The British Transplantation Society

October 16th and 17th, 1995

ROYAL COLLEGE OF PHYSICIANS
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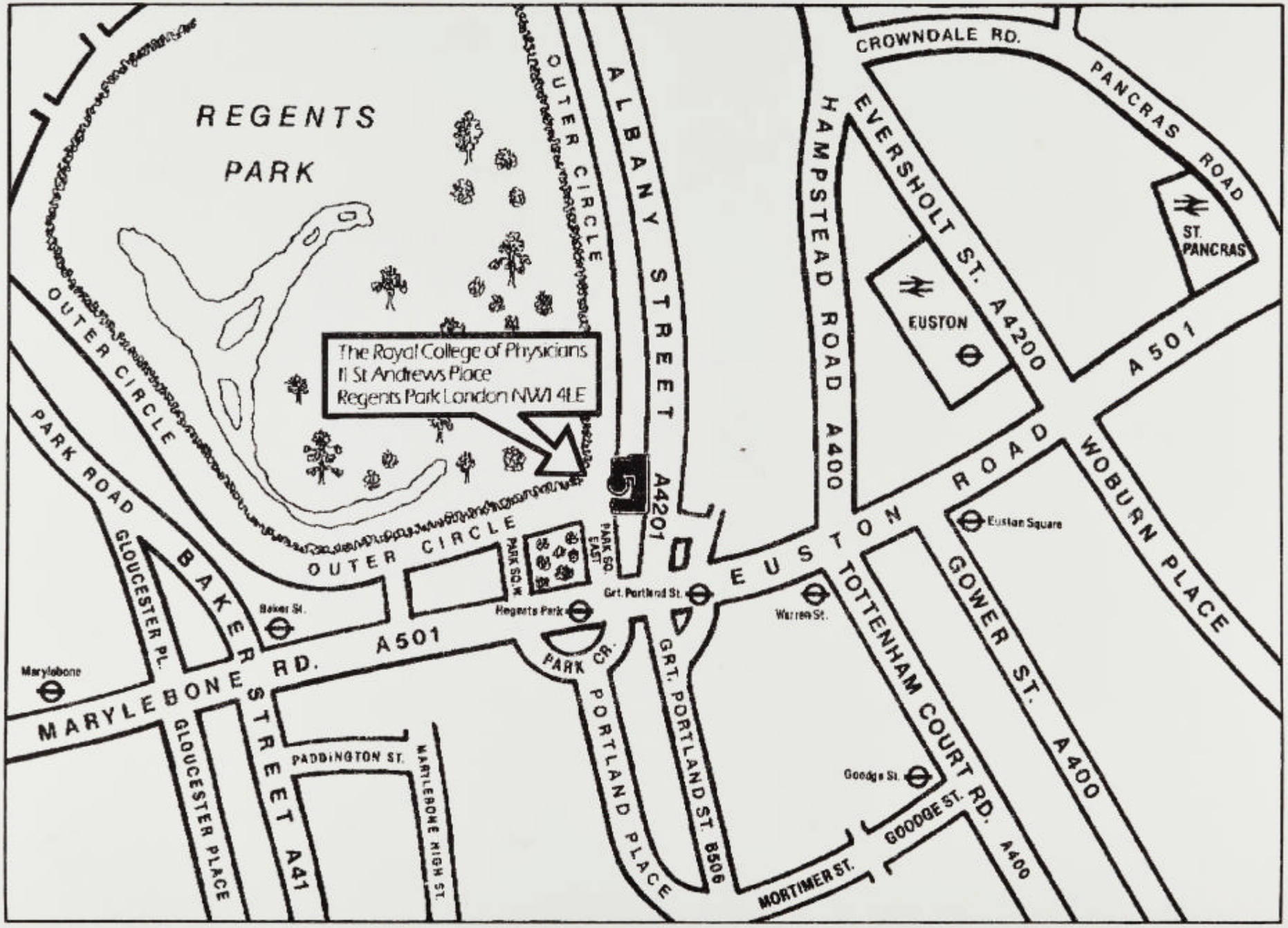
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95/10 TREASURER'S REPORT.

There was a positive balance of £68,000.

95/11 ARCHIVIST'S REPORT.

Professor Molly McGeown presented a full report of her progress with organising the **Society archives**. Cataloguing the archives would be funded by the Welcome Trust Contemporary Medical Archives. There was still a need to locate relevant records from 1972 and 1975-1979; all Members were asked to search their own files and forward records to Professor McGeown. There would be a summary of the Archivist's work published in the July 1995 Newsletter.

95/12 ANY OTHER BUSINESS.

a) Mr Peter Lodge asked if Society meetings qualified for **CME points**. Enquiries would be made to the Royal Colleges of Surgeons and Pathologists.

b) Mr RWG Johnson reminded Members that the Autumn 1977 meeting would be the **25th anniversary of the Society**. The President noted that the meeting should be a celebration.

ABSTRACTS SELECTED FOR PRESENTATION

LIVER TRANSPLANTATION (LT) FOR HEPATITIS B VIRUS (HBV) INFECTION - A DECADE'S EXPERIENCE.

M.M. Ahmed, A. Mesheikes, B-H.Ferraz-Neto, M.Hastings, E. Boxall*, E. Elias, P. McMaster and D.J. Mutimer.

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Refined patient selection and sustained post-transplant immunoprophylaxis with HBIg have improved graft and patient survival for HBV-associated liver disease. We describe the Birmingham Liver Unit experience since 1987.

Era 1 ('87-'90) - patients with replicative (HBeAg+ve) and non-replicative (anti-HBe+ve) infection were considered suitable for LT and immunoprophylaxis was given short-term. 11 patients were grafted - 4 perioperative deaths (unrelated to HBV recurrence), 3 late deaths (1,2,2 years post-LT; all HBeAg+ve pre-LT) due to aggressive HBV re-infection, 3 survivors (6,7,7 years post-LT) with HBV-infected cirrhotic grafts, 1 patient lost to follow-up.

Era 2 ('91-'95) - patients with non-replicative infection (including fulminant HBV) were considered suitable and long-term immunoprophylaxis was used. 14 patients were grafted- 1 perioperative death, 1 late death (aggressive HBV recurrence 8 months post-LT: HBeAg-ve but HBV DNA+ve pre-LT), 12 survivors (median 31, range 3-46 months follow-up). 5 survivors have HBV re-infection - immunoprophylaxis stopped because of allergic reactions in 3 patients, strategy failure in 2 patients. 7 patients have non-infected grafts and normal liver function 3-40 months post-LT.

Conclusions (1) Patients with replicative HBV infection are unsuitable transplant candidates - recurrent infection is associated with poor graft and patient survival. (2) Improved graft and patient survival is observed when patients with non-replicative infection receive long-term post-LT immunoprophylaxis, but better strategies are needed. (3) Innovative strategies, including the use of effective antiviral agents pre- and post-LT, are urgently required for patients with replicative infection.

TOTAL LYMPHOID IRRADIATION AS RESCUE THERAPY FOLLOWING CARDIAC TRANSPLANTATION

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Background: A proportion of cardiac transplant recipients develop allograft dysfunction without significant cellular infiltrate in endomyocardial biopsies and with normal coronary arteries at angiography. The mechanisms responsible for this presentation are unclear, but humoral factors may be important. Although it occurs less frequently than cellular rejection, this form of allograft dysfunction does not usually respond to standard forms of rejection therapy and is therefore associated with a poor prognosis . Some success has been reported with plasmaphoresis, immunoadsorbtion and cyclophosphamide therapy but the role of total lymphoid irradiation (TLI) in the management of this condition is unclear.

