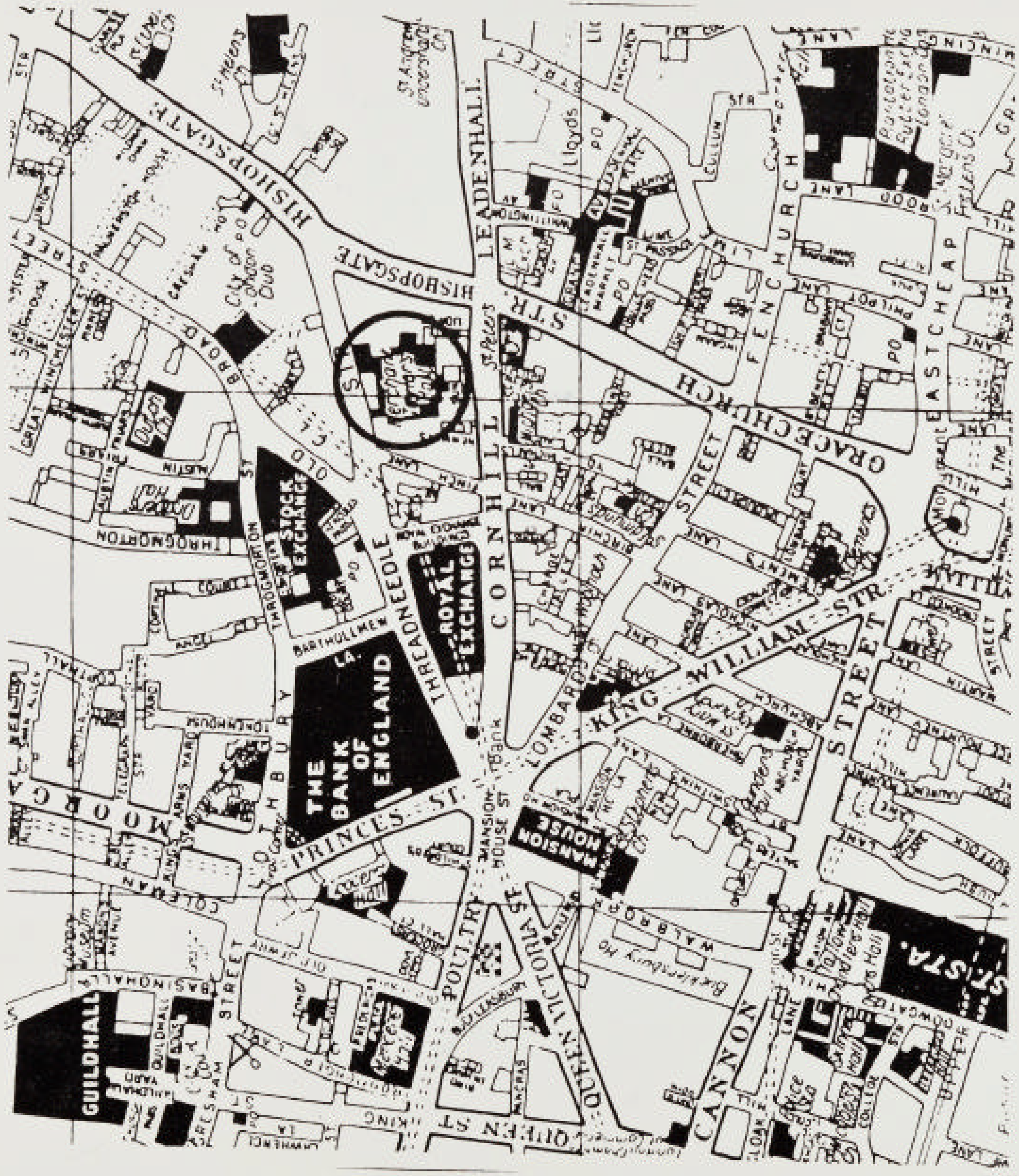
THE BRITISH TRANSPLANTATION SOCIETY

25th ANNIVERSARY MEETING

LONDON

14th and 15th October 1997

The Royal Society
6 Carlton House Terrace, London, SW1Y 5AG



BISHOPSGATE
LEADENHALL
GRACECHURCH
FENCHURCH
EASTCHEAP
BROAD
OLD
THROGMORTON
STOCK EXCHANGE
THREADNEEDLE
ROYAL EXCHANGE
CORNHILL
THE BANK OF ENGLAND
BARTHOLMEW
LOTHBURY
PRINCES ST
MOORGATE
COLEMAN
BASINGHALL
GUILDHALL
GRESHAM
POULTRY
QUEEN VICTORIA ST
MANSION HOUSE
LOMBARD
KING WILLIAM STR
WALBROOK
CANNON
ST. STA.
QUEEN ST
KING
STREET

KIDNEY DAMAGE AT RETRIEVAL - WHO CAN WE BLAME?

John L R Forsythe, F M Seeney, A Bakran, for the Kidney Advisory Group, UKTSSA

Introduction It is a common perception that kidney damage at retrieval is an increasing problem which is not always being reported. Many statements have been made about this:-

- damage is much more frequent when the liver team performs the retrieval
- damaged kidneys are often sent away
- kidney damage is more common in the older donor

The best available data was examined to investigate the truth of such statements and to examine the survival of kidneys which have been damaged at the retrieval operation.

Methods Data were examined on all kidneys donated over a 5 year period in the United Kingdom. The reports from the donor centre and the recipient centre were compared and the outcome of transplantation noted. Further examination of records revealed whether or not the retrieval had been performed by a liver team or a renal team. Figures for damage in those kidneys which had been held for local use were compared with kidneys sent to outside centres. Other factors which were felt to have a possible effect on the incidence of damage were examined, such as donor age and the experience of the donor transplant centre. Finally, Cox regression models were fitted to both the one year and 3 year transplant survival data in those kidneys which were transplanted despite recorded damage.

Results In the period from 1992 to 1996, 9,014 kidneys were retrieved. 359 of these kidneys were not transplanted - on 78 occasions this was because of organ damage. 1,726 kidneys were reported as damaged. Of the 1,630 transplanted and damaged kidneys, only 270 (16.5%) were reported as damaged by both the donor and the recipient centres. 95 out of 96 unused kidneys reported as damaged were noted by the donor centre.

