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# The British Transplantation Society

# LIVERPOOL

## 10th & 11th April 1990

Black-haired late 20s.

Single lung (20) transplants none are anaemic

PAPER 1

## PERSISTENT ANAEMIA AFTER HEART AND LUNG TRANSPLANTATION

Beverley J. Hunt, Ozay Halil, Sheela Amin and M. Yacoub.
Harefield Hospital, Middlesex, England.

We have noted a high prevalence of persistent anaemia in recipients of heart and lung transplantation (HLT), some requiring regular transfusion. Eighty-five currently surviving adult HLT recipients who received a transplant more than six months previously have been studied. All the patients receive cyclosporin and also azathioprine if it is tolerated.

Pretransplantation mean (+/–S.D.) haemoglobin (Hb) levels were 149(+/–33)g/l with mean MCV 86.6 (+/–3.9)fl. Post-transplantation mean Hb at six months were 108.3 (+/–13)g/l with mean MCV of 93.7 (+/– 6.6)fl; 27% of the patients had Hb levels less than 100g/l. A year post-transplantation 22% of the patients were running a steady haemoglobin level of between 6–10 g/l. At 18 months 25% still had steady-state Hb levels of less than 100g/l.

Further investigation, including erythropoeitin levels, showed (in all but two patients who had sideroblastic changes) the changes of anaemia of chronic disorders, although blood films and bone marrow aspirates also showed dyshaemopoietic changes which were more marked if the patient was receiving azathioprine.

In conclusion persistent anaemia is common after HLT. Although some of this anaemia may be due to azathioprine effect, the majority of patients also have an underlying anaemia which has some of the changes of anaemia of chronic disorders. The possible pathogenesis of these anaemias will be discussed.

ESR 35-118 all raised
TIBC ↓
Fer N/+
B12 & folate →
Reics <2%
Coombs neg
40% weakly pos auto antibodies

Approp EP for stan Hb
Marrow: dyserythro- & myelopoesis, haemopoesis

1. Anga.
2. Dyserythropoesis
3. "Anaemia of chronic disorders."

Azathioprine stopped at 4000 WBCs.
Pts not on aza had sign higher MCV

12

2yr surv approaches 70% falls off
Infection is main cause of death
Dense fibrosing aveolovitis: thought to be due to injection ∴ poss response to treatment

PAPER 2

## CELLULAR RESPONSES AFTER TREATMENT OF ACUTE LUNG REJECTION (ALR) IN HEART-LUNG TRANSPLANTS (HLT)

C.A. Clelland, T.W. Higenbottam, J.P. Scott, S. Stewart and J. Wallwork.
The Heart-lung Transplant Research Unit, Papworth Hospital, Cambridge CB3 8RE.

A clinical diagnosis of ALR in HLTs is confirmed by typical perivascular lymphocytic infiltrates on transbronchial lung biopsy (TBB). When bronchoalveolar lavage (BAL) is performed at the same time as TBB at fibreoptic bronchoscopy during ALR increased numbers of lymphocytes are recovered. We have followed up these observations by assessing alterations in profiles of inflammatory cells on TBB and BAL* before and after pulsed steroid treatment in 28 episodes of ALR in 21 HLT patients (*pairs of BAL samples obtained on 14 of the 28 occasions). Follow-up TBBs and BALs were taken about 3 weeks (mean 23.5 days) after the diagnosis was made and treatment instituted. The infiltrates on TBB were assessed semi-quantitatively on a scale of –, + or ++ and differential cell counts of BAL were made on stained cytocentrifuge preparations.

| | Cells on TBB | | % BAL cells recovered | |
|---|---|---|---|---|
| Cell type | ALR | TR | ALR | TR |
| Lymphocytes | 1.32 (0.66) | 0.75 (0.51) | 17.1 (17.3) | 7.4 (6.6) |
| Neutrophils | 0.61 (0.67) | 0.39 (0.62) | 14.4 (24.8) | 5.6 (13.0) |
| Eosinophils | 0.50 (0.63) | 0.18 (0.54) | 0.6 (1.8) | 0.2 (0.8) |

Data shown as mean (±SD) of semi-quantitative analysis of inflammatory cells on TBB and percentage of cell types in BAL. There is a general reduction in all cell types after treatment of ALR on both biopsy and lavage. The reductions are of a similar order and it appears that the BAL cell profile reflects the inflammatory infiltrate sampled at TBB.

Rejection = perivas. infiltration with plump lymphocytes, deposits of haemosiderin which is slowly removed.
The perivas infiltrate diminishes with anti rej therapy

People with frq acute rejection: multiple rejection: should be treated intensively
Nbd any info about matched — presumbed not mate
Sens. patients? — doesn't know

13

PAPER 3

## ASSESSMENT OF THE HAEMOSTATIC RISK FACTORS FOR THE DEVELOPMENT OF ISCHAEMIC HEART DISEASE IN HEART TRANSPLANT RECIPIENTS

Beverley J. Hunt, Helen Segal & M. Yacoub. Harefield Hospital.