Methods: Three patients who developed severe allograft dysfunction following orthotopic cardiac transplantation were successfully treated with TLI. Each patient developed bi-ventricular failure despite immunosuppression with cyclosporin A, azathiaprine, oral prednisolone, cyclophosphamide and intravenous methylprednisolone therapy. Endomyocardial biopsy specimens and coronary angiography were normal in each patient. TLI was given using standard mantle and inverted y fields over ten treatments to achieve a cumulative dose of 8Gy.

Results: Each patient had a significant improvement in clinical response and in ventricular performance following TLI which was well tolerated in each case. The patients remain well at eight, nine and twelve months respectively following completion of treatment.

Conclusions: TLI should be considered as adjunct therapy to conventional immunosuppression for cardiac transplant recipients who develop poor graft function in the absence of cellular rejection or coronary artery disease.

DUODENAL COMPLICATIONS AFTER WHOLE ORGAN PANCREAS (PA) TRANSPLANTATION (TX): FREQUENT BUT PROGNOSTICALLY FAVORABLE

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There is a broad spectrum of duodenal complications after bladder-drained PA TX using the duodenal segment technique; little is known of the effect of duodenal complications on the long-term prognosis of patients and PA grafts. **MATERIALS AND METHODS:** We studied incidence and outcome of duodenal complications after 373 bladder-drained (stapled duodenoscystostomy in 95%) whole organ pancreatico-duodenal transplants (7/85 thru 1/95). Complications were defined as early if they occurred within the first postoperative month, and late otherwise. Mean follow-up was 5.5 months (range, 3 to 108 months). **RESULTS:** 1. There were 42 ***duodenal leaks*** (11.3%); 12 early with a mean of 11.5 days (range, 1-28 days) and 30 late with a mean of 6.9 months (range, 1-36 months). The site of the leak was at the duodeno-cystostomy site (true bladder anastomotic leaks) in 15 cases, at the stapled proximal duodenal stump in 8, and at the stapled distal duodenal stump in 4. In the other 15 cases it was impossible to identify the exact site of the leakage because the patients were treated conservatively and the studies (Cystogram/CT Scan) were unable to identify the site. In 23 (55%) patients the leakage was oversewn; but 6 (14%). 4 patients had a recurrent leak requiring enteric conversion 2 to 12 months after the first leak. 12 (28%) patients with small leaks were treated conservatively with an indwelling catheter for 1 to 2 months with resolution of the leak. 2. Gross ***hematuria*** (defined as severe enough to require cystoscopy) occurred in 26 patients (7%), 10 early with a mean of 14 days (range, 7-21 days) and 16 later with a mean of 11.5 months (range, 1.5-60 months); 2 (8%) patients had an enteric conversion and 2 (8%) had a graft pancreatectomy. 3. Two patients (0.5%) had recurrent ***bladder stones*** requiring repeated cystoscopies and removal of stones encrusted at the site of visible staples. There were no graft losses. 4. Nine patients (2.4%) with ***recurrent UTI*** required cystoscopy and removal of stitching and staple material as the focus of infection. There were no graft losses or conversions. 5. ***CMV duodenal ulceration*** was identified in 6 (1.6%) patients; 2 patients had enteric conversion. **CONCLUSIONS:** Duodenal complications are common, but are not associated with a high rate of PA graft loss (8%); mortality from duodenal complications in our series was 0%. Early surgical intervention, including enteric conversion is safe and can decrease morbidity and mortality in this patient population.

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

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

CHANGING STABLE HEART TRANSPLANT RECIPIENTS FROM SANDIMMUNE TO NEORAL

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Purpose

Use of the currently available oral formulation of cyclosporin A (CyA), Sandimmune, is characterised by variable absorption. The new oral microemulsion formulation, Neoral, has been shown to provide more rapid absorption and greater bioavailability in renal transplant patients. We report a pharmacokinetic study to compare the two formulations in a cohort of 20 stable heart transplant recipients.

Methods

The 20 subjects had undergone orthotopic heart transplantation 12 to 88 months prior to the study. Our target trough CyA levels at this stage after transplantation are between 80 and 120ng/ml. Each patient had a CyA blood concentration profile carried out after their morning dose of Sandimmune. The following day they were converted to Neoral, at the same dose. One week later a further CyA profile was performed. Patients were then left on Neoral and were followed up for 6 months during which time their Neoral dose was adjusted on the basis of repeated trough levels.

Results

Neoral increased mean (±1 sd) CyA Cmax from 581(±196)ng/ml to 910(±268)ng/ml ($p<0.0001$, paired 2-tailed t-test), AUC from 2939(±590)ng/ml.hr to 3686(±575)ng/ml.hr ($p<0.0001$, paired 2-tailed t-test), and decreased mean Tmax from 134(±63)mins to 97(±28)mins ($p=0.03$, paired 2-tailed t-test). Although initial trough CyA levels were unchanged, after 6 months the mean CyA dose had been reduced, on the basis of repeated trough level measurements, by 11%. After 6 months there was no change in plasma creatinine levels, nor any increase in side effects of CyA.

Conclusion

In stable heart transplant patients, changing from Sandimmune to Neoral provides greater speed of absorption and bioavailability. In the long-term there was a reduction in CyA dose of 11%. There was no evidence of increased side-effects.

A CRITICAL ANALYSIS OF OUTCOME OF ATG THERAPY MONITORED BY CD3 COUNTING IN STEROID RESISTANT REJECTION.

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Monitoring CD3+ T cell levels during ATG treatment for renal transplant rejection may allow reduction in ATG dose without reducing its efficacy (Clark K R et al. Clin. Transplant. 1993; 6: 267-74). Dose reduction brings benefits in reducing risk of infection and cost. Ten consecutive patients who received (Merieux) 2.5 mg/kg/dose (or less) depending upon daily T-cell count by FACS analysis of peripheral blood lymphocytes: ATG was omitted on days when the CD3 count was < 50 cells/cumm (Group I). The outcome of these patients was compared with ten control recipients treated with ATG 2.5-5 mg/kg/day (as recommended by Merieux) continuously for 10-14 days without CD3 monitoring (Group II). The two groups were similar with regard to age, severity of steroid resistant rejection and anti-rejection prophylaxis.

Result	Group I	Group II
Mean follow-up (months)	7	13
Mean number of doses of ATG	4.4	8.9
Median dose of ATG (mg/kg)	6.6	18.7
Functioning grafts	8/10	8/10
Septicaemia	0	3
Death	0	1
Further rejections	7	3

Conclusion: In this small study, a reduction in ATG dose by CD3 monitoring does not appear to reduce the rate of reversal of rejection compared with case controls, and infections seem to be less common. Although graft survival is equal in each group, the higher (but not significant) rate of rejection gives cause for concern and merits further study.

SETTING STANDARDS FOR DONOR FAMILY CARE

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Donor family care is varied in the UK, depending largely on attitudes in the Intensive Care Unit (ICU) and those of regional procurement transplant co-ordinators (TC). All the ICUs in our area were offered a service with the TC visiting the ICU at the time of donation, meeting with the family, before or after the approach had been made, and then making arrangements for the donation. Afterwards each family received a letter of thanks and information; further information was only available if the family requested this. This study was devised to examine the needs of donor families at the time of death, immediately after donation and in the long term, to enable setting of standards. This could lead to a reduction in relative refusal.

Sixty families agreed to take part and 47 (78%) completed and returned their questionnaires. Of the 47 families, 57% did meet the transplant co-ordinator at the time of the death, another 31% felt that this would have been beneficial. Only three families were critical of the approach. More information post donation was requested by nine families, but the general content and timing of the letter was appreciated by most. 36% of families would have appreciated a home visit after the retrieval and 51% felt that a local support group would have been useful. 46% received letters of thanks from recipients and those who did not regretted this. Of those who had attended Thanksgiving Services, only 2 families felt it wasn't useful. 51% would have liked to meet recipients but agreed that this was not appropriate in the first year.