Of the 4,543 kidney donors, 3,108 also donated their liver. In the reduced data set where all information was available, the renal team caused damage to the kidneys in 26% of cases in a kidney only donor, and 21% in a multi-organ donor. The comparable liver team figure is 16.5%.

Approximately 14% of all kidneys kept locally were damaged. 30% of exchanged kidneys were damaged. 41 centres were involved in retrieving kidneys. Most notable were 5 centres where nearly all kidneys reported as damaged were sent away.

There is a clear indication that the proportion of damaged kidneys increases with increasing donor age for donors aged 40 years or more. 62% of the damaged organs were donated by donors in this category. Finally, the survival of damaged organs which were transplanted differed little from those which were not damaged, and difference was not statistically significant at one year or 3 years after the transplant procedure.

Conclusion There is significant under-reporting of kidney damage at organ retrieval. Damage is more likely to occur in the elderly donor where only kidneys are retrieved by a renal team. Such damaged kidneys are more likely to be exchanged than kept locally. It is worth working hard to preserve such damaged kidneys as graft survival figures are good.

VISUALISATION OF T-CELL MEDIATED CARDIAC ALLOGRAFT REJECTION IN VIVO

VAN MAURIK, A., JONES, N.D., MORRIS, P.J. and WOOD, K.J.

Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, UK.

The aim of this investigation was to study, *in vivo*, the response of alloantigen ($H2K^b$)-specific T cells to a $H2^{b+}$ cardiac allograft. The model we developed consisted of adoptive transfer of purified $CD8^+$ T cells from $H2K^b$-specific TCR transgenic mice (BM3.6; $H2^k$) into thymectomised, T cell depleted CBA/Ca ($H2^k$) mice. Before injection, the purified $CD8^+$ T cells were labelled, *in vitro*, with a fluorescein based dye, CFSE. It has been shown that the intracellular fluorescent label is divided equally between daughter cells upon cell division. One day after cell transfer, mice were transplanted with either a $H2K^{b+}$ or syngeneic ($H2^k$) cardiac allograft the day after transfer. Based on the sequential halving of the CFSE at each division step, the proliferative behaviour and the immunophenotype of K^b specific T cells with a defined division history were analysed by 4-colour FACS.

We found in short-term analysis that transgenic-$TCR^+/CD8^+$ T cells showed a distinct proliferative response, increase in T cell blasts, upregulation of CD44 and down regulation of CD62L (L-selectin) when a $H2K^{b+}$ heart was transplanted but not when a syngeneic graft was transplanted. These cells also showed an increase in Th1 associated cytokine production upon *in vitro* restimulation. Furthermore, 70 days after transplant, the transgenic-$TCR^+/CD8^+$ T cells were easily detectable, expressed a phenotype consistent with that of memory cells ($CD45RB^{lo}$) and showed a Th1 like cytokine pattern *in vitro* (IFNγ and TNFα production)

These data show that the activation of alloantigen-specific T cells can be followed *in vivo* in short term and long term experiments. This experimental approach provides a unique opportunity to study the mechanisms by which T cells respond to cardiac allografts in vivo.

CONVERSION FROM CYCLOSPORIN (NEORAL®) TO TACROLIMUS (PROGRAF ®) IN RENAL RECIPIENTS WITH FAILING GRAFTS DUE TO CHRONIC GRAFT NEPHROPATHY.

G Morris-Stiff, K Baboolal,V Balaji, K Ostrowski, A Ahmed, R Moore, C Darby, R Lord, WA Jurewicz.

Department of Transplant Surgery, University Hospital of Wales NHS Trust, Cardiff.

Introduction Chronic graft nephropathy (CGN) manifests as a progressive decline in graft function due to immunological (chronic rejection) and many non-immunological factors (e.g. nephrotoxicity). In such a scenario the maintenance therapy is clearly failing to maintain the graft. The introduction of tacrolimus has given an opportunity to investigate whether a change of medication may alter the course of CGN.

Methods From a cohort of renal graft recipients attending our department between February and April 1996 fourteen patients with CGN were identified and were converted from Neoral to Prograf at a dose of 0.15 mg/kg/day. Pre- and post-conversion renal function was determined by a calculated GFR using the Cockroft-Gould formula. There were a total of 1662 GFR estimations performed in these 14 patients. Statistical analysis was carried out using a regression technique with multilevel modelling.

Results There were 8 females and 6 males with a median age of 40 years. The median time from transplantation was 1750 days (range: 262-4226), the median serum creatinine 438Tmol/l (range 262-677) and the median GFR 1.4ml/min. All patients were extensively investigated and underwent a total of 43 ultrasound scans, 28 graft biopsies, 1123 (median 80/patient) serum creatinine measurements and 191 (median 10/patient) adjustments of Neoral dose in the 12-24 months prior to conversion. Two patterns of response have emerged during the 15 months follow up: (i) continuing deterioration of renal function with no deviation from the projected trend of GFR (n=9). Seven patients returned to dialysis between 6-42 weeks post-conversion, one died of a MI and only one patient remains dialysis independent; (ii) unequivocal change in the GFR trend line equation with reduced rate of deterioration in 1 patient and actual sustained improvement of GFR (reversal of the trend) in 4 patients. Serum albumin also improved from 35.4 to 41.0 g/l ($p<0.04$). There was no difference in the Neoral levels between groups at the time of conversion (92ng/ml vs.100ng/ml) but the tacrolimus level was higher the benefit group at 1 months post conversion (9.6ng/ml vs.13.3ng/ml)

Median GFR Pre-and Post-conversion

Time	*No benefit group(n=9)*	*Benefit group(n=5)*	*All patients(n=14)*
-6months	23.7ml/min	39.8ml/min	29.5ml/min
-1month	19.8ml/min	31.0ml/min	24.5ml/min
Conversion	18.7ml/min	26.2ml/min	21.4ml/min
+1month	19.8ml/min	33.2ml/min	24.6ml/min
End of study	13.5ml/min	30.2ml/min	19.5ml/min

All 5 patients that benefited from conversion to tacrolimus exceeded their estimated time of return to dialysis (projected GFR<10ml/min) by a median of 41 weeks (range: 29-52) and their grafts continue to function.

Conclusions Five out of 14 patients (36%) clearly benefited from replacing Neoral with Prograf. If these findings are confirmed in a prospective randomized trial it will be the first instance of effective treatment for chronic graft nephropathy.

