Renal Unit and [2]Quality & Audit Resource Centre, Nottingham City Hospital, Nottingham, NG5 1PB

Outcomes are being used increasingly to measure the effectiveness of health care interventions and are recognised by purchasers and providers of health care. However patient assessed outcomes need to be measured in addition to clinical outcomes. The aim of this study was to confirm objectively the clinical impression that transplantation does improve quality of life, by measuring patient assessed outcomes.

A two part questionnaire was designed incorporating the SF-36 generic health survey questionnaire and the disease specific renal quality of life questionnaire (RQLQ), both of which have been validated in previous studies. The SF-36 measures functionality, well being and overall health using the eight dimensions of physical and social function, physical and emotional role, general and mental health, energy and pain. The RQLQ measures seven dimensions relating to problems of life as a renal patient: diet, physical activity, work, sleep, leisure, psychosocial and treatment effects. The questionnaire was administered by post to 129 stable renal transplant patients (transplant > 1 year, creatinine <200Umol/l) and 92 dialysis patients on the transplant list (HD=31, CAPD=61), four monthly over a one year period. During the course of the study 21 dialysis patients were transplanted and these were analysed separately. The response rate was 85% for each of the three questionnaire administrations.

Using the SF-36 questionnaire, stable transplant patients scored significantly higher ($p<0.01$ Kruskal-Wallis) than HD or PD patients on the transplant list for all eight dimensions. For the RQLQ, transplant patients again scored significantly higher ($p<0.01$) for all seven dimensions compared with dialysis patients. For those patients who were transplanted during the course of the study, there was a significant improvement in scores for energy ($p<0.01$ Wilcoxon) and emotional role ($p<0.05$) using the SF-36 questionnaire; and physical activity ($p<0.05$), diet and leisure ($p<0.01$) using the RQLQ questionnaire.

This study gives objective evidence that transplant patients have an improved quality of life compared to dialysis patients, and that this effect can be measured very soon after transplantation. Patient assessed outcomes should be used as an adjunct to clinical outcomes and the information gained could be used to improve both quality of care for patients and to inform the contracting process between purchasers and providers.

NURSE PRACTITIONERS, HEALTH PROMOTION AND ANNUAL CLINICAL ASSESSMENT IN THE RENAL TRANSPLANT CLINIC.

Valentine AM, Russell S, Short CD, Hutchison AJ and Johnson RWG.

Renal Transplantation Unit, Manchester Royal Infirmary, Manchester.

In March 1996 we instituted an additional weekly transplant clinic, specifically designated to facilitate the discussion of health promotion issues and enable a comprehensive assessment to be made of medical problems. This clinic is staffed by two nurse practitioners and a member of the medical staff. The aim is to see all of our renal allograft recipients on an annual basis near the anniversary of their transplant operation. The patients are asked to bring a completed questionnaire outlining areas for discussion and the entire nurse/doctor consultation takes approximately 1 hour.

By the end of 1996 we had seen 388 patients, 236 males and 152 females, with a mean age of 45.3 years (range 19-74). Three hundred and twenty three (83%) were first allograft recipients, 57 (15%) second and 8 (2%) third or more. Seventy two patients were transplanted one year ago, 31 had received the allograft 15 or more years earlier.

Health promotion issues addressed included: (i) blood pressure control and other risk factors for ischaemic heart disease, (ii) dermatological, dental and neoplastic complications associated with immunosuppression and (iii) general advice on health and well being.

Total serum cholesterol was >5.2mmol/l in 74% of the patients but ≥6.9mmol/l in just 26%. Body mass index (BMI) was ≥ 25 in 63% male patients (compared to 45% in the general population). Respective figures for females were 58% and 36%. Only 22% of both male and female patients were smokers: for the general population these figures are 31% and 28%.

On skin examination, 7% (29) patients had Tinea versicolor, for which treatment was given in the clinic; (prevalence in general population is <1%). Warts were present in 26% (100) patients, some of whom were referred to their GP for treatment. A total of 61 patients in all were referred to a dermatologist for a variety reasons including suspected skin cancer and widespread warts.

When discussing dental hygiene, it was found that 71% patients regularly visit a dentist and 28% of these patients complained of gum problems. Of those not visiting a dentist 33% also complained of gum problems.

Annual cervical smears, as recommended had recently been performed in 58% of women. Both men (22%) and women (8%) complained of impaired sexual function. One hundred and thirty three referrals were made in all, 46% to the dermatology department.

Data to be obtained at subsequent annual reviews will enable us to plan interventional studies and ascertain if this integrated approach is beneficial to the health of our patients.

Antibody-Mediated accelerated rejection following immunisation with RT1.A^a α chain DNA. Evidence that T cells providing B cell help have been primed by the indirect pathway.

Pettigrew G, Lovegrove E, McLean J, MacDonald CM, Bolton EM, Bradley JA

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Aim: The contribution of the indirect pathway of allorecognition to graft rejection is poorly defined. We have shown previously that PVG RT1^u rats reject R8 A^a class I disparate heart grafts by CD4 T cell-dependent alloantibody mediated effector mechanisms. The aim of the present study was to determine whether indirect T cell recognition of class I alloantigen contributes to alloantibody production and graft rejection in this experimental model.

Methods: RT1^u recipients were immunised to A^a alloantigen and the ability of the immunisation protocol used to generate alloantibody to the intact A^a molecule and to accelerate rejection of A^a disparate heart grafts was examined. Animals were immunised in one of three ways:
1) Application of full thickness R8 skin allografts 7 days prior to heart transplantation.
2) Administration of peptides (21 or 24mers) corresponding to the hypervariable regions of the A^a molecule (100 μg peptide in CFA injected S/C 7 days before transplant).
3) Vaccination with DNA encoding either the membrane bound or soluble form of the A^a heavy chain. The genetic sequence for the full length or soluble A^a molecule was inserted in the pCMU-IV eukaryotic expression vector under the control of a human α globin promoter and an SV40 enhancer. The constructs were injected IM (200 μg) 12 and 7 days before transplantation.

Results: Immunisation with A^a skin allografts provoked a strong cytotoxic alloantibody response and markedly accelerated the rejection of R8 heart grafts (MST 2 days). Priming with A^a derived peptides did not generate a cytotoxic alloantibody response and peptide primed animals rejected R8 heart grafts only marginally faster than naive controls (MST 5 and 6 days respectively). However peptide primed animals displayed an accelerated alloantibody response after heart transplantation, indicating that T cell priming by the indirect pathway had occured. RT1^u rats injected with full length or soluble A^a α chain DNA constructs developed high levels of cytotoxic alloantibody, indicating that the soluble A^a molecule provided both a conformational B cell epitope and efficient T cell priming by indirect allorecognition. DNA vaccinated animals rejected A^a heart grafts as rapidly as animals immunised by skin grafting (MST 2 days).

Conclusions: These results highlight an important role for indirect T cell allorecognition in antibody-mediated allograft rejection. DNA vaccination studies results in production of sufficient MHC protein to stimulate strong T and B cell immunity and this approach may provide opportunities for manipulation of the alloimmune response.

ACUTE RENAL ALLOGRAFT REJECTION : β CHEMOKINE EXPRESSION AND DISTRIBUTION IN PARAFFIN-EMBEDDED BIOPSY SECTIONS ANALYSED BY CONFOCAL MICROSCOPY

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[*]Public Health Laboratory, Newcastle General Hospital, NE4 6BE.