In conclusion, we continue to offer to meet each family at the time of donation and have improved our follow up. Our families now receive three letters within the first six months, a home visit within the first month is offered and each recipient is encouraged to send a thank you letter to their donor family. We are currently examining the feasibility of setting up a local support group and shall continue to run biannual Thanksgiving Services where donor families and recipients can meet. We would hope that all TC s who deal with donor families would adopt these standards.

KIDNEY DONATION FROM PATIENTS OVER FIFTY FIVE: AN UNTAPPED SOURCE OF ORGANS

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The use of older patients as organ donors is an under-utilised source of kidneys for transplantation. Only a fifth of all kidney transplants in the UK are from donors over 55, despite the higher incidence of brain stem death in this age group. This is an audit of ten years experience of kidney transplantation from donors aged 55 years or more, harvested in a single unit, and transplanted either locally or in one of 21 other centres via UKTSSA. Demographic details of donor and recipient, graft and recipient survival, immediate and 12 month function were requested by postal questionnaire.

57 donors age 63(55-82) years, median (range) provided 107 kidneys, of which 59 (55%) were used locally. Data has been collected on 105 (98%) of the recipients, median age 48.5 (21-73), 64% male.

53% of grafts achieved immediate function, according to local criteria. 43% of grafts with delayed function were working at one year. 68% of all grafts were functioning at one year with median creatinine of 216 (122-659) mmol/l. At the time of the audit 71% were still alive with the time to death of the other 29% median 1.2 years, range 6 days to 7.2 years. 46% had transplant function at time of death. Time to graft failure independent of recipient death was median 68 days (1 day-10.1 years). No pattern emerged of causes of graft failure or recipient death.

The sub group of local patients showed better but non significant immediate function (58%) and one year graft survival (78%). One year serum creatinine 217 (124-604) in recipients with surviving grafts was the same as the group as a whole.

These data suggest that elderly cadaveric donors may have slightly reduced graft survival compared to a younger donor group, but that this may be acceptable in the face of a generalised shortage of organs for donation.

COMPLICATIONS OF URETEROVESICAL ANASTOMOSIS IN KIDNEY TRANSPLANT PATIENTS: THE MINNESOTA EXPERIENCE

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*Transplant Unit, St Mary's Hospital, London, UK

We reviewed urologic complications of 1183 consecutive primary or secondary renal transplants performed with bladder anastomoses at the University of Minnesota Hospital between 1985 and 1993. The Politano-Leadbetter (PL) technique of ureteroneocystostomy was used in 410 patients: the multistitch (MS) extravesical technique modified from the methods of Witzel, Sampson and Lich in 295; and the extravesical single-stitch (SS) technique in 478. Urologic complications occurred in 81 patients (6.8%). Of the complications, 68 (5.7%) were early (<4 months) and 13 (1.1%) late; 32 (7.8%) were after PL, 17 (5.8%) after MS and 32 (6.7%) after SS. A total of 13 patients had an *anastomotic leak*, 7 (1.7%) after PL, 4 (1.4%) after MS and 2 (0.0004%) after SS; 49 patients had a *ureterovesical obstruction*, 16 (4.0%) after PL, 12 (4.0%) after MS and 21 (4.2%) after SS; 5 patients had a *ureteropelvic obstruction*, 2 (0.5%) after PL, 2 (0.7%) after MS, and 1 (0.2%) after SS; and 14 patients had *hematuria*, 7 (1.7%) after PL, 1 (0.34%) after MS, and 6 (1.3%) after SS. Of the 81 patients with urologic complications, one (1%) resolves spontaneously, 30 (37%) were treated with temporary percutaneous nephrostomy, 17 (21%) with dilatation and stent; the 14 (17.3%) with hematuria were treated via cystoscopy; 19 (23%) required reoperation. Only 2 (2.5%) patients lost their graft. For both cadaver and living donor recipients, there was no difference between techniques for early and late complications of leakage, stricture and hematuria. Each technique has certain advantages and each should be in every surgeon's repertoire.

TRANSPLANTATION OF PEDIATRIC KIDNEYS INTO ADULT RECIPIENTS - A TWELVE YEAR EXPERIENCE

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Because of the extreme organ shortage we have systematically transplanted kidneys from children less than 10 years of age into adults. This paper outlines our experience in 62 such kidneys which have been followed up for up to 12 years. Fifty-three of the 62 kidneys came from donors 1-5 years of age (Group 1), and 9 from donors aged 5-10 years (Group 2). The cold ischemia time before transplantation was 17-61 hours. Graft preservation was with UW solution in 51, Eurocolline solution in 9, and hypothermic perfusion in 2. The recipients were aged 20-62 years, and weighed 42-120 kg. Kidneys were removed from multi-organ cadaver donors and were transplanted using microsurgical techniques when indicated. Primary non-function was seen in 5% in Group 1, but none in Group 2. Delayed function was 37% and 22% in Group 1 and 2,respectively. Overall patient survival was 95%, 93%, 86%, and 80% at 1, 2, 5, and 10 years. Graft survival was 83%, 76%, 50%, and 45% at the same periods. There was no significant difference in patient or graft survival among Group 1 and Group 2. During the study period, 19 grafts were lost: 8 due to acute rejection, 3 to primary non-function, 2 each for patient death, chronic rejection, and recurrence of disease. One graft was lost due to Cyclosporine toxicity and another due to renal artery thrombosis. Vascular necrosis of the ureter was seen in 3 patients, but all were salvaged by re-operation. Monitoring of these grafts with ultrasound and clearance studies showed rapid anatomic and functional hypertrophy over a period of 3-6 months, with a mean serum creatinine falling from 4.8 at 10 days to 2.2 at 90 days. It is concluded that small pediatric kidneys can provide excellent function in adult recipients and because of organ shortage they should always be used as single not as enblock grafts.

THE EPIDEMIOLOGY OF LYMPHOCYTOTOXIC AUTOANTIBODIES IN PATIENTS WITH RENAL FAILURE AWAITING TRANSPLANTATION

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Lymphocytotoxic autoreactive antibodies (AAB) are identified by their reactivity with peripheral blood lymphocytes but not with lymphocytes from patients with chronic lymphocytic leukaemia and confirmed by a positive cytotoxic crossmatch with autologous lymphocytes. AAB are usually IgM antibodies and their reactivity can be abrogated by reduction with dithiothreitol (DTT). As AAB cause a false positive crossmatch it is imperative that they are identified in patients awaiting renal transplantation so that a DTT crossmatch can be performed. Despite several studies, neither the target antigen(s) nor the risk factors associated with production of AAB have been clearly defined. We have studied the incidence of AAB in 79 patients (21 female, 58 male) from one dialysis unit who were on the transplant waiting list in April 1995 and analysed demographic data relating to primary renal disease, number of blood transfusions, recorded peritonitis, mode of renal replacement therapy (HD or CAPD) and previous transplant status.

Of the 79 patients studied (53 listed for primary, 26 for retransplant), 40 (50.6%) had AAB. Of that 40, 26 (65%) had received at least one previous transplant. There was a highly significant correlation between previous transplants and the production of AAB (Chi-square $p<0.0001$).

Of the 40 patients with AAB, 14 (6 female, 8 male) had not had a previous transplant. For this group there was no apparent association between the production of AAB and any of the parameters studied.

We conclude that there is a clear association between transplantation and the production of AAB with all of the patients awaiting a retransplant having AAB. The study should now be extended to larger numbers to determine the additional risk factors in non-transplanted patients.