ANALYSIS OF CLASS I MHC EPITOPES WHICH PROVIDE COGNATE T CELL HELP FOR ANTIBODY-MEDIATED GRAFT REJECTION.

Lovegrove E, Pettigrew G, Bolton EM, Bradley JA.

University Department of Surgery, Western Infirmary, Glasgow G11 6NT.

Introduction. We have shown previously that MHC class I-disparate PVG.R8 rat heart grafts in PVG.RT1^u recipients are rejected by CD4 T cell-dependent alloantibody-mediated effector mechanisms. It is likely that CD4 T cells recognise the allogeneic class I after it has been processed and presented by recipient APC, ie, by the indirect pathway. In this study we sought to identify the dominant T cell epitopes presented during processing of the RT1.A^{av1} molecule in this experimental model.

Methods. A series of 18 overlapping peptides (15mers), spanning the entire α1 and α2 domains of the RT1.A^{av1} molecule, were emulsified in complete Freund's adjuvant and injected either singly, or in combination, into the hind footpads of RT1^u rats (100 μg per peptide per rat). Seven days after immunisation, one group of rats was sacrificed and the ability of their T cells to proliferate in vitro to the different peptides was determined. A further group of peptide-primed animals was given R8 heart grafts. The kinetics of graft rejection and the production of circulating cytotoxic anti-A^{av1} antibody were determined.

Results. In vitro T cell assays confirmed that proliferation was specific for the peptide with which the animal had been primed. Rats immunised with all 18 peptides rejected R8 heart grafts more rapidly than control animals primed with CFA alone (MST 4 days and 6.5 days respectively). When animals were primed with individual peptides, several different peptides resulted in accelerated rejection. These corresponded to the hypervariable region of the a helix of the α1 domain and a shorter, non-polymorphic area of the β sheet of the α2 domain. The same peptides invariably primed for an accelerated anti-A^{av1} antibody response following heart grafting. Combinations of immunogenic peptides did not act in synergy, and tolerogenic peptides were not identified. Interestingly, the ability of a particular peptide to promote accelerated rejection did not correlate with the T cell proliferative response to that peptide in animals primed with an A^{av1}-disparate graft.

Conclusions. Several different T cell epitopes within the rat A^{av1} class I molecule prime T cells to provide cognate help for alloantibody production, and thereby accelerate rejection of class I-disparate heart grafts. The immunogenic epitopes identified, by an in vivo functional readout, correspond predominantly to the most polymorphic regions of the class I molecule. Surprisingly, epitopes derived from consensual sequences of donor and recipient MHC may also be immunogenic.

SIROLIMUS IN CLINICAL LIVER TRANSPLANTATION: A PILOT STUDY

CJE Watson, RY Calne, PJ Friend, NV Jamieson, TW Frick, A. Gimson*, G Alexander*.

University of Cambridge Departments of Surgery and Medicine*, Addenbrooke's Hospital, Cambridge, CB2 2QQ

Sirolimus (rapamycin) is a new immuno-suppressant which is synergistic with cyclosporin in the immunosuppression of kidney allografts. This pilot study evaluates sirolimus in liver transplantation.

Patients and Method:s
Patients undergoing orthotopic liver transplantation received one of two combinations of sirolimus and half-dose cyclosporin. Protocol A comprised sirolimus (2mg/m^2 increasing to 4mg/m^2 at 14 days), Neoral cyclosporin (levels 100ng/ml), and Prednisolone; Protocol B omitted prednisolone. At 10 weeks all patients were on sirolimus monotherapy.

Results:
Eleven patients were studied (A4, B7). Only one episode of acute rejection was seen; this responded to steroids. Two patients discontinued cyclosporin early, both as a result of neurological side effects; they continued on sirolimus monotherapy. Three patients discontinued sirolimus, one for hyperlipidaemia one with pneumocystis pneumonia: both subsequently died from unrelated causes (graft versus host disease and recurrent hepatoma). The third patient discontinued sirolimus because he disliked its taste.

Of the remaining 8 patients one died at seven days from overwhelming chest sepsis in the face of initial poor graft function. Four are on sirolimus monotherapy at between 70 and 684 days, and three are on cyclosporin and sirolimus less than 70 days since transplant.

Only one patient suffered an adverse event (hyperlipidaemia) attributable to the sirolimus. Significant infections were seen and resulted in the change in the protocol. These infections included pneumocystis carinii pneumonia, staphylococcal pneumonia, herpes simplex, cytomegalovirus and wound infections.

No nephrotoxicity or diabetogenic effects were seen. The neurological side-effects seen could be attributed to high levels of cyclosporin. Sirolimus was also shown to increase the blood concentration of cyclosporin.

Conclusions:
- Sirolimus combined with cyclosporin provides potent immuno-suppression of liver allografts
- Sirolimus monotherapy is adequate for maintenance therapy.
- Side-effects of sirolimus are uncommon and reversible on cessation of therapy.

FK506 AS PRIMARY IMMUNOSUPPRESSIVE THERAPY IN RENAL TRANSPLANTATION

Preston R, Ball S, Cairns T, Hakim N, Palmer A and Taube D

Renal and Transplant Units, St Mary's Hospital, Paddington, London

Although the large multicentre trials comparing FK506 with cyclosporin show that FK506 reduces the incidence and severity of acute rejection in renal allograft recipients there are few published single centre studies, particularly in the UK. In this study, we report our experience over the past 2 years with FK506 in renal transplantation.
73 patients [43 male, 30 female], mean age 43.5 years, including 9 re-transplants and 6 live related donor transplants received FK506 [0.15 mg/kg/day] and prednisolone [0.5 mg/kg/day tapering to 0.15 mg/kg/day at 3 months]. The dose of FK506 was adjusted to maintain trough levels of 10 -15 ng/ml. In addition 44 patients received azathioprine [1mg/kg/day] and 4 received mycophenolate mofetil [1-2 g/day]. Anti-lymphocyte preparations [ALG/ATG] were administered to 4 highly sensitised patients prophylactically. The mean number of HLA antigen mismatches was 2.8.
Mean follow up was 14.4 [range 3-24] months. Mean initial hospital stay was 15.7 [range 6-57] days. 23 patients (31%) experienced 30 discreet episodes of acute rejection. 4 patients with vascular rejection or steroid non responsive cellular rejection required ALG/ATG therapy. Patient and allograft survival and allograft function are shown in the table below.