Previous studies using immunohistochemistry and *in situ* hybridisation have shown that, in tubulitis associated with acute cellular rejection, intratubular T cells actively proliferate and become fully activated *in situ*. This immunohistochemical study in renal transplant biopsies has attempted to establish some understanding of the involvement of the β chemokines RANTES, MCP-1, MIP-1α and MIP-1β in recruiting T cells to the intratubular site.

Paraffin-embedded biopsy sections, representing all stages of acute cellular rejection, were treated for conventional indirect immunofluorescence to detect the selected chemokines. Scanning laser confocal microscopy, which permits computer-driven analysis, was used to provide a measure of fluorescence intensity resulting from binding of FITC-labelled secondary antibody. Cells secreting individual chemokines could be identified and distribution of chemokines throughout the interstitial matrix was established. Within the limits of the staining method it was possible to obtain a semi-quantitative assessment of individual chemokine activity at different points in biopsy sections.

Peak fluorescence intensity for RANTES (up to a pixel intensity of 220 units compared with <50 in normal kidney) was found in the cytoplasm of tubular epithelial cells (TEC), suggestive of the production of RANTES by TEC. The distribution of such staining varied from isolated tubules or groups of tubules in cases of minimal to moderate acute cellular rejection to extensive TEC staining over the whole tubular area in a case of severe acute cellular rejection with prominent tubulitis. In addition, there was widespread distribution of RANTES in the matrix around tubules, although fluorescence intensity was lower than in the cytoplasm of TEC. Peak fluorescence intensity for MIP-1β also reached ~200 pixel units in the cytoplasm of TEC in some cases but was generally less widely distributed and less intense in the interstitial matrix. The pattern of staining for MCP-1 followed that of RANTES at a reduced level (peaking at ~120-150 pixel units), whilst MIP-1α appeared to be expressed mainly by isolated intraglomerular cells and cells of the interstitial infiltrate.

The results indicate that the β chemokines, RANTES, MIP-1β and MCP-1 are variably expressed and secreted by graft TEC and are distributed throughout the interstitial matrix during acute cellular rejection. The high concentration of chemokines on the basolateral surface of TEC suggests a mechanism for specific chemotactic recruitment of T cells to the tubular epithelium.

The findings have implications for the design of early immunomodulatory intervention strategies in renal allograft rejection.

MODULATION OF ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION AND LYMPHOCYTE ADHERENCE USING ANTISENSE OLIGONUCLEOTIDES.

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DEPT. SURGERY, UNIVERSITY NEWCASTLE UPON TYNE.

Introduction- Endothelial-leucocyte adhesive interactions are crucial to both the afferent and efferent phases of the rejection response and can be upregulated by the cytokine TNFα. Antibodies specific for ICAM-1 have been used to increase allograft survival in an animal model but their immunogenicity limits prolonged or repeated use. One alternative is to obtain direct and specific genetic downregulation of membrane receptors involved in intercellular adhesion by administration of appropriate antisense oligonucleotides thereby suppressing the rejection process by reducing lymphocyte-endothelial cell interactions.

Methods-Experiments were performed with confluent cultures of the immortalised human endothelial cell line (EA.hy 926) on 24 well plates. Cells were incubated at 37ºC with 25-200nM ICAM-1 antisense oligonucleotide (ISIS1939) in serum free media for 4-6hrs and the detergent lipofectin was used (15µg/ml) to enhance cellular uptake. Control experiments were performed with ICAM-1 sense construct. Cells were then transferred to serum containing media for incubation with recombinant TNFα for 24 hours and adhesion molecules quantified. Resting cells, cytokine stimulated and oligonucleotide treated cells were labelled with ICAM-1 monoclonal antibody and counterstained with FITC conjugated reagents, analysed by flow microfluorimetry and adhesion molecules quantified by use of standard curves generated with FITC conjugated beads. Lymphocyte adhesion to resting, cytokine stimulated and oligonucleotide treated monolayers was measured using a sensitive flow cytometric assay.

Results-Endothelial cell ICAM-1 expression was significantly reduced by the administration of 25-200nM ICAM-1 antisense oligonucleotide (paired t-test p< 0.05) and no significant reduction was seen with the control sense construct.There was a gradual and progressive increase in effect with increasing concentration of antisense oligonucleotide . The effect was abrogated by the absence of lipofectin. Lymphocyte adhesion to ICAM-1 antisense oligonucleotide treated endothelial cells was significantly reduced (MannWhitney p<0.05) with >50% inhibition of adhesion.

Conclusion-Antisense oligonucleotide mediated downregulation of adhesion molecule expression (ICAM-1) and subsequent disruption of the endothelial-leucocyte interaction has been demonstrated. Future studies should address the effect of this modulation on functional lymphocyte behaviour. Expansion of these techniques to include additional adhesion molecules and the effect on endothelial and graft parenchymal cells would contribute to defining the role of antisense oligonucleotides in the armamentarium against allograft rejection.

ADMINISTRATION OF EXOGENEOUS IL2 PREVENTS THE DEVELOPMENT OF SPONTANEOUS TOLERANCE IN THE RAT MODEL OF LIVER TRANSPLANTATION BETWEEN DA AND PVG BUT DOES NOT REVERSE LONG TERM ESTABLISHED TOLERANCE.

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Spontaneous acceptance of liver allografts uniformly occurs in the DA to PVG rat model despite there being a full MHC class I and II mismatch whilst, in contrast, heart and kidney allografts performed in the same combination undergo typical acute rejection. To assess whether this spontaneous tolerance is the consequence of an induced deficiency of T cell help at the time of first exposure of allo-antigen in the host, we studied the effect of exogeneous recombinant IL2 (rIL2) injections following orthotopic liver transplantation in this rat combination.

Daily injections of rIL2 (3×10^5 U) were given subcutaneously in 6 liver transplant recipients starting on completion of the transplant. This treatment resulted in death of the animals due to liver failure by the 5th or 6th postoperative day. Histological studies of the graft revealed severe acute cellular rejection with a greater than 50 fold increase in serum alanine transferase (ALT). Immunohistology revealed expression of IL2 receptor on graft infiltrating lymphocytes in the treated animals which was not present in untreated animals. The same doses of rIL2 given for 10 days to recipients of syngeneic liver grafts resulted in no change to the standard postoperative course of uniform survival, weight loss and temporary elevation of ALT levels.

The effect of the exogeneous IL2 administration was limited to the immediate postoperative period. Doses up to 10^6 IU had no effect when given 3 to 6 months post transplant in 6 animals with long term liver allograft acceptance.

Our results demonstrate that the initial process which results in the spontaneous induction of tolerance to a liver allograft in the DA to PVG rat model can be abrogated by exogeneous IL2 injection. This strongly suggests that an induced deficiency of T cell help is present at an early stage of allograft recognition in this model of liver allograft acceptance. The mechanism by which this occurs is still unknown.