THE ROLE OF INTERLEUKIN 12 IN T CELL POLARISATION IN MURINE GRAFT-VERSUS-HOST DISEASE

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The immunopathology of graft-versus-host-disease (GvHD) can readily be studied by inoculating F1 hybrid mice with parental T lymphocytes and here we have utilised (C57Bl/6 x DBA/2) F1 (BDF1) mice as hosts, in which the outcome of the GvHD depends on the parental cells used. Thus, DBA/2 cells injected into BDF1 mice results in a chronic, immunostimulatory disease characterised by increased IL-4 production and autoimmunity, while injection of C57Bl/6 cells induces an acute form of GvHD with enhanced levels of IFN-γ production, early lymphoid hyperplasia, followed by immunosuppression and death.

This suggests that the distinct pathologies which develop in BDF1 mice given either C57Bl/6 or DBA/2 parental cells may reflect differential activation of T helper (Th) cell subsets. Since IL-12 plays an important role in the early polarisation of Th cell responses in other systems, we have investigated the role of IL-12 in determining the outcome of the GvHR in BDF1 mice.

Depletion of IL-12 reduced the early increases in NK cell activity, lymphocyte proliferation and splenomegaly seen in acute GvHD, but had no effect on the chronic disease. Treatment with IL-12 antibody also down-regulated the levels of Th1 associated cytokines produced by acute GvHD spleen cells and prevented the profound immunosuppression seen later in the disease. Furthermore, administration of rm IL-12 to mice which normally develop the chronic disease, resulted in an acute GvHD-like syndrome, in which early weight loss and mortality were observed together with activation of anti-host CTL and increased levels of IFN-γ. Our findings support the view that early production of IL-12 is required for the development of an acute GvHD and this cytokine may be a useful target for immune modulation of clinical GvHD.

SPECIFIC AND NON SPECIFIC EVENTS FOLLOWING INTRATHYMIC INJECTION OF ALLOANTIGEN USING TCR TRANSGENIC MICE.

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We have recently shown that specific deletion of alloreactive CD8 single positive (SP) and double positive (DP) thymocytes can be detected 9 days after intrathymic injection of BL/10 ($H2^{b+}$) splenocytes into an adult K^b specific TCR transgenic mouse (BM3.6). Here we present data using a RAG negative variant of the BM3.6 mouse examining the the various roles played by intrathymic injection, operative stress and persistence of donor cells in the observed deletion of DP and CD8 SP thymocytes.
Injection of medium or operative stress alone had little effect with preserved thymic morphology, near normal thymocyte numbers and background levels of apoptosis as detected using an in situ method.
However, intrathymic injection of splenocytes [syngeneic (CBA), third party (BALB/c(K^{b-})) or BL/10(K^{b+})] resulted in an early reduction in thymocyte numbers with associated disordered architechture and increased levels of apoptosis. This effect was most dramatic when K^{b+} cells were injected.
The early non specific depletion was maximal by day 7 following injection, after which time thymi injected with third party cells recovered rapidly and completely. In marked contrast deletion of both the CD8 SP and DP thymocyte compartments was evident for up to 28 days in thymi injected with K^{b+} cells.
By day 75 after injection these thymi had recovered a naive type FACS profile. This correlated with the persistence, in the thymus, of K^{b+} donor cells which could easily be detected, using immunohistochemistry, up to 28 days after injection but were undetectable by day 75.
We conclude that in this model there are early events leading to both specific and non specific depletion of thymocytes but subsequent repopulation is specifically determined by the haplotype of donor cells persisting in the thymus during recovery.

ANALYSIS OF THE MULTI-ORGAN RETRIEVAL SCHEME FOR THORACIC ORGANS AND LIVERS

UKTSSA on behalf of the Users Multi Organ Retrieval Audit Group

UK Transplant Support Service Authority, Bristol, UK

Prior to November 1993, thoracic organs and livers were offered in strict rotation, and the centre accepting the organ was responsible for the organ retrieval. This often resulted in delays and excessive travelling for retrieval teams. The multi-organ allocation scheme, introduced in November 1993, assigned a geographic zone to each designated liver and thoracic transplant centre. The zonal centre has first refusal of organs from the zone but is also obliged to retrieve those which cannot be used locally.

An initial analysis of the six months April - September 1994 compared with the same period the preceding year revealed that while donor numbers declined (from 462 to 429) the number of donors providing both livers and thoracic organs increased by 16% and 8% respectively, as did the number of transplants (187 to 202, 110 to 124 and 316 to 334 for hearts, lungs and livers respectively). Exchange rates between centres also increased dramatically from 14% to 29% for hearts, 11% to 43% for lungs and 26% to 51% for livers. Ideally, under this scheme each donor hospital and renal transplant team should deal with one liver and one thoracic retrieval team. In 1993, 25% of hospitals providing thoracic donors and 29% of those with liver donors were visited by two or more retrieval teams compared with 9% and 13% respectively in 1994. Distances travelled by retrieval teams also decreased. In 1993, an average of 1.6 Regional Health Authority boundaries were crossed when travelling to a thoracic retrieval (1.5 for liver retrievals) compared with an average of 0.8 boundaries crossed in 1994 (0.9 for liver retrievals).

While these efficiency improvements cannot be solely attributed to the zoning, transplant centres and donor hospitals prefer the scheme and consider it a success. This paper will extend the analysis to include activity in the second year of operation to establish whether these effects have been sustained.

SPLIT LIVER TRANSPLANTATION - LOGISTICAL DIFFICULTIES, SURGICAL PROCEDURES AND RESULTS.

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The shortage of paediatric ITU beds and paediatric donors results in longer waiting times for paediatric recipients. Split liver transplantation (SLTx) may be one way of reducing deaths on waiting lists. Methods: Up to July 1995, 15 patients (12 paediatric, 3 adult) underwent 15 SLTx from 8 donor livers (7 left segmental grafts, 8 right grafts). 66% (10/15) of grafts were carried out as urgent or super-urgent procedures. Prior to bench splitting, cholangiography was performed to define segmental biliary anatomy. The left graft comprised segments II and III (n=7), and the right graft comprised segments IV-VIII in 3 cases and V-VIII in 5 cases. Logistical dificulties prevented the recipient procedures to be carried out in parallel, and all SLTx were done sequentially. The median cold ishaemia time (CIT) for the first graft was 697 (437-921) mins, and for the second graft was 1025 (902-1326) mins. An additional arterial reconstruction (donor iliac artery interposition or aortic conduit) was necessary in 6/15 grafts, and a donor iliac vein interpostion was required for only 1/15 portal venous anastomoses. The left segmental graft was implanted leaving the recipient IVC in situ, and the right graft implanted with the donor IVC. Biliary reconstruction was by means of a left hepaticojejunostomy (7), choledochojejunostomy (4), and choledocho-choledochostomy (4).
Results: The median day 1 AST was 908 (544-2345) iu/L. The overall patient survival was 73% at a median follow-up of 10 (1-33) months. There were four deaths, two related to poor initial graft function. One patient underwent regrafting at 7 months for chronic rejection, and two patients developed biliary strictures.
In conclusion, these results encourage increased use of SLTx. The sharing of split livers between centres may decrease CIT periods with consequent improved early graft function..