Mth post txp	% patient survival	% graft survival	mean plasma creatinine [umol/l]	mean FK506 level [ng/ml]
3 [72]*	100	95.8	165	10.2
6 [71]	100	95.8	158	10.2
12 [64]	100	95.8	154	10.2
18 [18]	98.6	94.5	152	8.9

* number of patients available for follow up
One patient died a year post transplantation with disseminated carcinoma of the breast, having been disease free for 5 years pre transplantation. Two grafts were lost from untreatable renal artery stenosis and one from a perioperative renal vein thrombosis. There were no graft losses due to rejection. Mean FK506 dose was 0.13 mg/kg/day. Reversible nephrotoxicity occurred in 9 and neurotoxicity in 1 patient. Serious infections occurred in 20 patients [wound 2, pyelonephritis 5, pneumonia 2 and CMV 12]. New onset diabetes mellitus occurred in 9 patients. Thirty two patients required anti-hypertensive medications.
This study shows that FK506 is a very effective immunosuppressive agent in renal transplantation and that our results are comparable with the multicentre trial data.

ADENOVIRAL GENE DELIVERY OF A SOLUBLE TNF RECEPTOR MOLECULE: POTENTIAL FOR MODULATING CORNEAL ALLOGRAFT SURVIVAL

S A Rayner[1], A J T George[1], D F P Larkin[2,3]
[1]Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, [2]Department of Pathology, Institute of Ophthalmology & [3]Moorfields Eye Hospital, London.

Adenoviral gene transfer to the corneal endothelium is an efficient method of expressing putative therapeutic genes in corneal endothelium. This has potential value in modifying the course of corneal graft rejection.

Measurement of tumour necrosis factor (TNF) levels in the anterior chamber of rabbits undergoing corneal allograft rejection has indicated high levels, with peaks as high as 10 ng/ml in the aqueous humour. In order to block the action of this cytokine, we decided to examine the production by rabbit cornea of recombinant soluble TNF p55 receptor fused to a mouse Fc region. This molecule was encoded by cDNA inserted into an E1-deleted adenovirus termed AdTNFR. *Ex vivo* production from rabbit corneas of the TNF receptor-Ig molecule (TNFR-Ig) was demonstrated by blocking the activity of both recombinant rat TNF and rabbit TNF in aqueous sampled during a rejection episode, using an L929 bioassay. Production of a TNF-blocking molecule was demonstrated for at least 21 days.

We then proceeded to test this construct in a transplantation model. Donor Dutch Belted rabbit corneas were transduced with $1.5x10^7$ pfu AdTNFR or control Ad0 (E1-deleted but no cDNA insert) and then transplanted as orthotopic grafts in New Zealand White recipients. Graft survival time was compared with control allografts. Median survival time of donor corneas transduced with Ad0 (n = 5) was 16 days, AdTNFR (n = 8) was 18 days, and unmodified control donor corneas (n = 14) was 22 days.

In summary, we have demonstrated *ex vivo* production of TNFR-Ig using this gene delivery strategy. *In vivo*, however, no prolongation of graft survival was seen using the above protocol. One possibility is that the adenoviral vector has an inflammatory effect *in vivo* which counteracts any therapeutic benefit of this gene product. Alternatively, gene expression may be too short to block TNF activity at the critical time. We are investigating these possibilities, both delivering the therapeutic gene using less inflammatory non-viral vectors and examining the timing of gene delivery in relation to transplantation.

RENAL TRANSPLANTATION IN SPINA BIFIDA AND OTHER PATIENTS WITH ABNORMAL LOWER URINARY TRACTS.

Augustine T; Campbell B A; Asderakis A; Riad HN; Parrot NR; Pearson RC; Johnson RWG.

Renal Transplant Unit, Manchester Royal Infirmary, Oxford Road, M13 9WL

AIM: The aim of this retrospective study was to evaluate the technical problems and outcome of renal transplantation in patients with spina bifida and other abnormalities of the lower urinary tract with urinary drainage into a resting ileal conduit.
METHODS: The study group consisted of **26** patients who had **27** kidney transplants for a variety of conditions causing ESRD including spina bifida (13), posterior urethral valves (3), congenital uretero-vaginal fistulae (2), reflux nephropathy (3), bladder neck obstruction(1) ectopia vesicae (1) sacrococcygeal teratoma (1), tuberculosis of the renal tract (1), and post laminectomy neurogenic bladder(1). This group formed 2% of the 1356 renal transplants done between August 86 and March 97 in our unit and comprises one of the largest single centre experience with transplantation into an ileal conduit. All these patients had a resting ileal conduit created between 2 months to 17 years before being activated on the transplant waiting list. There were 14 males and 12 females with an age range of 1yr 11mths to 63 yrs. (mean 26.52 yrs). There were 22 cadaveric and 5 live related grafts. The actual surgical implantation procedure was similar to other routine transplants but the ureter was anastamosed to the ileal conduits over a stent. Standard immunosuppression was used.
RESULTS: All patients in this group have had multiple previous operations with an average of 3.4 procedures per patient. 20% of patients were wheel chair bound and 18% had kyphoscoliosis making surgical access difficult. Vascular dissection was more difficult due to fibrosis from previous operations. One, two and three year patient survival figures were 96.2%, 96.2%, 92.3%, respectively comparing favourably with national figures of 93.2%, 90.8% and 77.8%. (UKTSSA). The 2 deaths in the series were due to PCP and NHL. One two and five year graft survival figures were 96%, 95.2% and 44% compared to national figures 87.3%, 85.0% and 76.9%. Five grafts have failed so far. They were due to renal vein thrombosis, chronic transplant nephropathy, pyelonephritis and urolithiasis. Surgical complications included three urinary leaks, two small bowel obstructions, one subphrenic abscess and a transplant kidney volvulous, all of which responded to surgical intervention with excellent subsequent renal function. There was an acute tubular necrosis rate of 14.8%, and an acute rejection rate of 34.6%. There were an average of 2 urinary tract infections per patient which responded to antibiotics. In the two patients with recurrent urinary tract infections (> 5 episodes) there was narrowing of the uretero-ileal anastamosis with subsequent graft loss due to nephrolithiasis and pyelonephritis.
CONCLUSION: Renal transplantation in patients with spina bifida and other conditions with abnormal lower urinary tracts where the transplant ureter is anastamosed to a resting ileal conduit, in our experience gives excellent results in spite of the multiple associated comorbid factors. Kidney transplantation should be actively considered at an earlier stage in the multidisciplinary treatment of this subgroup of patients.