GENOMIC CHARACTERISATION OF THE HLA CLASS I REGION AND IDENTIFICATION OF NEW GENES

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In addition to containing the genes encoding the major transplantation antigens, recent work has shown that the major histocompatibility complex (MHC) contains many other genes involved in the immune response. The complete characterisation of this 4Mb region is thus of fundamental interest to transplant immunologists. We have developed strategies to identify new genes in the 2Mb class I region, the least well studied part of the complex. HLA class I region cosmids were isolated from a chromosome 6 library by hybridisation with unique class I region probes and overlapping YACs. Positive clones were restriction fingerprinted by double digestion with Sau3A/HindIII, end labelled with ^{32}P and resulting fragments separated on 4% denaturing polyacrylamide gels prior to autoradiography. Autorads were analysed by a suite of computer programs to identify overlapping clones and assemble them into contigs. Using this method 627 cosmids were isolated, of which 252 have been assembled into 56 contigs. We then chose 4 cosmids to investigate the potential for identifying new class I region genes by sample sequencing, a process in which short stretches of random genomic sequence are generated from cosmids and compared with sequences deposited in nucleotide databases. Cosmids were sonnicated to produce fragments of 0.5-1kb, subcloned and sequenced using an automated sequencer. Sequences were compared with nucleotide sequences deposited in the Genbank databases using the BLASTN algorithm. A number of potential new class I region genes were identified, including a cDNA with homology to the *tre* oncogene, the trans-activating factor *SC1* and a member of the interferon inducible *1-8* gene family. These observations suggest that sample sequencing is an efficient method for identifying new MHC genes and extend previous observations that the class I region contains a variety of genes other than those encoding HLA antigens.

A NON-VIRAL VECTOR SYSTEM FOR EFFICIENT GENE TRANSFER TO CORNEAL ENDOTHELIAL CELLS VIA MEMBRANE INTEGRINS

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Genetic manipulation of allografts to suppress their ability to induce rejection is a promising approach for controlling rejection responses. A key to this approach is the development of appropriate DNA vectors. We are studying non-viral DNA vector systems based on synthetic peptides containing an integrin-binding segment for cellular targeting and a polylysine segment for DNA binding. Two such peptides have been tested for their ability to deliver the β-galactosidase reporter gene to the corneal endothelial cells of the rabbit, pig and man. One vector was based on an integrin-binding peptide defined by a phage display library, and the other vector was based on the integrin-binding domain of the venom of the American pit viper *Crotalus molossus molossus*.

Corneas were cultured overnight and then exposed to the DNA/peptide vector under a variety of conditions involving different DNA concentrations, different chloroquine concentrations, pre-incubation or not with chloroquine, different times of exposure, presence of serum and presence of polyanion buffers. Expression of the β-galactosidase gene was tested after 3 further days in culture.

We report that approximately 30% of corneal endothelial cells can be transfected with our optimal protocol. Transfection is dependent on the presence of chloroquine and is inhibited by polyanion buffers such as Hepes. Effects of the treatment on viability of the endothelium was examined by confocal microscopy. Viability of the corneal endothelium was excellent, except if corneas were incubated at 500μM or higher concentrations of chloroquine for prolonged periods (24 hours).

We conclude that synthetic peptides containing an integrin targeting moiety and a DNA binding moiety are promising as simple and highly versatile DNA vectors for corneal transplantation.

RE-TRANSPLANTATION IN THE RAT AORTIC MODEL CAN PREVENT, HALT OR RETARD THE DEVELOPMENT OF CHRONIC REJECTION

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AIMS: The non-immunosuppressed F344 to Lewis renal transplant model supports the importance of immune mechanisms in the development of chronic allograft rejection. The aims of this work were:
(1) to validate the aortic model for the vascular changes of chronic rejection in the F344 to Lewis strain combination
(2) to perform re-transplantation experiments to determine the importance of a continuing allo-immune environment to the progression of chronic rejection.

METHODS: Segments of thoracic aorta were transplanted from F344 rats into the abdominal position of Lewis recipients with similar F344 isograft controls. The aortas were retrieved at 2, 4 and 6 months post-implantation and examined by standard histology, computerised morphometry and immunohistochemistry.
A second series of aortic transplants were performed but these recipients underwent re-operation after 3, 14, 28 or 84 days when a segment of the graft was excised and the remaining graft re-anastomosed. Half of the excised segment was retained for analysis and the other half was transplanted back into an F344 recipient syngeneic with the original donor. Re-transplanted and re-anastomosed segments were both retrieved at 6 months from the date of primary implantation. There were 8 rats in each experimental group. No immunosuppression or antibiotics were used at any stage.

RESULTS: The aortic allografts underwent acute rejection which was maximal at 1 month before declining. Smooth muscle actin positive intimal thickening first developed at 1 month post-transplantation and was progressive. There were no such changes in isograft controls.
Re-transplantation at 3 or 14 days prevented the subsequent development of intimal thickening in aortas but re-anastomosed grafts developed features of chronic rejection similar to simple allografts after 6 months. After 1 month aortic allografts developed only minimal intimal thickening but this progressed in re-transplanted segments but less than in simple allografts after 6 months. Allografts excised at 3 months post-implantation displayed significant intimal thickening which had not progressed when re-excised 3 months after re-transplantation.

CONCLUSIONS: The F344 to Lewis aortic transplant is a simple and reliable model for the vascular features of chronic allograft rejection and supports a major immunological contribution to it's pathogenesis. The development of chronic rejection can be prevented, halted or retarded by altering the immunological environment of the graft. This offers hope for the prevention of chronic rejection in clinical transplantation by immunomodulation.

LINKED EPITOPE SUPPRESSION IN VIVO: IMPLICATIONS FOR PRETRANSPLANT ALLOANTIGEN DELIVERY

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Introduction Allograft survival can be prolonged by administration of donor alloantigens before transplantation in many experimental models. In humans, blood transfusion is the commonest form of alloantigen pretreatment currently being used. However, for recipients of organs from cadaver donors, it is not possible to predict the identity of the organ donor in advance. Therefore, it is highly unlikely that all the alloantigens expressed by a cadaver organ donor will be represented in the alloantigen pretreatment inoculum. We have previously shown that it is not necessary to expose the recipient to the full complement of donor alloantigens to induce long term survival of a subsequent fully allogeneic cardiac allograft. Here, we investigated the in vivo mechanism responsible for this phenomena.

Methods Unresponsiveness to the mouse MHC class I molecule K^b was induced in CBA.Ca ($H2^k$) recipients by administration of bone marrow cells from transgenic CBA mice, CBK ($H2^k + K^b$) and 2 doses of depleting anti-Cd4 monoclonal antibody 28 days before transplantation of fully allogeneic and F_1 vascularised cardiac allografts.

Results All recipient CBA mice pretreated with bone marrow cells expressing the single alloantigen K^b accepted C57BL/10 ($H2^b$) cardiac allografts indefinitely. C57BL/10 allografts express K^b plus other alloantigens, namely D^b, IA^b and C57BL/10 minor histocompatibility (miHC) antigens. The long term survival of the C57BL/10 allografts in CBA recipients previously exposed only to K^b was not due to the lack of immunogenicity of D^b, IA^b and C57BL/10 miHC antigens; all C57BL/10 allografts were rejected by CBK recipients, MST = 7 days (D^b, IA^b and C57BL/10 miHC antigens being the alloantigens barrier here). Inducing unresponsiveness recipients to K^b also allowed cardiac allografts bearing K^b and 3rd party alloantigens, (CBKxBALB/c) F_1 ($H2^{k,d} + K^b$), to be accepted. In contrast, allografts that did not express K^b, such as (CBAxBALB/c) F_1 ($H2^{k,d}$) or BALB/c ($H2^d$) were rejected acutely, MST = 6 and 7 days respectively. Furthermore, when recipients pretreated with CBK bone marrow cells were grafted with a BALB/c heart and a CBK heart simultaneously, the BALB/c hearts were rejected (MST = 10 days) while the CBK hearts were accepted. These data suggest that the 'new' alloantigens have to be expressed by the same organ as K^b. By contrast, in the maintenance phase, ie. after transplantation recipients with long term surviving (CBKxBALB/c) F_1 hearts (>100 days) were found to accept BALB/c hearts indefinitely while 4th party B10.S ($H2^g$) grafts were rejected (MST = 7.5 days) indicating that the allografts bearing K^b could tolerise recipients to other alloantigens expressed by the transplanted heart.