COMPARISON OF SHORT &LONG TERM OUTCOME OF KIDNEY (K) TRANSPLANTS (Txs) WITH SINGLE VS. MULTIPLE RENAL ARTERIES (MRAs)

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We compared, retrospectively, the incidence, risk factors and outcome of K Txs with single vs. MRA. **PATIENTS AND METHODS:** 998 adult primary or secondary K recipients were transplanted between 12/1985 and 6/1993. All grafts were anastomosed to the external or internal iliac artery. The average age was 44 ± 4.6 years; 62% of the patients (Pts) were male and 38% female; 504 received a cadaver and 494 a living related donor graft; 952 (95%0 were primary and 46 (5%) first reTXs. The Pt population was divided into 3 groups, according to the presence of MRAs and their reconstruction: **Group A**, a single donor artery with a single anastomosis (n=835) to the recipient (REC) vessel, either end-to-end (n=132), or end-to-side (n=703); **Group B**, MRAs (either on an aortic patch or after bench reconstruction on a single artery) with a single anastomosis (n=112) to the REC vessel; **Group C**, MRAs with multiple anastomoses to the recipient vessels (n=51). **RESULTS:** No significant difference between the 3 groups was found for Pt survival (p=0.39) and graft survival (p=0.90) as well as the incidence of postoperative ATN (p=0.73), hypertension (p=0.76), rejection episodes (p=0.21), and mean creatinine levels at 1, 3 and 5 years after Tx (p=0.45). The rate of urologic complications (ureteral obstruction, urinary leak) was not different between the groups (p=0.123). The overall incidence of vascular complications (thrombosis, stenosis, bleeding, aneurysm) was 4.6%, including 2.2% early (within 10 days postTX) and 2.4% late. The presence of MRAs with single or multiple anastomoses did not increase the incidence of early and late vascular complications (Group A = 4.3%, Group B = 14%, Group C = 3.9%) (p=0.395). Early vascular complications caused 20 graft losses (2% of entire series): arterial (n=4) and venous (n=10) thrombosis, bleeding (n=1), and infarction with patent TX vessels (n=5); no Pts died as a consequence of these complications. All renal artery (0.4% of entire series) and vein (1%) thrombosis occurred in Group A Pts and TX nephrectomy was required in all cases. The most common vascular complication in all groups was renal artery stenosis (2%);**CONCLUSIONS:** 1. K Txs with MRAs can be performed with results similar to those obtained utilizing grafts with single arteries. 2. MRAs with single vs. multiple anastomoses to the Recs vessels did not influence the vascular complication rate or graft outcome.

IMMEDIATE NEUTROPHIL INFILTRATION POST RENAL TRANSPLANTATION -RELATION WITH ADHESION MOLECULE INDUCTION, COLD ISCHAEMIA TIME AND EARLY GRAFT FUNCTION

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During the period of ischaemic storage of the donor kidney, a number of toxic substances accumulate within the graft, so that upon reperfusion the endothelium becomes susceptible to damage. In vitro models have shown that reperfusion injury involves neutrophil infiltration via selectin-mediated adhesion to endothelium.

An immunohistochemical analysis was performed on wedge biopsies obtained from transplanted cadaver kidneys (n=23) at two time points: (i) after perfusion and storage but before implantation and (ii) immediately before wound closure. For comparison with the cadaver group, pre-anastomosis biopsies were obtained from 12 LRD transplants and post-anastomosis biopsies were available from 4 of these transplants. Cryostat tissue sections were stained with mAb against P-Selectin, E-Selectin and leucocyte markers, using an indirect immunoperoxidase technique. Sections were evaluated and assigned semi-quantitative grades..

High levels of endothelial E-Selectin expression were observed in 65% of the pre-transplant biopsies from cadaver donors, whereas E-Selectin was absent from the LRD kidneys (P=0.002). P-Selectin expression was observed in only 19% of the pre-anastamosis biopsies. Again, no LRD grafts showed expression of P-Selectin.

Pre- and post-anastomosis biopsies of each patient were compared to determine changes which may occur following reperfusion. There were no obvious changes in the level of interstitial $CD3^+$ or $CD14^+/CD68^+$ leucocytes, nor in the transcriptionally dependent expression of E-Selectin. Upon reperfusion, a high incidence of neutrophil infiltration into the glomeruli was observed and this was significantly associated with P-Selectin induction on the microvascular endothelium (P=0.008). None of these changes were noted in any of the LRD patients. The incidence of neutrophil influx into the glomeruli was progressively linked to the length of cold ischaemia time, occurring in 100% of patients with CIT >30 hours (P=0.003). This influx was found to be related to graft function at 3 months; there was a greater incidence of neutrophil infiltration in patients with a serum creatinine level >165, compared with those with a lower level of creatinine (P=0.023).

Our results strongly suggest that a long cold ischaemia time is associated with an increase in the level of neutrophil infiltration after reperfusion. This early influx adversely influences graft function at 3 months, suggesting that the effects of reperfusion injury are more than immediate. We propose that by reducing the length of cold ischaemia we may reduce reperfusion injury and therefore improve long term graft function.

THREE YEAR EXPERIENCE OF KIDNEY TRANSPLANTATION FROM ASYSTOLIC CADVERIC DONORS

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As a result of a continuing shortage of donor organs for renal transplantation we have established a non-heart beating donor (NHBD) organ retrieval programme. This paper presents our experience over a 36 month period.

Asystolic donors were referred from two sources: patients dying following failed attempts at resuscitation in the A&E department (n=22) and from deaths on the medical wards as a result of intracerebral haemorrhage (n=2). In-situ kidney perfusion and cooling were achieved using an aortic double balloon triple lumen catheter inserted via femoral artery cut down.

The programme resulted in 44 kidneys being retrieved over the 3 year period. Thirty NHBD kidneys were transplanted locally, 8 were used in other UK centres and 6 kidneys were not used. The median (95% C.I.) warm and cold ischaemic times were 25 (20-29) mins and 16.5 (14.6-18) hours respectively. Donor ages ranged from 25 to 63 (median 48) yrs. Post-operative dialysis was required in all patients for a median interval of 22 days (range 7-63). Twenty-six transplants (87%) functioned with four primary non-functions (13%). The median (95% CI) serum creatinine at 24 months was 231 (169-302). There has been one graft failure due to rejection and one death with a functioning graft. One and two year graft survival rates were 91 and 87 per cent respectively. During the period under study 140 kidney transplants were performed in this unit and NHBD organs accounted for 21% of our total programme.

In conclusion, NHBD kidneys yield acceptable renal function and graft survival at two years follow-up and have proved a valuable additonal source of transplant kidneys.

WORK-LOAD GENERATED BY SETTING UP A NON-HEART BEATING KIDNEY TRANSPLANT PROGRAMME

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A non-heart beating donor (NHBD) kidney transplant programme was set up 3 years ago in an attempt to improve local transplant numbers. This report describes the total work-load which has been generated by the programme.

NHBDs were referred from 2 sources: the Accident and Emergency Department of a local hospital (failed resuscitations following cardiac arrest) and ward patients dying from intracerebral haemorrhage. Donor selection criteria included age <60 years and warm ischaemic time < 40 minutes. A rapid response retrieval team was established consisting of 1 or 2 transplant co-ordinators and 1-2 surgeons. The local A&E department is not in the same hospital as the Transplant Unit and the team had to travel to all the referrals made. Organsiation of a NHBD retrieval involves 5 stages: Referral by appropriate clinicians and mobilisation of the team, obtaining consent from relatives, in situ kidney perfusion, referral to the coroner and transfer to an operating theatre for the retrieval procedure.

Over a three year period a total of 73 referrals have been made, 64 from the A&E department and 9 from the wards. Organ procurement was performed in 24 cases (33%) and resulted in the retrieval of 44 kidneys. Reasons for failure to achieve organ procurement were: refused consent 13 (18%), relatives unavailable to ask for consent 9 (12%), technical problems with catheter insertion or perfusion 10 (14%), transplant staff unavailable 1 (1%), long asystolic period 8 (11%) and donor unsuitable for other reasons 8 (11%).

Of the 44 kidneys retrieved 30 were transplanted locally, 8 were transplanted at other centres in the UK and 6 were discarded or used for research because a suitably matched recipient could not be found. Locally transplanted NHBD kidneys represent 20% of the total transplant programme during the time period under study. NHBD kidneys are a good source of additional organs for transplantation but only one third of referrals result in a successful procurement procedure and the setting up of a programme is labour intensive and requires a highly committed staff.