HUMAN T CELL RESPONSES TO PORCINE AND HUMAN ENDOTHELIAL CELLS ARE SENSITIVE TO CYCLOSPORINE A AND FK506 BUT ONLY HUMAN ENDOTHELIAL CELLS BECOME RESISTANT IN THE PRESENCE OF B7.

Batten, P., McCormack, A., Yacoub, M. and Rose, M.

National Heart and Lung Institute, Imperial College at Harefield Hospital, Middlesex, UK.

Human endothelial cells have been shown to be effective at activating T cells to proliferate and release IL-2. However, release of IL-2 by human T cells in response to PHA in the presence of human endothelial cells (EC) as accessory cells has been reported to be resistant to inhibition by cyclosporine A (CSA). In view of this finding we have investigated the sensitivity of human T cell responses to both CSA and FK506 directly, using human EC and porcine EC as stimulators, as well as T cell responses to PHA and MLR.

1. B7-independent responses of T cells to HUVEC and PAEC.

The direct response of human T cells to HUVEC and PAEC is highly sensitive to CSA with regard to both proliferation and IL-2 release. Similar results were also obtained for FK506. The sensitivity to CSA was further demonstrated by the differences in the ID_{50} between HUVEC and PAEC (6ng/ml and 16ng/ml respectively). Similar results were obtained for FK506, the ID_{50} being 0.025ng/ml and 0.45ng/ml respectively. In contrast, inhibition of T cell responses to PHA in the presence of CSA and FK506 were relatively resistant (ID_{50}=300ng/ml and 0.26ng/ml respectively), whereas inhibition of T cell responses in an MLR were more sensitive than the PHA responses (ID_{50}=38ng/ml for CSA) but more resistant than the responses seen with the endothelial cells. In addition, inhibition of T cell responses to PAECs were significantly more resistant than to HUVECs in the presence of both immunosuppressive drugs.

2. B7-transfectants increase HUVEC resistance to cyclosporin A and FK506.

In the presence of B7-transfectants (MHC class II negative mouse fibroblasts, DAP.B7), CSA or FK506 sensitive HUVECs became highly resistant in a dose-dependent manner. The ID_{50} was increased from 10ng/ml to 1500ng/ml (CSA). This was further corroborated by the total inhibition of the T cell response by the anti-B7 chimeric protein, CTLA-4-Ig, giving an ID_{50} similar to that without DAP.B7 (ID_{50}=7.5ng/ml). T cell responses to DAP.B7 alone did not stimulate any proliferation. In addition, the inclusion of this costimulatory molecule also augmented the T cell response to both HUVEC and PAEC in the absence of CSA. However, the addition of DAP.B7 to PAECs did not significantly increase CSA or FK506 resistance. This may well reflect the involvement of porcine B7/human CD28 interactions in the human T cell response to PAECs.

In conclusion, direct stimulation of human T cells by human and porcine endothelial cells is sensitive to CsA and FK506, but can be reversed by the addition of B7 to HUVECs only.

EARLY SINGLE CENTRE EXPERIENCE WITH MYCOPHENOLATE MOFETIL (MMF) IN CADAVERIC RENAL TRANSPLANTATION.

G Morris-Stiff, V Balaji, K Ostrowski, R Moore, C Darby, R Lord, WA Jurewicz.

Department of Transplant Surgery, University Hospital of Wales NHS Trust, Cardiff.

Introduction The aims of this study are to report a preliminary single centre experience with MMF in two clinical situations: (a) treatment **of recurrent rejection (RR)**, (b) *de-novo* use in **sensitized patients**.

Methods Data was obtained from 100 consecutive adult cadaveric renal recipients. **RR** was defined as either a lack of response to high dose steroid pulse therapy (SPT) or recurrent deterioration of function with histological evidence of acute rejection (Banff criteria). Patients with RR failing to respond to further treatment with SPT or other agents were switched from azathioprine to MMF at a dose of 2000 mg/day. Sensitized patients (PRA>50%) were randomized to either Neoral or tacrolimus-based triple therapy with **MMF** prescribed instead of azathioprine at a dose of 2000 mg/day.

Results ***MMF for RR*** Of 100 patients (all on triple therapy based on Neoral [n=50] or tacrolimus [n=50]), 41 developed primary acute rejection. Eighteen of these patients suffered recurrent rejections only five of which (27%) responded to SPT. The majority 13/18 (83%) had further rejections and required additional therapy. In 9 of these 13 cases MMF was used. Anti-rejection therapy prior to MMF consisted of: SPT (n=9, median 8 days), OKT3 (n=2) and Neoral/tacrolimus switch (n=2). In 6 cases no further rejection was seen and all have good graft function with a median creatinine (Cr) of 138 µmol/l (range 103-197 µmol/l). Of the remainder, two had additional treatment with SPT (Cr-122 µmol/l) or a change from tacrolimus to Neoral (Cr-317 µmol/l) and one recipient underwent graft-nephrectomy.

De-novo MMF in sensitized patients

Primary MMF Therapy	MMF + Tacrolimus + Steroid (n = 3)	MMF + Neoral + Steroid (n=2)
No Rejection	2	0
1 Rejection Episode	1	0
Recurrent Rejection	0	2
Change of Primary Agent	0	2
Graft Loss	0	0
Median Current Creatinine	138 µmol (90-154 µmol/l)	222 µmol (152-292 µmol/l)

Conclusions MMF appears effective both as a primary agent in highly sensitized patients and in the treatment of recurrent rejection episodes and further studies are ongoing in both areas to better define the role of MMF in the context of modern immunosuppressive regimens. We observed that the majority of patients (83%) who develop recurrent rejection while receiving Neoral or tacrolimus based maintenance triple therapy continue to experience further rejections. We interpret this as a failure of maintenance therapy to sustain the graft and recommend a switch from azathioprine to MMF.