Conclusions These data provide clear evidence for linked epitope suppression in the induction of operational tolerance in vivo. Tolerance induced to a single MHC molecule can act as a 'key'. This key will allow organ grafts expressing other alloantigens as well as this key alloantigen to be accepted. It is conceivable that cadaveric organs could be matched to the HLA antigen(s) to which potential organ graft recipients had been made unresponsive. If linked epitope suppression is a generalised phenomenon, then only one HLA match between the organ donor and the list of tolerised antigens should be sufficient to promote unresponsiveness to the transplant organ.

PENTOXIFYLLINE, NEUTROPHIL DEPLETION AND PRESSURE CONTROL IN LUNG REPERFUSION INJURY.

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Leucocyte depletion, Pentoxifylline and pressure control attenuate reperfusion injury but have not been directly compared. We investigated these interventions in a porcine model of left single lung transplantation. Donor lungs were preserved with modified Euro-Collins for a mean ischaemic time of 18.6 hours. Gas exchange, pulmonary vascular resistance and lung compliance were assessed over a 12 hour period. Sampling cannulae in the pulmonary artery (PA) and vein (PV) enabled measurement of leucocyte sequestration in the lung graft. Partial occlusion of the contralateral PA allowed manipulation of the pulmonary artery pressure in the transplanted lung.

Four groups of 5 animals each were investigated. Group A was reperfused at a mean pulmonary artery pressure (PAP) of 25mmHg, Group B was reperfused at a mean PAP of 45mmHg for 10 minutes before reducing the pressure to the same as Group A. Group C was reperfused at a mean pressure of 25mmHg for 30 minutes with leucocyte deplete blood from an extracorporeal circuit incorporating a Pall BC1-B filter. Group D was reperfused at 25mmHg with the addition of intravenous Pentoxifylline (loading dose 20mg/kg, then 2mg/kg/hr).

Leucocyte sequestration was observed in the first 10 minutes after reperfusion in Groups A and B with maximal sequestration occurring at 2 minutes (Group A -PV count 55.6% of PA count, Group B - 43.9%). Both leucocyte filtration and Pentoxifylline abolished sequestration (PV count 95.26% of PA count). Scheffe analysis of variance using summary measures showed lung compliance was lowest in Group B. Low pressure reperfusion (Group A) significantly improved compliance (p=0.002) as did Pentoxifylline and leucocyte filtration (p<0.0001).

Pulmonary venous oxygen tension in the allograft lung was worst in Group B (p<0.001). Group A was better (p<0.001), but Groups C and D were superior to all other groups (p<0.001). Pulmonary vascular resistance was also greatest in Group B, lower in Group A, but lowest in Groups C and D (P<0.001).

There were no differences in any of the outcome measures between Groups C and D.

We conclude that low pressure reperfusion modulates reperfusion injury. The addition of a period of leucocyte depletion or Pentoxifylline in the recipient confers significant additional benefit but no differences between these interventions in terms of their effectiveness could be demonstrated.

INHIBITION OF MHC CLASS II USING RIBOZYME CONSTRUCTS DIRECTED AT CIITA MRNA

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In man, MHC Class II is expressed on normal, resting vascular endothelial cells whereas in the rodent it is not. Mounting evidence shows that the MHC class II positive human vascular endothelial cells are able to stimulate by direct recognition unprimed allogeneic T cells. Suppression of endothelial cell MHC class II expression might therefore markedly reduce the ability of grafts to induce rejection responses.

The class II specific transactivator CIITA has been shown to be essential for expression of all the MHC class II α and β chain genes. The aim of this study was to determine if MHC class II expression could be reduced using ribozymes against CIITA mRNA. Three ribozymes against CIITA were synthesised and cloned into a eukaryotic expression vector. Two of the ribozymes were shown to be non-functional and were used as transfection controls. Transient as well as stable transfection of both epithelial and endothelial human cell lines with these ribozyme constructs were performed and this was followed by gamma interferon class II induction. The level of expression of class I and class II was analysed by fluorescence activated flow cytometry with mouse monoclonal antibodies at 24, 48 and 72 hours post transfection. The third ribozyme showed greater than 90% reduction of MHC class II in the transfected cells compared to normal and mock transfected controls. RNA studies are now underway.

LAMIVUDINE USE FOR RECURRENT HEPATITIS B POST LIVER TRANSPLANT FOLLOWING FAILURE OF HBIG PROPHYLAXIS.

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The use of prophylactic hepatitis B immune globulin (HBIG) has reduced the incidence of recurrent HBV infection and improved survival in HBsAg positive liver transplant recipients. Patients with evidence of active viral replication at the time of transplantation (HBeAg or HBV DNA positive) have the greatest risk of recurrent infection despite immunoprophylaxis. Effective therapeutic options for recurrent disease are limited. Lamivudine, the (-) enantiomer of 3'-thiacytidine, is an inhibitor of HBV reverse transcriptase and may be effective in the treatment of chronic active hepatitis B, but its role in liver transplantation is undefined.
We report the successful use of lamivudine in 2 patients with recurrent HBV infection following failure of prophylaxis with HBIG which was administered to maintain trough levels > 100 IU in each case.
Patient 1 was HBsAg positive, HBeAg, HBV DNA negative pre-transplant. His HBsAb level became undetectable 5 months post transplant, HBsAg and DNA were negative with normal liver function tests (LFTs). 2 weeks later he became HBsAg and DNA positive (246 pg/ml) with histological evidence of recurrent hepatitis B. His immunosuppression was reduced and lamivudine 100 mg/day was administered. He had a hepatitic flare which gradually resolved. He became HBV DNA negative at week 4 and HBsAg negative at week 8 of treatment and remains so 3 months later.
Patient 2 was HBsAg positive, HBeAg negative, HBV DNA 10.5 pg/ml pre-transplant. 7 months post transplant his HBsAb became undetectable and HBsAg was positive with normal LFTs and HBV DNA 643pg/ml. He subsequently developed granulomatous hepatitis on biopsy, with positive staining for surface and core antigen. Initial treatment with famciclovir 250mg tds for 10 months was unsuccessful with persistence of HBV DNA and deterioration of LFTs and histology. On transfering to lamivudine 100mg/day he became HBV DNA negative at week 8, his LFTs returned to normal and remain so 1 year later.
Recurrent HBV infection post liver transplant despite immunoprophylaxis with HBIG may be treated successfully with lamivudine.

IDENTIFICATION OF THE MECHANISMS INVOLVED IN THE MAINTENANCE OF TOLERANCE FOLLOWING TRANSPLANTATION USING H2K^b-SPECIFIC TCR TRANSGENIC MICE.

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AIM: Intrathymic (IT) injection of alloantigen coincident with transient peripheral immunomodulation can result in the induction of adult tolerance in vivo. In a previous study we used H2K^b-specific TCR transgenic mice (BM3.6) to determine the fate of donor MHC class I specific thymocytes after IT injection of H2K^{b+} splenocytes. The study showed that prolonged IT deletion of H2K^b-specific Cd8$^+$ thymocytes was required for the induction of tolerance following IT injection of H2K^{b+} leukocytes and depletion of peripheral T cells. The aim of the present study was to determine whether IT deletion was sufficient to maintain tolerance or whether peripheral mechanisms were also involved.