CLINICAL AND PHYSIOLOGICAL RESPONSES TO TOTAL PARATHYROIDECTOMY AFTER RENAL TRANSPLANTATION

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Despite improvements in medical management of secondary hyperparathyroidism, parathyroidectomy is still necessary in some renal transplant recipients. The operative strategy in this situation remains controversial and in particular total parathyroidectomy has been criticised over concerns that the aparathyroid state will lead to adynamic bone disease and difficulties in therapeutic management. We describe our experience of total parathyroidectomy in a series of 21 renal transplant patients.

Total parathyroidectomy was performed either in the 5 year period prior to renal transplantation (n=15) or between 6 and 33 months following transplantation (n=6). The indications for surgery were severe bone disease, uncontrolled hypercalcaemia (tertiary disease), soft tissue calcification and grossly elevated PTH levels. Surgery consisted of 4 gland parathyroidectomy and included transcervical thymectomy where necessary. All patients have been followed up with regular biochemical profiles and skeletal surveys.

The clinical and biochemical success rates at 24 months follow up were 86 and 100 % respectively. Detailed skeletal surveys demonstrated evidence of partial or complete bone healing in 95% of patients followed to 2 years. Post-operative PTH levels (measured by a sensitive immunoradiometric assay) demonstrated residual parathyroid function in 17 patients (81%). There were no cases of persistent or recurrent hypercalcaemia in the series. Postoperative i.v. calcium infusion was necessary in 12 patients (57%). Vitamin D analogues were required in 81% of patients at 2 years follow up. There were no cases of recurrent laryngeal nerve palsy or permanent voice change after surgery.

There has been no evidence of adynamic bone disease and no therapeutic difficulties in any of our patients and the data presented suggests that total parathyroidectomy is a safe and effective procedure in dialysis patients who are awaiting a kidney transplant or in renal transplant recipients.

CAUSES OF GRAFT LOSS IN RENAL TRANSPLANT RECIPIENTS - TEN YEARS EXPERIENCE

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A successful outcome of renal transplantation is defined as long-term maintenance of normal renal function, complete patient rehabilitation and avoidance of major morbidity from the transplant operation and medical therapy aimed at preventing rejection. However, grafts are lost annually from various causes and reduction of graft loss is desirable and an attainable goal. We have reviewed retrospectively the causes of allograft loss from the cohort of renal transplant recipients grafted over the last decade with a view to identifying preventable factors contributing to graft failure. Over a period of 10 years between January 1985 and December 1994, 701 transplants (684 kidney and 17 combined kidney and pancreas) were performed and the causes of allograft loss is shown in the table below.

Causes of graft loss	Number
Acute rejection	68(30%)
Death with function	63 (27.8%)
Technical - vascular and ureteric problems	29 (12.8%)
Chronic rejection	28(12.4%)
Recurrent renal disease	14 (6.2%)
Hyperacute rejection	12 (5.3%)
Unknown	12 (5.3%)
TOTAL	**226**

226 out of 701 (32.2%) grafts have failed over the period of 10 years and acute rejection (30%) was the major cause of graft failure, followed by death with function (27.8%). 378 out of 701 (54%) patients had acute rejection episodes leading to graft failure in 68(18%) cases. The median time to graft loss due to acute rejection was 108 weeks posttransplant (range 1-417 weeks) and 42% of the grafts lost to this cause occurred within first 6 weeks posttransplant. The median time to graft loss from chronic rejection was 4.4 years (range 62 days - 9 years). 22 out of 28 (78%) of the graft lost from chronic rejection had undergone treatment for acute rejection episodes in the past (P=0.005). The major cause of death was cardiac in origin (39%) followed by septicaemia (23%) and malignancy (8%). There was no significant difference in the actuarial graft and patient survival under monotherapy and triple therapy immunosuppression. The actuarial graft survival was 85%, 65%, and 45% and the patient survival was 97%, 83% and 74% at 1, 5, and 10 years respectively.

Since acute rejection and death from cardiovascular cause are the leading causes of renal allograft loss, attention should be focused on these aspects in order to reduce graft loss. New immunosuppressive agents and regimens appear to offer a decreased incidence of acute rejection and may therefore promote long term graft survival.

EOSINOPHILIA IN RENAL ALLOGRAFT REJECTION

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Recent reports have suggested that significant eosinophilia within blood and transplant kidney is predictive of rejection with a poor prognosis. Most have been based on transplant kidney FNA and there has been no correlation with histological grades of rejection. This study was designed to determine whether graft eosinophilia is of prognostic significance, independant of grade of rejection, and if peripheral blood eosinophil counts are useful in monitoring response to anti-rejection therapy.

A SNOMED search revealed that of 413 renal transplant biopsies performed in our unit between 01/07/93 and 01/03/95, 165 showed acute rejection, 34 (21%) with a vascular component (Banff grade 2) and 7 biopsies from 6 patients were coded as containing a heavy infiltrate of eosinophils. In 5 of the 6 patients with graft eosinophilia biopsies revealed vascular rejection. All patients with acute vascular rejection were subsequently reviewed and follow up was available on 19. The presence of eosinophilia was found to be an adverse prognostic factor in this group; 9 of 10 patients with either blood or graft eosinophilia suffered severe / irreversible rejection episodes as compared to 4 of 9 patients without eosinophilia (p=0.05). Blood eosinophil counts fell rapidly with pulse steroid therapy in all but two patients, who had a persistent eosinophilia and developed active chronic vascular rejection within 6 months of transplantation.

We conclude that a heavy graft infiltrate of eosinophils is associated with vascular rejection and is a poor prognostic factor. Persistent blood eosinophilia may indicate progressive vascular rejection.

EVALUATION OF AN ELISA ASSAY FOR THE DETECTION OF HLA CLASS I SPECIFIC ANTIBODIES.

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We have evaluated the QUIKSCREEN ELISA kit, (GTI, Wisconsin) which uses HLA class I antigens, purified from a pool of donor platelets, immobilized on microtitre plates. Binding of HLA specific alloantibodies is detected by the addition of alkaline phosphatase conjugated anti-human IgG, IgM and IgA followed by p-nitrophenyl phosphate disodium as a substrate.

776 sera from renal and thoracic organ transplant patients were tested by both QUIKSCREEN and a standard complement dependent lymphocytotoxic assay (CDC). 655 (84.4%) sera gave concordant results of which 106 (13.7%) were concordant positives and 549 (70.7%) were concordant negatives.

In the discordant group 88 sera were negative by QUIKSCREEN and positive by CDC of which 83 were shown to be due to non- HLA or autoreactive antibodies. The remaining 5 sera contained 3 commonly assigned specificities (HLA-A1, A3, B8). 33 sera were positive by ELISA but CDC negative and these are under further investigation by flow cytometry for non-complement fixing antibodies.

13 HLA class I specific phenotyping sera (9 alloantisera, 4 mouse monoclonal antibodies) were similarly tested. The 9 alloantisera used for phenotyping (specific for HLA-A1, A10 + A34, A30, B7, B13 {2 sera} B62 and B22) were positive and as predicted the 4 monoclonal antibodies were negative.

In conclusion, we found the kit to be a rapid 'user friendly' method for screening large numbers of sera for the presence of HLA class I specific alloantibodies, although low representation of certain HLA antigens in the platelet pool may result in occasional false negative results. Use of the kit would enable the identification of sera which require further extensive screening, concentrating limited time and resources on a much smaller cohort of samples.

TUMOR NECROSIS FACTOR-α IN HUMAN CARDIAC TRANSPLANTATION.