RETRANSPLANTATION IN THE UK AND REPUBLIC OF IRELAND, 1987-1996

Belger, M A on behalf of the transplant community of the UK and Republic of Ireland, UKTSSA, Bristol UK

During the past ten years, 1987-1996, over 26,000 solid organ transplants have been undertaken in the UK and Republic of Ireland: 13.3% of these were for recipients who received their second or subsequent transplant. 3497 retransplants were reported: 2733 kidney, 623 liver and 141 cardiothoracic. The analysis undertaken looks at the characteristics of these retransplant recipients and their donor factors and highlights differences between them and recipients receiving their first transplant. Where sufficient data were available, survival analyses have also been undertaken.

The mean age of recipients of the 623 liver retransplants was 33.0 years compared with 40.1 years for first liver transplant recipients. 28% of retransplants were in paediatric recipients (under 18 years), while the proportion of first transplants carried out in paediatrics during 1987-1996 was only 16%. When comparing survival time of first and second liver transplants, a significant difference in outcome was apparent: first grafts had a one year survival of 64% compared with 46% for regrafts (Log-rank test, p=0.0001).

Cardiothoracic retransplants comprised 51 heart, 34 heart/lung and 56 lung transplants. Despite noticeable differences between characteristics of heart, heart/lung and lung recipients generally, there were few differences between first and retransplant recipients for each transplant type.

Kidney retransplants represented 15.8% of all kidney transplants undertaken during 1987-1996. Comparison of the mean recipient age for first transplant and retransplant recipients showed a significant difference (42.9 years for first transplant recipients; 37.5 years for retransplant recipients). Improved HLA matching for retransplants was also apparent.

Survival analysis was undertaken on 1244 adult retransplants undertaken between 1986 and 1993 in 23 centres in the UK. Results showed that one year retransplant survival has improved significantly throughout this time period (67% in 1986 to 84% in 1993). A multivariate analysis of cadaveric kidney retransplant survival using Cox's proportional hazards model showed graft year, recipient and donor age and HLA matching to be significant, but one of the most statistically significant factors affecting retransplant survival time was the survival time of the first graft. A longer transplant survival time for the first transplant was associated with a longer regraft survival time.

This study of the characteristics of first and retransplant recipients and their donors shows that retransplant recipients tend to be younger and in the case of liver and heart/lung transplants are more likely to be female. For liver transplantation survival of regrafts is significantly worse than first transplant survival but in the case of kidney transplantation, the younger and better matched recipients selected for retransplant may at least partially explain the comparable one year transplant survival estimates for first and retransplants (81% and 78% respectively for the 1986-1993 dataset of 23 centres).

NON-VIRAL STRATEGIES FOR GENE DELIVERY TO THE CORNEAL ENDOTHELIUM: PROSPECTS FOR MODULATING GRAFT REJECTION.

Andrew J.T. George[1], Carolina V. Arancibia-Cárcamo[1], H.Barbaros Oral[1,2], Dorian O Haskard[2], D. Frank P. Larkin[1,3]

[1]Department of Immunology, Division of Medicine and [2]Division of Cardiovascular Medicine, Imperial College School of Medicine at the Hammersmith, Hammersmith Hospital, Du Cane Road, London, W12 0NN, United Kingdom.
[3]Department of Pathology, Institute of Ophthalmology, Moorfields Eye Hospital, London, EC1 2PD, United Kingdom.

Gene transfer to the corneal endothelium has potential for the prevention or reversal of corneal allograft rejection. Previous work has concentrated on using adenoviral vectors to modulate gene transfer to endothelium. While such vectors are very efficient, they have a number of theoretical and practical disadvantages, both for experimental and clinical applications. These include the necessity to clone the gene of interest into the viral vector and also the inflammatory and immunogenic nature of the viruses *in vivo*. We have therefore used lipoadenofection, in which plasmid DNA is delivered using a combination of liposomes and adenovirus, to transfer marker genes both to endothelial cell lines and to the cornea. This study shows that this approach is efficient, with around 40-50% of an endothelial cell line (EAhy 926) being transduced. This contrasts to lipofection, in which around 7% of the cells are transduced. In the cornea, gene expression is limited to the endothelium, with no expression seen in the epithelium. Expression is short term, with maximal expression being seen between days 3-10, falling to undetectable levels after 28 days.

We have investigated the delivery of a gene construct containing an inducible promoter that is activated by tumour necrosis factor (TNF) and shown that expression of this gene occurs only when TNF is present. Thus expression of the chloramphenicaol acetyl transferase (CAT) marker gene is increased 9-10 fold following TNFα stimulation. As TNF is present in aqueous humour during allograft rejection, and this is in contact with the corneal endothelium, this inducible promoter has the potential to restrict expression of a therapeutic gene to rejection episodes in the cornea.

The major important advantages of the lipoadenofection strategy demonstrated in these studies are a) the feasibility of moderately efficient transfer of exogenous DNA without the necessity of cloning into recombinant viral vectors and b) the feasibility of conditional promoter control of transcription following lipoadenofection, a facility found to be poorly conserved in orthodox adenoviral mediated gene transfer to other cell types. Lipoadenofection therefore has potential in the development of gene based approaches to a number of disorders of corneal endothelium, in particular modulation of allograft rejection.

PROSPECTIVE RANDOMISED STUDY COMPARING TACROLIMUS (PROGRAF®) AND CYCLOSPORIN (NEORAL®) AS PRIMARY IMMUNOSUPPRESSION IN 80 CONSECUTIVE ADULT CADAVERIC RENAL TRANSPLANTS AT A SINGLE INSTITUTION.

G Morris-Stiff, K Ostrowski, V Balaji, R Moore, C Darby, R Lord, WA Jurewicz.

Department of Transplant Surgery, University Hospital of Wales NHS Trust, Cardiff.

Introduction The role of primary immunosuppression with tacrolimus is well established in hepatic transplantation however, its role in renal transplantation is less well defined and requires further evaluation.

Methods In an ongoing study, 80 patients transplanted in 1996 received Prograf or Neoral as primary immunosuppression in a triple therapy regimen. Tacrolimus was commenced at 0.2 mg/kg/day and cyclosporin at 8 mg/kg/day, both drugs were administered in two divided doses and adjusted according to clinical response and 12 hour trough blood levels.