METHODS: Cytotoxic T lymphocyte (CTLp) and helper T lymphocyte (HTLp) frequencies of H2^b reactive leukocytes isolated from the spleens of tolerant mice were determined by limiting dilution analysis (LDA). The spleen, lymph node and thymus of tolerant TCR-transgenic mice were analysed by flow cytometry. Events following transplantation of the cardiac allograft were followed by flow cytometric analysis of peripheral blood.

RESULTS: Here we show that tolerance can be induced in adult BM3.6 mice by IT delivery of 5x10^7 H2K^{b+} leukocytes combined with transient peripheral T cell depletion, as demonstrated by the indefinite survival of H2K^{b+} cardiac allografts. LDA of spleen leukocytes from the tolerant TCR transgenic mice 100 days after transplantation revealed a pronounced reduction in both helper and cytotoxic T cell precursor frequencies in the periphery compared to naive controls. This reduction in the frequency of H2K^b reactive cells correlated with the reduced numbers of Cd8$^+$ T cells in the periphery of all of the tolerant mice analysed (88% reduction compared to naive, age-matched BM3.6 mice).
        Analysis of the thymi of tolerant mice revealed that most thymi showed no evidence of IT deletion or donor cell persistence 100 days post-transplant, suggesting that peripheral mechanisms were paramount in maintaining tolerance in this model. This hypothesis was confirmed and extended by analysis of peripheral blood taken at several time-points between pretreatment with IT injection + T cell depletion and transplantation of a H2K^{b+} cardiac allograft. The results showed that following pretreatment with IT injection and T cell depletion (but no cardiac transplant) IT deletion and donor cell persistence was lost between 27-55 days after pretreatment as evidenced by the increase in the percentage of Cd8$^+$ T cells in the peripheral blood (due to thymic export). However, in the presence of the cardiac allograft, despite thymic export of Cd8$^+$ T cells, there was no increase in the percentage of Cd8$^+$ T cells in peripheral blood indicating that most recent thymic emigrant (RTE) Cd8$^+$ T cells were deleted soon after export to the periphery.

CONCLUSIONS: Together these results demonstrate that long term unresponsiveness can be achieved in adult mice by the prolonged IT deletion of alloantigen-specific thymocytes before transplantation. Interestingly, our findings suggest that deletion of thymocytes is only necessary during tolerance induction, i.e. before transplantation, and that the maintenance of tolerance after transplantation occurs mainly by the deletion of RTE Cd8$^+$ T cells although other non-deletional mechanisms are also operational.

HEPARIN INHIBITS PRODUCTION OF MCP-1 BY ENDOTHELIUM FOLLOWING TREATMENT WITH IFN-γ: CONSEQUENCES FOR LOCAL T CELL ACTIVATION

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Interferon-γ (IFN-γ) plays a central role in the inflammatory response. This cytokine augments the immunogenicity of endothelial cells by upregulation of class I MHC antigens and adhesion molecules, and by induction of class II MHC antigens and chemokines such as monocyte chemotactic peptide-1 (MCP-1).
        Previous studies by this group have demonstrated that heparin, a heavily sulphated glycosaminoglycan, inhibits the induction of class II MHC antigens and upregulation of ICAM-1 expression that result from treatment of endothelial cells with IFN-γ in vitro.
        In the present study, the effect of heparin on the ability of IFN-γ to induce production of MCP-1 by endothelium was investigated. As a member of the C-C chemokine subfamily, MCP-1 is a potent chemoattractant for monocytes and T cells. This chemokine also induces important adhesive interactions during T cell migration into tissue.
        It was shown that treatment for 24 hours with 100U/ml IFN-γ significantly increased the expression of MCP-1 by cultured human lung microvascular endothelial cells. The presence of 250μg/ml heparin during stimulation with IFN-γ completely inhibited this increase in MCP-1 production.
        Further experiments were performed, in which the effect of MCP-1 on leukocyte activation was investigated. Freshly isolated peripheral blood mononuclear cells were treated with 15ng/ml MCP-1 for increasing lengths of time. Cell lysates were then prepared and analysed by SDS-PAGE and subsequent western blotting. Stimulation with MCP-1 was found to significantly increase phosphorylation of phosphoinositide 3-kinase (PI 3-kinase), with optimal activation occurring after treatment for 10-20 minutes. The amount of PI 3-kinase protein present was unaffected by MCP-1 treatment. Activation of PI 3-kinase commonly occurs following triggering of T cell surface molecules that modulate the early events in T cell responses.
        By inhibiting endothelial expression of MCP-1 in the presence of IFN-γ, heparin reduces the chemotactic stimulus for attraction of leukocytes towards these cells. Furthermore, inhibition of MCP-1 production diminishes the potential for T cell PI 3-kinase activation. We have previously shown that heparin reduces the potential immunogenicity of endothelium in the presence of IFN-γ, by preventing increased expression of MHC antigens and adhesion molecules. Here we suggest that by reducing endothelial output of MCP-1 following treatment with IFN-γ, heparin may also inhibit T cell recruitment and extravasation, and may remove the stimulus for triggering of T cell signalling pathways. These observations may go some way to explaining reports that heparin has immunomodulatory properties, both in vivo and in vitro.

CROSSREACTIVITY BETWEEN HLA SPECIFIC ANTIBODIES AND SLA. SHOULD WE PERFORM A PIG LYMPHOCYTE CROSSMATCH FOR SENSITIZED PATIENTS IN FUTURE XENOTRANSPLANT PROGRAMMES?

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A prerequisite for heart and kidney transplantation is to ensure the absence of immunological sensitization to the donor. Failure to do so almost invariably results in hyperacute or acute rejection, mediated by preformed antibodies or primed T cells. Pretransplant recipient sensitization is determined by screening for HLA specific antibodies and performing a donor lymphocyte crossmatch.

HLA antibodies (Ab's) are often crossreactive, demonstrating reactivity against multiple HLA specificities. This is due to shared antigenic epitopes and sequence homology between HLA alleles. SLA also shares remarkable sequence homology with HLA and may therefore be recognised by HLA specific Ab's. Such donor specific sensitization to SLA could indicate immunological priming to potential xenogeneic pig donors, analogous to a positive HLA crossmatch in human donor transplants. If true, this may indicate a requirement to select an "SLA" crossmatch negative pig donor when planning future stategies for xenotransplantation.

Methods: Natural xenoreactive anti-pig antibodies were absorbed from 12 sensitized and 8 non-sensitized patient sera using pig red blood cells (P-RBC). Absorption of IgM and IgG reactivity towards Gal-α-1:3-Gal was confirmed by ELISA. Absorbed and unabsorbed sera were tested for IgG antibody binding to human and pig lymphocytes by flow cytometry (FC). Reactivity was then correlated with the presence or absence of HLA specific Ab's.

Crossreactivity of HLA Ab's with SLA was investigated by further absorption of sera using pooled human platelets. Subsequent IgG binding to pig lymphocytes following removal of HLA specific Ab's was again determined by FC.

Results: Absorption of natural xenoreactive antibodies did not significantly alter the IgG anti-HLA reactivity. Following P-RBC absorption, only those sera with IgG HLA specific Ab's had a positive crossmatch with pig lymphocytes. Absorption of HLA class I specific Ab's also removed the IgG binding to pig lymphocytes, suggesting crossreactivity with SLA.