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The presence of TNF alpha mRNA and protein was determined by *in situ* hybridisation using digoxygenin (DIG) labelled complementary DNA probes and immunohistology in 34 endomyocardial biopsy (EMB) specimens taken from thirteen patients at various times after transplantation. Cytospin preparations of Chinese Hamster Ovary (CHO) cells transfected with the Human TNF-alpha gene, were used as positive controls for both mRNA and protein product expression. Blood samples were taken after each biopsy and serum levels of the TNF alpha protein analysed using specific enzyme-linked immunosorbent assays.
TNF alpha mRNA transcripts were present in 22/34 EMB examined, of which eight also contained protein. In all cases protein expression in the graft was within the first six months of Transplantation, but did not relate to the grade of rejection in that or subsequent biopsies. In most cases serum levels of TNF alpha were undetectable (< 30 pg/ml). Of the 9 positive samples, 7 were obtained within the first 3 months. This early presence of TNF-alpha in the serum may reflect therapy with anti-thymocyte globulin. The lack of correlation between TNF alpha in the graft and rejection suggests that this cytokine is not critical in the rejection process, and that serum TNF alpha levels have no diagnostic or prognostic value in cardiac transplantation. This study has important implications in the use of cytokine serum analysis for the study of cytokine profiles within the tissue and ultimately in the prediction of cardiac allograft rejection.

T-LYMPHOCYTES INFILTRATING RENAL ALLOGRAFTS EXPRESS A LIMITED USAGE OF T CELL RECEPTOR Vβ GENES

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Background&Aim: Allograft rejection involves the recognition of foreign histocompatibility antigens expressed by the graft *via* T cell receptor (TCR). This recognition elicits a series of immune effector mechanisms that result in the destruction of the foreign graft. The preferential usage of certain TCR Vβ genes has been well established in several major histocompatibility complex (MHC) - restricted immune responses. However, Vβ usage among allogeneic responses remains unclear.

We have previously reported the predominant usage of certain TCR Vβ genes by allogeneic T cell clones and lines derived in vitro from mixed lymphocyte cultures between DA ($RT1^a$) rat responder and LEW ($RT1^l$) stimulator LN cells. In this report, we examined the clonality of the TCR β-chain gene repertoire involved in the rejection of renal allografts disparate for major and minor histocompatibility antigens in LEW-to-DA rats.

Methods: Orthotopic renal transplantation was performed using DA rats as recipients for LEW kidneys using microsurgical techniques. T cell lines were established from graft-infiltrating lymphocytes (GIL) that were isolated on day 5 postgrafting. Total RNA was extracted from established T cell lines and the reverse transcription-polymerase chain reaction (RT-PCR) performed using a universal Vβ and a Cβ oligonucleotide primers. The PCR product was cloned into pCR-script vector and the TCR β chain was characterised by direct sequencing of the plasmid DNA using the dideoxy chain termination method.

Results: Among established T cell lines, only four Vβ gene families 3.1, 5.1, 8.3, and 16 were expressed. A direct sequencing analysis of 14 cDNA cloned TCR V-(D)-J regions revealed that 60% of T cells expressed a member of the Vβ 3.1 gene family. A smaller, yet significant number (20%) of cells expressed TCR using Vβ 8.3. In contrast to a predominant usage of Vβ, six different Jβ were used and a high degree of N-region sequence diversity was identified by expanded T cells.

Discussion: The results of the present study demonstrate that Vβ 3.1 is the dominant Vβ segment in this graft model. we do not know whether the Vβ 3.1 positive T cells play a role in the rejection process. in addition to the expression of Vβ 3.1, we also detected significant elevation in the level of Vβ 8.3, which we previously reported in the established kidney alloreactive T cell lines. Taking all the results together, there is clearly restricted Vβ usage in renal allograft-infiltrating lymphocytes. This may provide the opportunity for prevention of renal allograft rejection by specific immunosuppression with anti-Vβ antibodies.

PROLONGED SURVIVAL OF PIG CARDIAC XENOGRAFTS IN PRIMATES

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Controlling complement activation may provide a method of avoiding hyperacute xenograft rejection. One strategy for inhibiting cross species complement activation is to produce animals transgenic for human regulators of complement activation (RCA). We have produced pigs transgenic for one such RCA - Decay Accelerating Factor (DAF). Hearts from heterozygous offspring of founders expressing supra human levels of DAF both on their tissues and endothelial cells have been transplanted heterotopically into unmodified Cynomolgus monkeys (N = 8). None of these hearts were hyperacutely rejected. Survival in these untreated monkeys was for a median of 5.1 days. Histology at the time of rejection showed a variable picture of cellular and humoral rejection . Controls (N = 10) survived for a median of 1.6 days. Histology showed classic humoral rejection. Hearts from DAF transgenic pigs have been transplanted into immunosuppressed Cynomolgus monkeys (N = 10). Immunosuppression was Neoral to trough levels of 300 ng/ml, Cyclophosphamide on alternate days sufficient to reduce the white cell count (but not below 2 x 10^6/ml) and steroids at 1 mg/kg reducing to 0.1 mg/kg by day 20. Mean survival is currently >35 days with 2 hearts still beating. Normal pig hearts transplanted into immunosuppressed monkeys were hyperacutely rejected.

Conclusion : Hearts from pigs transgenic for human DAF are not hyperacutely rejected by Cynomolgus monkeys. Long term survival can be achieved with clinically applicable immunosuppression.

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THOMSEN-FRIEDENREICH AND P^k ANTIGENS IN PIG-TO-HUMAN XENO-TRANSPLANTATION

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Galili antigens (Galα1-3Galβ1.....) are of major importance in pig-to-human xeno-transplantation. Absorption and inhibition studies using these antigens suggest however that other antigen/antibody systems may be important. Human IgG and IgM bind not only to Galili glycolipids from pig aorta but also to Galα1-4Galβ1-4Glc (P^k) and to Galβ1-3GalNAc (Thomsen-Friedenreich, 'T'). Whilst humans are known to have IgG and IgM anti-T, natural anti-P^k antibodies have not previously been described. We have tested 12 human sera (3 from each of the 4 ABO blood groups) in a P^k-BSA ELISA. All sera had IgG and IgM anti-P^k, titre $\geq$ 1 in 64. In addition, antibodies adsorbed from human serum onto P^k and T immunoadsorbents (ChemBioMed) have been eluted and found by ELISA to bind to pig aortic endothelium in primary culture. Using monoclonal antibodies or the above eluates, we have to date demonstrated the presence of T antigen in pig pancreatic islets and within the medulla of pig kidney, and P^k antigen on pancreatic vessels. Both specificities therefore belong to the human natural antibody repertoire. T and P^k antigens should not be overlooked in designing strategies for pig-to-human xenotransplantation.

DEVELOPMENT OF SPECIFIC IMMUNO-ADSORBENTS FOR THE REMOVAL OF XENOREACTIVE HUMAN ANTIBODIES.

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Galili antigens (which terminate in the disaccharide Galα1-3Gal) are established as major xeno-antigens on pig endothelium. We and others have shown that toxic concentrations of soluble Galili antigens can block binding of human natural antibodies to pig endothelium. For future clinical use extra-corporeal antigen-specific immunoadsorption may be a more effective and less toxic method. We have developed a Galα1-3Gal di-saccharide-sepharose column and tested its ability to remove Galili-reactive antibodies. An ELISA was used in which the solid phase antigen was either the terminal di- (Galα1-3Gal), tri- (Galα1-3Galβ1-4GlcNAc) or full penta-saccharide (Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc), which is found in pig vascular endothelium. Results are shown in the table, and expressed as the number of sera binding to the antigen post-immuno-adsorption/number binding pre-adsorption.