Results

	PROGRAF (n=40)	NEORAL (n=40)	Statistical Analysis
Post-Transplant dialysis	5 (12%)	7 (17%)	n.s.
Serum creatinine at 3 months (median)	128μmol/l	135μmol/l	n.s.
Rejection - Number of Patients	16(40%)	13 (33%)	n.s.
- Number of episodes	26	22	n.s.
Graft Losses (including deaths)	0	6	$\chi^2 < 0.02$
Cytomegalovirus infection	0	4	n.s.
New cases of diabetes mellitus	3	2	n.s.
Serum cholesterol (median at 3 months)	5.6 mmol/l	6.6 mmol/l	n.s
Anti-hypertensive Index	1.90	1.95	n.s

At a follow-up of 3 months, 39/40 patients are still receiving tacrolimus, one having converted to cyclosporin due to tacrolimus enteropathy. Five patients in the cyclosporin group were converted to tacrolimus due to refractory rejection with satisfactory outcome in 4 and 1 graft failure.

Conclusion We conclude that Prograf at the starting dose of 0.2mg/kg/day and with trough levels lower than previously recommended represents an effective and safe therapy as a primary immunosuppressive agent following cadaveric renal transplantation and appears to have a better side-effect profile than the new formulation of cyclosporin (Neoral).

IMMUNOSUPPRESSIVE EFFECT OF SPLENECTOMY ON HDAF TRANSGENIC PIG TO PRIMATE RENAL XENOTRANSPLANTATION

Zaidi A, Bhatti FNK, Schmoeckel M, Tolan M, Waterworth P, Cozzi E, Chavez G, Wallwork J, White DJG and Friend PJ.

The Imutran/Novartis Laboratories, Department of Surgery, University of Cambridge and Papworth Hospital, UK.

AIM : To evaluate the effect on survival and xenoantibody response of splenectomy in a life-supporting model of transgenic pig to primate renal xenotransplantation.

METHODS : Kidneys from hDAF (human decay-accelerating factor) transgenic pigs were transplanted orthotopically into *Cynomolgus* monkeys. Simultaneous bilateral nephrectomy was performed. Nine recipients underwent concomitant splenectomy (Spx) and seven did not (NoSpx). Both groups were immunosuppressed with cyclophosphamide, cyclosporin A (trough level 300-400ng/ml) and steroids. Daily urine output, urine biochemistry and regular blood sampling for haematology, biochemistry and anti-pig antibody levels was performed. Rejection was defined post mortem when all kidneys were examined histologically with H&E staining and immunohistochemistry for C3, C4, C9, DAF, IgG and P-selectin.

SURVIVAL : NoSpx - 6,7,8,13,16,27, 35 days (median 13 days)
Spx - 5,6,9,18,>22,>35,>37,56,78 days (median >22 days)
p=0.067

Hyperacute rejection did not occur. Induced haemolytic anti-pig antibody titres were consistently lower in the Spx group despite significantly lower doses of cyclophosphamide (mean 4.12mg/kg/day in Spx group *vs* 8.61mg/kg/day in NoSpx group). Plasma biochemistry, fluid balance and acid-base balance were well maintained in both groups.

CONCLUSION : i) hDAF transgenic porcine kidneys can provide life-supporting function in primates into the third post-operative month ii) Splenectomy decreases induced xenoantibody response and increases survival.

WORK-LOAD GENERATED BY A LIVING DONOR KIDNEY TRANSPLANT PROGRAMME - THE TRANSPLANT CO-ORDINATOR'S PERSPECTIVE

WARD K, ELWELL R, BELL PRF, NICHOLSON ML

RENAL TRANSPLANT UNIT, LEICESTER GENERAL HOSPITAL, LEICESTER LE5 4PW, UK

Transplant teams under their duty of care must ensure that live kidney donation involves minimal risks, is truly voluntary and that both parties have been educated to facilitate informed consent. The process of working up a living donor therefore takes a considerable amount of time and the extra work-load created has received little attention. This paper describes the work-load generated by a live donor programme over the three years from 1994-1996.

Potential living donors were referred to a specialised transplant co-ordinator by consultant nephrologists. A pro-active attitude was adopted by nephrologists in suggesting living donation as an option to patients and their families. This included related and non related donors.

The organisation of a live donor renal transplants involved 7 stages:

1 Co-ordinator counselling and ABO grouping
2 Tissue typing
3 Consultant Surgeon counselling and examinations
4 Cytotoxic cross match (including flow cytometry)
5 Donor GFR and IVU
6 Donor angiography and review by Consultant Nephrological and Surgical staff
7 Hot test/ULTRA application

At most stages some referrals were found to be inappropriate and further investigations were sometimes indicated.

Over the three year period 87 potential donor-recipient pairs (174 patients) were referred to the co-ordinator; 163 patients were tissue typed and 52 pairs reached stage 3. Donor nephrectomy was performed in only 25 cases: 24 of these were related and 1 unrelated. 35 potential transplants were lost between stages 1 and 2 (14 were ABO incompatible); 27 pairs were lost (or remained in the work up process) between stages 3-7. Donor unsuitability was the major cause of loss in these stages. For those patients proceeding to stage 3, the eventual transplantation rate was higher for related pairs (24/43, 56%) compared with unrelated pairs (1/9, 11%; $p = 0.025$, Fisher's exact test).

Live donor transplantation represented 20% of the total transplant programme over the 3 years. Only 28% of initial referrals resulted in transplantation. Living donation can be a valuable source of kidneys but the programme is labour intensive and the attrition rate high. A highly motivated team is essential and, if living donation is to be increased in the UK, extra resources will undoubtedly be required.