Conclusion: The results indicate that some HLA specific Ab's crossreact with SLA expressed on pig lymphocytes, giving a positive "SLA" crossmatch. These data suggest that it may therefore be necessary to select an "SLA" crossmatch negative pig donor when considering xenotransplantation in recipients sensitized to HLA.

INFLUENCE OF COMPLEMENT INHIBITION ON THE B CELL RESPONSE IN EXPERIMENTAL ALLOGRAFT REJECTION

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The induction of an alloantibody response against organ transplants is a strong determinant of graft loss. Since complement facilitates the induction of antibody responses to T-cell dependent antigens, we asked if complement was important in the induction of the alloantibody response.

We transplanted rat kidney allografts into fully MHC mismatched recipients in which complement activation was inhibited by daily injection of soluble recombinant human complement receptor type 1 (sCR1). This regimen achieved >90% systemic C inhibition and markedly reduced the tissue deposition of C3 and C5b-9 when compared to saline treated controls (n=21 rats/group).

Subset analysis of spleen cells expressing CD25 in control v. treated rats at day 5 showed: *CD5 pan T cells*, 79.1% $\pm$ 4.1% v. 37.9% $\pm$ 7.8%; *CD4*, 71.7% $\pm$ 8.08% v. 47.4% $\pm$ 3.6%; *CD8*, 55.7% $\pm$ 10.5% v. 46.4% $\pm$ 10.2%; and *B cells*, 71.5% $\pm$ 6.6% v. 53.2% $\pm$ 11.9%. This reduction in the proportion of activated T and B cells coincided with a reduction in plasma antibody binding activity against donor MHC class I specific target cells ($4.4 \times 10^3 \pm 0.3 \times 10^3$ v. $75.0 \times 10^3 \pm 30.4 \times 10^3$ Molecular Equivalence of Soluble Fluorochrome, p<0.001), and against cells sharing minor histocompatibility antigens with the donor. Antibody and complement deposition was reduced in the grafts of sCR1 treated animals together a reduction in vascular injury and a prolongation of graft survival. Loss of efficacy of sCR1 after 7 days of treatment was associated with a rising titre of antibodies against sCR1 and eventual graft loss.

These results suggest the synthesis of antibody against donor MHC Class I and other allospecific determinants on rat kidney is complement dependent. Complement inhibition therefore might provide a useful adjunct therapy in the prevention of allospecific antibody formation in humans.

Anti-CD4 and anti-CD2 monoclonal antibodies synergise to induce tolerance to rat cardiac allografts in the high responder DA to Lewis strain combination.

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Recent evidence suggests that the use of combinations of mAbs directed against different T cell surface molecules may be particularly effective in promoting allograft survival. In this study we targeted the T cell molecules CD4 and CD2 and examined the ability of mAbs against these molecules to induce tolerance to cardiac allografts in the high responder DA (RT1^a) to Lewis (RT1^l) rat strain combination-a model notoriously resistant to tolerance induction.

In the reciprocal low responder Lewis to DA combination, mAbs directed against CD4 (OX38 16mg/kg) and CD2 (OX34 10mg/kg), were each able to induce long term graft survival when administered to graft recipients preoperatively (MST>100 days, n=8/group). However in the high responder rat strain combination (DA to Lewis) neither anti-CD4 nor anti-CD2 therapy alone led to long term graft survival (anti-CD4 MST=18 days, n=5; anti-CD2 MST=19days, n=6) and all animals ultimately rejected their grafts. However when anti-CD2 and anti-CD4 mAb were given in combination, using the treatment schedule outlined above, they had a strongly synergistic effect on allograft survival (MST=49 days, n=17) and eight of seventeen recipients (47%) retained their heart grafts indefinitely. Permanently engrafted animals (> 100 days) after anti-CD4 plus anti-CD2 mAb treatment showed donor specific tolerance, accepting a second DA cardiac allograft (n=4) but rejecting a third party PVG graft (n=3). The synergy between anti-CD2 and anti-CD4 was not seen when anti-CD2 was given in combination with the mAb R73 directed against the αβTCR, where cardiac allograft survival in the high responder DA to Lewis strain combination was no greater than when anti-CD2 is used alone (MST=15 days, n=7).

T cells from long term tolerant animals following anti-CD4 plus anti-CD2 therapy showed normal *in vitro* proliferation to both donor (DA) and third party (PVG) stimulator cells. However production of IL2 by lymphocytes from tolerant animals in response to donor stimulators was markedly impaired. Tolerant animals showed reduced levels of cytotoxic T cells and cytotoxic alloantibody levels were undetectable.

These results demonstrate that mAbs to CD4 and CD2 synergise to induce transplant tolerance in a stringent model of allograft rejection and suggest that tolerance in this model may be asssociated with a defect in the IL2 pathway.

EVIDENCE FOR IMMUNOREGULATION BY CD4 T CELLS IN THE INDUCTION OF SPECIFIC IMMUNOLOGICAL UNRESPONSIVENESS TO ALLOANTIGENS IN VIVO

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Donor-specific unresponsiveness to allogeneic heart allografts in mice can be induced by pretreatment (28 days) with a donor-specific blood transfusion (DST) under the cover of a depleting or non-depleting anti-Cd4 antibody. We studied whether tolerance, in this model, is due to the presence of Cd4$^+$ T cells which regulate immune responses towards the graft. For the analysis of the allospecific HTLp and CTLp frequencies limiting dilution was performed. C3H/He mice received 50µg of purified YTA 3.1.2 (anti-Cd4) i.v. 28 days and 27 days before transplantation. The second dose of the mAb was combined with 250 µl i.v. transfusion of heparinized C57BL/10 whole blood. For flow cytometric purification of Cd4$^+$ splenocytes two different protocols were used: sorting based on a negative population and on a Cd4$^+$ population.

Analysis of splenocytes from mice, at the time of transplantation, i.e. 28 days after treatment, showed that both donor-specific and third-party reactive IL-2 producing T helper precursor (HTLp) frequencies were reduced both after treatment with anti-Cd4 alone as well as after combined treatment with anti-Cd4 and DST. In contrast, cytotoxic T lymphocyte precursor (CTLp) frequencies were only reduced against donor specific targets after the combined anti-Cd4/DST treatment; responses to third-party alloantigens were comparable with those found in naive mice. After anti-Cd4 treatment alone the donor-specific and third-party specific CTLp frequencies were unchanged.

Next Cd4$^+$ T cells were purified from the spleen 28 days after the different treatments by cell sorting. When Cd4$^+$ T cells were purified from mice treated with the anti-Cd4 treatment alone and added to naive splenocytes in vitro, the donor-specific- and third-party-specific CTLp frequencies remained unchanged. Interestingly, addition of Cd4$^+$ T cells derived from mice after the tolerogenic antibody/DST treatment showed a selective reduction in the donor-specific CTLp response, whereas the response of the naive cells to third-party alloantigens remained unchanged. Moreover, the data for donor-specific responses showed a bimodal distribution, indicating the presence of regulatory Cd4$^+$ T cells.

The existence of such donor-specific regulatory Cd4$^+$ T cells was only detectable in vitro after the combined with donor-specific transfusion and anti-Cd4 monoclonal antibody therapy; not after either pretreatment only. Moreover, in preliminary experiments using an in vivo adoptive transfer protocol designed to mimic the in vitro assay, adoptive transfer of Cd4$^+$ splenocytes purified from mice 28 days after combined treatment with DST (C57BL/10) and anti-Cd4 mAb resulted in prolongation of C57BL/10 cardiac allografts. Cd4$^+$ cells purified from the spleens of mice treated with anti-Cd4 mAb 28 days earlier has no effect on graft survival.