	Antigen		
Ig Class:	**di-saccharide**	**tri-saccharide**	**penta-saccharide**
IgM	0/11	2/11	4/11
IgG	0/4	0/4	7/8

Di-saccharide was therefore insufficient to consistently remove anti-pentasaccharide. In addition we have shown that di-saccharide sepharose is also insufficient to remove all natural antibody binding to pig aortic endothelial cells in ELISA, and we have therefore developed tri-saccharide and pentasaccharide columns. Preliminary data show them capable of removing all anti-penta-saccharide antibody from 5 sera tested to date. Although the biological role of these antibodies is yet to be defined, this study shows that we have developed specific immuno-adsorbents capable of removing all known anti-Galili antibodies.

Complement Inhibition Using Soluble Complement Receptor 1 (sCR1) in the Allograft Immune Response

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Complement (C) promotes the activation of B and T cells, and mediates the effector arm of the humoral immune response. To examine the possible influence of C on the allograft response, we investigated an unsensititized model of rat renal allograft rejection, using sCR1 to inhibit C at the level of the C3 and C5 convertases. Lewis to DA renal transplant recipients were treated with 25mg/kg sCR1, or saline (control) daily (n=15 in each group), and sacrificed on days 1-5 post transplant. Transplant organs were examined histologically, and by semi-quantitative immunochemical analysis. Splenocytes were double stained for activation (*CD25; IL-2R*) and subset markers, and analyzed by FACS. The generation of the alloantibody response was measured by determining Ig binding to donor type CD5 T cells using flow cytometry.

Treated rats displayed >90% inhibition of plasma C activity (CH_{50}), and a marked reduction in tissue C3 and C5b-9 deposition. In controls v treated animals, sCR1 produced a reduction in the area of infiltrating leukocytes within the grafts at 5 days post transplant, as follows: *CD45*, 69 ± 7.4 v 49 ± 10.7%; *CD5*, 14.6 ± 6.4 v 6.8 ± 2.7%; *CD4*, 11.4 ± 1.7 v 7.4 ± 1.8%; *CD8*, 8.5 ± 0.9 v 4.8 ± 1.0 %; *CD25*, 14.9 ± 3.9 v 10.1 ± 3.5%; and *CD11b* macrophage/dendritic cells, 25.7 ± 5.3 v 12.3 ± 4.7%. There was a striking reduction in vascular injury (endothelial lifting and swelling, thrombosis, haemorrhage and infarction) in the treated animals. Analysis of per cent of splenocyte subsets expressing IL-2R in control v treated rats showed the following changes by day 5: *CD5*, 79.1 ± 4.1 v 37.9 ± 7.8%; *CD4*, 71.7 ± 8.08% v 47.4 ± 3.6%; *CD8*, 55.7 ± 10.5 v 46.4 ± 10.2% ; and *CD45 B cell specific isoform*, 71.5 ± 6.6 v 53.2 ± 11.9%. Preliminary data show binding of alloantibody to donor type CD5 cells was detectable in both groups from Day 1, and increased in the untreated group from Day 4 to a greater degree than in the sCR1 treated animals.

The results suggest that C inhibition with sCR1, in an unsensitized model of allograft rejection, was able to suppress the vascular and cell mediated components of tissue injury. The data support not only a role for C in antibody and possibly cell mediated cytotoxicity in the graft, but also suggest a role in the primary immune response leading to both T cell and B cell activation.

TOLERANCE INDUCED BY DONOR BONE MARROW AND SYNGENEIC BONE MARROW EXPRESSING A SINGLE DONOR CLASS I MHC MOLECULE

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Bone marrow cells (BMCs) may be a useful vehicle for pre-transplant alloantigen delivery to induce tolerance. Administration of donor BMCs has been shown to prolong graft survival in human and animal models. Moreover, BMCs can promote a state of macro or microchimerism which may be important both in the induction and maintenance of tolerance. In this study, we have investigated the ability of BMCs expressing a single donor MHC class I molecule to induce specific unresponsiveness in vivo.

Two main protocols were investigated. Recipient CBA ($H2^k$) mice were either given BMCs alone iv at various times before a heterotopic C57/BL10 ($H2^b$) cardiac transplant (protocol A), or BMCs (day -27) in combination with an anti-CD4 monoclonal antibody YTA3.1 at day -28 and -27 before transplantation on day 0 (protocol B). BMCs were either fully allogeneic from C57/BL10 donors or from transgenic CBK mice (CBA+K^b) which express the donor class I molecule K^b as a transgene.

When $5x10^6$ fully allogeneic, donor ($H2^b$) BMCs were used alone (protocol A), they were ineffective when given 14 days before or at the time of transplantation. In contrast, long term graft survival (LTGS) was achieved in 66 and 75% of recipients when the same dose were given 27 and 42 days before transplantation. Interestingly, when the ability of CBK ($H2^k$+ K^b) BMCs to induce LTGS was evaluated using protocol A, they were found to be more effective. $5x10^6$ as well as $5x10^7$ CBK BMCs were able to induce 100% LTGS when administered 14 or 27 days before transplantation, When pretreatment with BMCs was combined with anti-CD4 (protocol B), doses as low as $5x10^5$ cells were found to be effective with both donor and CBK bone marrow. Again, CBK BMCs were found to be relatively more effective in inducing LTGS in 100% of recipients.

In conclusion, we have found that BMCs are suitable vehicles for alloantigen delivery before transplantation either alone or in combination with anti-CD4 monoclonal antibody therapy. Pretreatment with BMCs expressing a single donor class I molecule is sufficient, and in this model, may be more effective than when donor BMCs are used. The addition of anti-CD4 to the pretreatment protocol reduces the number of BMCs required to achieve LTGS by 100 fold. These data confirm that it is not necessary to expose the recipient to the full complement of donor major and minor histocompatibility antigens to induce unresponsiveness.

ANTI-ENDOTHELIAL CELL SURFACE ANTIBODIES IN SERA FROM CARDIAC AND RENAL TRANSPLANT RECIPIENTS.

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While the aetiologies of recurrent coronary artery disease (CAD) after cardiac transplantation and chronic rejection following renal transplantation remain ill defined, it has been hypothesised for both organs that an early initiating event causes injury to graft endothelial cells leading to a generalised arteriosclerosis. There are also strong similarities in the pathology of both conditions. In this preliminary study we used FACS analysis to look for evidence of anti-endothelial cell surface antibodies (anti-EC) in sera from both cardiac and renal transplant recipients pre and post transplantation. Of 23 cardiac recipients, who subsequently developed coronary artery disease (CAD), 61% had anti-EC antibodies of IgM isotype and 13% of IgG isotype post transplantation.of 15 cardiac recipients who did not develop CAD, 14% had IgG anti-EC antibodies and 14% IgM anti-EC antibodies post transplantation. There was little evidence for the presence of IgG or IgM anti-EC antibodies in cardiac recipients pre transplantation. Of 5 renal transplant recipients whose transplants failed due to chronic rejection 60% had IgG anti-EC antibodies and 40% IgM anti-EC antibodies post transplantation. Of 4 renal transplant recipients whose long term transplant failure could not be attributed to any known immunological cause, 25% had IgG anti-EC antibodies and 75% IgM anti-EC antibodies post transplantion. There was no evidence of anti-EC antibodies in sera from 5 patients with normally functioning grafts nor in sera from 2 patients whose grafts had undergone acute rejection. The anti- EC antibodies detected do not appear to be anti-HLA or anti-blood group antibodies. All sera were negative for common IgG autoantibodies although 4.5% of cardiac sera were ANCA positive. The potential significance of these anti-EC antibodies to the development of chronic rejection after renal transplantation and to CAD following cardiac transplantation will be discussed.

TRAVEL

The Royal College of Physicians is located in the South East corner of Regent's Park, close to Marlebone Road in London.

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