A SHORT COURSE OF FLT-3 LIGAND PREVENTS THE THERAPEUTIC EFFECT OF DONOR BONE MARROW IN TRANSIENTLY IMMUNOSUPPRESSED CARDIAC ALLOGRAFT RECIPIENTS

Ajai Khanna MD FRCS, Mary A. Antonysamy Ph.D., Raymond J. Steptoe Ph.D., William A. Rudert Ph.D., Vladimir M.Subbotin MD. Ph.D., Angus W. Thomson Ph.D. DSc.
Thomas E. Starzl Transplantation Institute and Department of Surgery, University of Pittsburgh, PA 15213, USA

The influence of the novel hematopoietic growth factor Flt-3 ligand (FL), with or without concomitant systemic immunosuppression, on microchimerism, anti-donor immune reactivity and cardiac allograft survival, in mice given allogeneic bone marrow (BM) was investigated. Normal C3H ($H\text{-}2^k$) mice received 50 x 10^6 unmodified B10 ($H\text{-}2^b$) BM cells alone or with Flt3 Ligand (FL; 10 mg/day), tacrolimus (2mg/kg/day) or both agents for 7 days. Donor MHC class II$^+$cells (IA^{b+}) in recipient spleen were quantitated by immunohistochemical analysis, and donor class II DNA in BM by PCR. BM + FL + tacrolimus led to an 8-fold increase in donor cells and enhanced donor DNA compared to the BM + tacrolimus group, and a 490-fold increase in donor cells compared to BM alone. Donor cells were rare in all other groups. Heart allograft recipients (C3H) given perioperative B10 BM and a 13-day course of tacrolimus, exhibited markedly extended graft survival times (MST: 42 days) compared to recipients given tacrolimus alone (MST: 22 days). Addition of FL (10 mg/day; 7 days) to BM + tacrolimus reversed the beneficial effect (MST: 18 days). Administration of BM alone or BM + FL, resulted in uniform early heart graft failure (< 8 days).
Functional assays performed 7 days post BM transplant and 15 days post heart transplant revealed maximal anti-donor MLR and CTL activities in the BM and BM + FL treated groups, with minimal activity in all groups given tacrolimus. These studies demonstrate that FL dramatically augments microchimerism under cover of tacrolimus, with attendant abrogation of anti-donor T cell responses.The reduced heart graft survival times following tacrolimus withdrawal may be attributable to the potent capacity of FL to augment numbers of potential stimulatory APC, in particular functional dendritic cells and anti-donor effector mechanisms that remain to be fully characterized.

TOWARDS A TRANSPLANT RELEVANT GENOTYPE[1]

Hart,K.W., Bunce, M.B., Hill*,.A.V. and Welsh, K.I.

Transplant Immunology, Churchill Hospital, Headington, Oxford OX3 7LJ

*Molecular Immunology Group, Institute of Molecular Medicine, Oxford

Limited SSP typing for HLA was first described in abstracts published from Guy's Hospital in London (BSR) and Upjohn in the US (ASHI) in 1987 and patented by ICI in 1989. It was the work of Olerup and Zetterquist, however, who first described its clinical application to MHC class II. We extended the technique in the rapid HLA-A,B,C,DR,DQ typing of bone marrow and solid organ donors (BTS 1994) and for ABO and secretor status (BTS 1996). The modifications described here improve the resolution and the signals obtained from such procedures.

Simple salt and solvent extraction abrogate the need for proteinase K in the DNA purification. A combination of increased redundancy and "haplotyping technology" negates the need for control amplification . The effect of primer dimer is minimised by the pre-titering of primer pairs and a dual temperature readout enables discrimination of primer dimer from product utilising differences in T_m. Addition of one of the intercalating fluorescent dyes Yo-Pro-1 (pre-PCR) or SyBr Green (post-PCR) quantitates the difference between positives and negatives. The result is read straight from the PCR-plate in a Cytofluor Series 4000 multi-well plate reader and directly computerised. Validation has produced results which are in 100% concordance with those derived from agarose gel electrophoresis.

The method allows rapid detection of polymorphisms in other genes which are of possible relevance to transplant outcomes, namely cytokines, TAP, HSP-70, HLA-DP, selectins and enzymes involved in the metabolism of immunosuppressant drugs.

THE EFFECT OF ISCHAEMIA-REPERFUSION INJURY AND DENERVATION ON THE DEVELOPMENT OF CYCLOSPORIN NEPHROTOXICITY

J Mitchell, AG Pockley, AM El Nahas, AT Raftery, RFM Wood, Dept. Surgical Sciences, Sheffield.

This study investigated the effect of ischaemia-reperfusion and denervation on the development of CyA nephrotoxicity. CyA nephrotoxicity is mediated via myofibroblasts, cells which express cytoplasmic contractile proteins such as alpha smooth muscle actin (α sma) when activated. The state of activation of these cells is controlled by cytokines such as transforming growth factor beta (TGFβ). This study was devised to assess the effects of the components of transplantation, namely ischaemia-reperfusion and denervation on the development of CyA nephrotoxicity.

CyA was made into a microemulsion with Cremaphor (Sigma) and administered by continuous subcutaneous infusion using an osmotic minipump (Alzet). Adult male rats were used and divided into 4 experimental groups. Group 1 received CyA at 12.5 mg/Kg/day. Group 2 underwent left unilateral nephrectomy. Those in group 3 were subjected to 45 minutes of warm ischaemia of the right kidney. In group 4 the right kidney was denervated by stripping of the perivascular tissue and phenol ablation of the right renal artery. All animals received CyA as in group 1. Further control animals underwent identical surgical protocols in the absence of CyA. All groups underwent biopsy at 4 and 8 weeks. Blood was taken at the time of biopsy for the measurement of serum CyA and urea and electrolytes.

Tissue sections were stained with H&E, PAS and Masson's trichrome. Paraffin sections were stained using monoclonal antibodies to α sma and TGFβ using a streptavidin-biotin horseradish immunoperoxidase reaction. Sections were then studied using light microscopy and the grade of severity of fibrosis and the density of staining with immunohistochemistry assessed by counting the number of stained objects in 20 adjacent fields in a grid.

	4 weeks (αsma)	8 weeks (αsma)	4 weeks (TGFβ)	8 weeks (TGFβ)
CyA alone	4.2±1.3 p=0.02	27.2±8.8 p=0.003	0 ns	4.9±0.9 p=0.002
nephrectomy	10±3.8 p=0.009	17.2±7.6 p=0.01	4.4±2.7 p=0.02	6.6±1.9 p=0.001
ischaemia-reperfusion	17.2±3 p=0.002	25±2.9 p<0.001	5.7±2. p=0.01	8.1±1.8 p=0.001
denervation	5.8±1.5 p=0.003	14.3±2.1 p=0.006	4.35±4.3 ns	9±0 p=0.001

Ischaemia and denervation significantly increased fibrosis in CyA nephrotoxicity (Table: paired t test). Nephrectomy also increased fibrosis. These results suggest that denervation and ischaemic injury potentiate the toxic effects of cyclosporin on the kidney.