These data suggest a clear role for alloantigen- specific Cd4$^+$ T cell regulation in the induction of transplantation tolerance following combined treatment with donor antigen (DST) and anti-Cd4 antibodies. The characterization of these regulatory cells may shed more light on the application of protocols which aim to induce donor-specific immunological tolerance in vivo.

THE PROGRESSION OF CHANGES IN ARTERIES FOLLOWING COLD STORAGE PRESERVATION IN UW AND COLLINS SOLUTION IN A SYNGENEIC AORTIC TRANSPLANT MODEL

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Accelerated graft arteriosclerosis is the leading cause of kidney and heart transplant failures. The pathogenesis is uncertain and probably multifactorial however there is increasing evidence pointing to cold ischaemia time being a critical factor.

It was the aim of this study to document changes over time associated with cold storage preservation of a rat aortic segment and follow the changes after syngeneic transplantation, to determine if preservation-reperfusion injury resulted in long-term injury to transplanted vessels.

The infra-renal aortae of 18 Wistar rats were stored in either UW or Collins (C) solution at 4°C for 24 hours, then processed for electron microscopy. A control group were not stored. Aortic transplants were performed in 168 Lewis rats. Donor aortae were either transplanted immediately after flushing or after 24 hours of cold storage. Grafts were harvested after 1 hour, 1 day, 1 week and 1, 3, 6 and 12 months.

Preservation in UW solution produced minimal ultrastructural evidence of injury, whereas C solution produced a severe injury consisting of gross cytoplasmic and mitochondrial swelling with peripheral clumping of the nuclear chromatin. The endothelial lining remained intact in both groups. Reperfusion resulted in damage to both grafts, however the total amount of damage to the Collins stored graft was worse due to pre-existing preservation injury. The combined preservation-reperfusion injury consisted of endothelial denudation, leucocyte and platelet adherence, medial smooth muscle cell swelling and the formation of large intra- and extra-cytoplasmic vacuoles. Re-endothelialisation occurred by 1 day and 1 week in UW and C groups respectively. Leucocytes remained in the intima and returned to control values at 1 week. The medial swelling and vacuolisation recovered by 1 day, however at 1 week (C) and 1 month (UW) there were patchy areas of smooth muscle cell death, replaced by pools of proteoglycan. These areas worsened with time and were still present at 1 year. They did not occur in control grafts. There was no neointimal hyperplasia, however proteoglycan accumulated in the intima in all groups with time.

These findings show that preservation-reperfusion injury occurs in transplanted vessels and results in long-term structural alterations in the vessel wall in both well and poorly preserved grafts. This will alter vessel wall homeostasis and thus could be a factor in the development of accelerated graft arteriosclerosis.

GENOTYPIC VARIATION IN THE TGFβ1 GENE: ITS ASSOCIATION WITH TGFβ SYNTHESIS, FIBROTIC LUNG DISEASES AND GRAFT FIBROSIS AFTER LUNG TRANSPLANTATION

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Transforming growth factor beta (TGFβ) promotes the deposition of matrix in wound healing and scar formation. It is a prime candidate for the development of fibrosis in transplanted tissues. We have therefore studied the regulation of TGFβ production in transplant patients in order to understand the process of graft damage, particularly in the transplanted lung.

We have identified allelic variants of the TGFβ1 gene in the promoter, the protein leader sequence and in the encoding region for the mature cytokine. This presentation will focus on leader sequence variants which are associated with the ability of the individual to produce high or low amounts of TGFβ1. In the general population 81% are homozygous for the high producer allele, and produce the greatest amount of TGFβ1 in vitro. Heterozygous (high/low) genotypic people account for 18% of the normal population and produce approximately half the amount of TGFβ1 in vitro compared with high producers. Individuals homozygous for the low producer allele are rare (1%). In patients requiring lung transplantation for a fibrotic lung condition there is a very significant increase in those having the high TGFβ1 genotype. For example, 100% of patients transplanted for cystic fibrosis are homozygous high TGFβ1 producers ($p<0.001$). When all lung transplant patients are included, an analysis of biopsy specimens shows that the presence of TGFβ1 in the graft and the development of fibrosis are both significantly associated with the high TGFβ1 genotype ($p<0.002$). After lung transplantation, in terms of patient survival, inheritance of the low producer TGFβ1 allele is protective ($p=0.033$).

We conclude that the production of TGFβ is under genetic control, and that this in turn influences the development of lung fibrosis and the outcome after lung transplantation. We think that TGFβ1 genotype is of prognostic significance. The implications will be discussed.

DIFFERENTIAL EFFECTS OF CYCLOSPORIN A AND TACROLIMUS ON THE PRODUCTION OF TGF-β: IMPLICATIONS FOR THE DEVELOPMENT OF OBLITERATIVE BRONCHIOLITIS AFTER LUNG TRANSPLANTATION

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Cyclosporin A (CsA) and tacrolimus (FK506) both prevent T cell proliferation by inhibition of calcineurin, a calcium dependent phosphatase which is vital for assembly of a range of transcription factors including NFAT. The effect of these agents on parenchymal cells within an allograft is less well understood. In this study we compared the effect of these two drugs on production of the fibrogenic growth factor TGF-β by primary human lung small airway epithelial cells (SAEC).

SAEC were propagated in serum-free medium and showed a doubling time of around 30 hours. Addition of a polyclonal anti-TGF-β antibody at 80μg/ml increased the rate of proliferation (p<0.01) by blocking the autocrine effect of TGF-β. Titration of exogenous TGF-β1 into the cultures indicated that the ID_{50} for SAEC proliferation was 600pg/ml. When CsA was added at 2μg/ml (a concentration which inhibited lymphoproliferation in mixed leukocyte cultures), SAEC proliferation was inhibited by 60% (p<0.01). This inhibitory effect was antagonised (50%; p<0.01) by addition of the anti-TGF-β antibody at 80μg/ml. ELISA demonstrated a significant increase in the production of TGF-β following treatment of SAEC with CsA. Addition of immunosuppressive doses (2ng/ml) of tacrolimus to SAEC cultures had no effect on proliferation and did not augment production of TGF-β.

The development of obliterative bronchiolitis is a common cause for failure of lung allografts. However, it has been reported that immunosuppression with tacrolimus is associated with a lower incidence of this fibrotic disease than is observed for patients receiving CsA. The results of the current study provide a basis for understanding these differences in terms of differential regulation of the production of TGF-β, and suggest a mechanism by which some immunosuppressive drugs might promote fibrosis and chronic graft loss.

HLA ANTIGEN MATCHING FOR FIRST CADAVER KIDNEY TRANSPLANTATION AVOIDS POST-TRANSPLANT SENSITISATION.

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Recipients of cadaver kidneys are exposed to donor HLA antigens and so are at risk of becoming sensitised by forming antibodies to those antigens. Repeat renal transplantation was performed in the UK at a rate of 17.1% between 1984 and 1993 and in our centre 14.9% of all transplants done in that period were re-grafts. Two strategies have been reported to facilitate transplantation of highly sensitised patients; the definition of acceptable HLA mismatches or removal of circulating antibody by combined plasmapheresis and cytotoxic drugs. Neither of these strategies have proved to be particularly successful nor widely accepted. We suggest a third more realistic approach is to prevent sensitisation in the first instance by minimising the donor / recipient HLA mismatch.

In our centre, from 1989 to 1995 we adopted a minimal HLA mismatch approach for the 761 first cadaver kidney transplants (90.8% adult recipients) that were preformed. Their graft survival at one and five years was 86.5% and 76.9% respectively. Serum samples for post-transplant panel reactive antibody (PRA) screening were available for 64 of 88 adults whose transplants failed. In sensitised patients with >60% PRA, 10/12 had HLA-A mismatches (RR 2.1) and 11/12 had HLA-B mismatches (RR 4.1). The proportion of patients with HLA-DR mismatches was the same in sensitised and non-sensitised groups reflecting our allocation policy. Transplants failed in 21 "Beneficially" matched recipients but none had post transplant PRA > 20%. Of the 34 patients with PRA > 10%, 23 had antibodies with specificity for HLA antigens often those mismatched with the donor kidney. Rejection episodes and the time waited for a repeat transplant were both increased in sensitised patients (see table). There were only 6 recipients (0.9%) who became highly sensitised (>80% PRA) and 5 of these each had 3 HLA-A, -B, -DR mismatches.

Post-tpx % PRA	Number of patients.	PERCENT OF PATIENTS	
		Rejection episodes	Waited >12 months for repeat tpx
0	18	27.8	28.6
<11	12	36.4	50.0
11-60	22	50.0	56.3
>60	12	72.7	88.9

We show that sensitisation to HLA alloantigens in failed kidney transplantation is directly related to the degree of HLA mismatch at time of first transplant. By minimising such mismatches the degree of sensitisation can be controlled making successful retransplantation a realistic possibility.

SEROLOGICAL CYTOMEGALOVIRUS SURVEILLANCE IN CARDIOPULMONARY TRANSPLANTION IS NOT INDICATED

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OBJECTIVE: Cytomegalovirus (CMV) infection in cardiopulmonary transplant patients increases early morbidity and has been implicated in allograft vasculopathy. We investigated the value of monitoring anti-CMV IgM and IgG seroconversion in the first 6 months after transplantation to predict patients at risk and enable early CMV treatment.

METHODS: The IgM/IgG status of 86 patients who underwent cardiopulmonary transplantation over a four year period was studied. CMV IgG status was ascertained prior to transplantation and the IgG/IgM titres measured serially after transplantation.

RESULTS: Follow up to 6 months is complete for all patients (mean age 38, range 17-61). There was 1 death from CMV pneumonitis in 20 episodes of CMV infection requiring treatment in 15 (17.4%) patients, 4 of whom were high risk mismatches (donor IgG +ve and recipient IgG -ve). Of the 20 episodes of symptomatic CMV infection, 3 were preceeded by IgM seroconversion within the 14 days prior to the development of symptoms. On 7 occasions IgM seroconversion was first detected while investigating an established illness. There were 7 episodes of CMV infection where IgM had become positive more than 14 days prior to the development of symptoms (range 21-121 days), and a further two episodes where IgM remained negative in IgG -ve patients despite a biopsy or culture proven CMV infection. IgM seroconversion occurred on 42 occasions in asymptomatic patients.

CONCLUSIONS: The use of serological surveillance to predict CMV disease in cardiopulmonary transplant patients is limited and attention should be directed towards establishing specific diagnostic markers of infection.

THE ATTITUDE OF THE STAFF OF ONE ACCIDENT AND EMERGENCY DEPARTMENT TOWARDS NON-HEART BEATING KIDNEY DONATION

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With rising kidney transplant waiting lists and declining numbers of brain stem dead (BSD) organ donors in the UK some transplant units are showing interest in the revival of non-heart beating (NHB) donation. NHB donors are found in (i) the Intensive Care Unit or Ward environment where relatives consent can often be gained prior to death and (ii) the Accident and Emergency Department (A & E) where this is often not the case. This paper examines the attitude of the nursing and medical staff of one A & E towards NHB donation using a double balloon triple lumen catheter in situ preservation technique.

Questionnaires were sent to 18 day duty A & E nurses listed on a single weeks off duty, six middle and senior grade A & E doctors and eight doctors on the Resident Medical Officer (RMO) rota who attended the A & E to supervise cardio-pulmonary resuscitation.

The questionnaire requested a response to:-

Attitudes towards:- brain stem dead donation, kidney transplantation, NHB donation, issues of consent, the role of A & E staff in donation, the requirement to assist with NHB donation.

Knowledge of:- waiting lists for kidney transplantation.

Experience in:- making the request for organ or tissue donation participation in NHB donation.

Results - None of the staff were opposed to kidney transplantation, 71% felt it very worthwhile. Knowledge of kidney transplant waiting lists was poor with only 14% and 10% knowing the UK and local figures respectively.

Only one felt that the responsibility for organ procurement was singularly down to the Transplant Team. 86% supported the programme of organ donation from BSD organ donors and 95% felt the NHB donation procedure was acceptable. Opinions were divided about aortic cannulation prior to relative's consent or carrying of a donor card. 67% felt the most appropriate person to make the request for donation to be the Transplant Coordinator and 48% had in the past made a request for organ or tissue donation. 71% were freely willing to assist with NHB donation when required and 38% had assisted in the past.

Conclusion - The staff in this A & E are generally in favour and willing to assist with organ procurement and donation. They feel kidney transplantation to be worthwhile. Despite accepting the NHB donation procedure, there is some difference of opinion surrounding cannulation and perfusion prior to relatives consent or the present of a donor card.

This survey has helped in the preparation of a teaching programme for staff as a NHB donation programme was implemented.

RENAL CONSEQUENCES OF CHANGING CYCLOSPORIN FORMULATION IN THORACIC TRANSPLANT PATIENTS .

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Objectives. The nephrotoxicity of Cyclosporin A is well documented. A new formulation with improved absorption (*Neoral*) has been introduced to overcome the unpredictable absorption rates of the standard preparation (*Sandimmun*). We have investigated the effects of conversion on renal function in 38 cardiac/lung transplant recipients, previously stabilised on Sandimmun for at least 3 months.

Methods. Sandimmun and Neoral were exchanged on a milligram per milligram basis, with subsequent dosage adjustment according to whole blood trough levels. Blood levels were evaluated using a well-validated, specific monoclonal immunoassay (*EMIT*). Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated pre-transfer and four weeks post-transfer using standard ^{51}Cr-EDTA and ^{123}Iodohippuran probes.

Results. The transfer led to an insignificant reduction in GFR and ERPF. Cyclosporin dosage was decreased by less than 2%, but Cyclosporin trough levels 4 weeks post-transfer rose by 16%.

	Pre-transfer	4 wks post-transfer
GFR (mean)	64.7 ± 26.4	62.3 ± 25
ERPF (mean)	387 ± 183	360 ± 152
CyA (mean)	188 ± 58.8	203 ± 63.9

Seven patients suffered minor symptoms, e.g. headaches or tremor, related to increased trough levels.

Conclusion. We conclude that despite improved absorption, conversion from Sandimmun to Neoral does not affect renal function or blood flow in the stable cardiac/lung transplant population.

Travel to Nottingham

From M1 motorway

Leave motorway at **Junction 25** to join **A52** to **Nottingham**. Turn right at **The Priory** roundabout (about 4 miles from M1), then left at next roundabout to enter the University's **West Entrance**. The **East Midlands Conference Centre** and **Rutland Hall** are signposted from there (see back cover). There is ample car parking.

By rail

Frequent train services run to Nottingham from London St Pancras and other places around the country. It will be easiest to get a taxi from the station, although the 38 bus runs from the Old Market Square, a 10 minute walk from the station.

Access to the University of Nottingham