Epidemiological studies have shown that in the normal population the risk of ischaemic heart disease (IHD) is increased in those with high fibrinogen and factor VIIc. Factor VIIIc has also shown an association. These haemostatic variables were assessed in 114 heart transplant recipients (age range 19-63) who were transplanted more than one year previously, as they have a high risk of accelerated coronary sclerosis.

The transplant recipients had higher fibrinogen levels (4.49+/–1.04g/l compared with age-matched healthy controls (3.20+/–78g/l, p<0.001), factor VIIc (1.09 +/– .29u/ml v 1.0+/–.2u/ml, p<0.05), factor VIIIc (1.05 +/–.4u/ml v 0.88+/–.2u/ml, p<0.01). Twenty-three who had required transplantation for a cardiomyopathy (age range 19-54) had similar Factor VIIc and VIIIc levels to the healthy controls, but still had significantly higher fibrinogen levels (p<0.05). Patients who were originally transplanted for IHD, were compared to a control group of patients with IHD (age range 45-68). Again levels were significantly higher in the transplanted group; fibrinogen (4.8+/–.7g/l v 4.0+/–.83g/l), factor VII and factor VIII levels (1.10+/–.4g/l v .90+/–.32g/l, p<0.001).

The 26 recipients with accelerated coronary sclerosis had the highest levels of Factor VIIc and factor VIIIc (p<0.05).

In conclusion, fibrinogen levels are raised after heart transplantation, and factor VIIc and VIIIc are increased in those transplanted for IHD. Transplant recipients with accelerated coronary sclerosis had the most marked prothrombotic changes.

16% of pts treated with aza + cyA



PAPER 4

## ENDOTHELIAL CELL HAEMOSTATIC FUNCTION IN HEART TRANSPLANT RECIPIENTS.

Beverley J. Hunt, Helen Segal & M. Yacoub. Harefield Hospital.

Endothelial cells take an active part in haemostasis, producing von Willebrand's factor (vWF), antithrombin III (ATIII), tissue-plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI). Poor fibrinolytic activity and high levels of PAI and vWF have been associated with atheromatous coronary artery disease. In view of the high incidence of accelerated coronary sclerosis in heart transplant recipients we have measured these endothelial products in 114 heart transplant recipients (HTR) receiving cyclosporin +/– azathioprine, after their first post-transplant year.

The HTR had raised vWF antigen (ELISA) levels with a mean (+/–S.D.) of 1.94 (+/–.96) I.U/ml versus levels of 1.00 (+/– .313) (p< 0.001) in 36 age matched controls. ATIII (I.L. chromogenic assay) levels were also increased at 1.13 (+/– .31) I.U/ml v. controls 0.99 (+/– .19) I.U/ml (p<0.01).

Fibrinolysis was studied in 60 HTR: mean t-PA antigen levels (Biopool) were raised at 8.2 (+/– 3.5)ng.ml versus the control group at 4.4 (+/– 1.97 (p0.001), as was functional plasma PAI activity (Kabi Diagnostics) at 11.4(+/– 6.6) A.U/ml versus 6.8 (+/– 2.9) A.U/ml (p<0.001) The net effects of these increased levels were decreased fibrinolysis for euglobulin clot lysis time in 114 heart transplant recipients showed mean times of 313 (+/–79) mins v. 195 (+/–101) mins in the controls (p<0.001).

In conclusion there is pertubation of endothelial cell haemostatic function after heart transplantation. The aetiology and implications of these findings will be discussed.

PAPER 5

## LONG-TERM IMPROVEMENT IN RENAL FUNCTION USING NIFEDIPINE IN CYCLOSPORIN-ASSOCIATED HYPERTENSION

A.J.B. Kirk, I. Omar, J.H. Dark, Freeman Hospital, Newcastle upon Tyne.

We have previously reported the beneficial effect of nifedipine in protecting against the nephrotoxic effects of cyclosporin (CyA) in our heart, lung and heart-lung recipients.* This study was carried out using the same cohort of patients to investigate the long-term effects of nifedipine therapy.

The patients in the original NIFEDIPINE treated group (n=17) continued on that therapy. Of those who were receiving PRAZOSIN (n=29), only 12 remained on this at long-term review.

Late follow-up was at a mean period of 28 months post-transplant. Creatinine clearance in the PRAZOSIN group was significantly worse than the pre-transplant values, as described previously. (63.9 ml/min [SE 5.3] v 51.9 [1.9]; $p<0.05$).

Whilst no significant difference was noted at 12 months follow-up in the NIFEDIPINE treated group, at late follow-up creatinine clearance had improved. (66.7 [6.7] v 75.6 [3.9]; $p<0.05$). No difference in CyA levels, dosage or total dose were noted between the two groups.

These findings of improved renal function using nifedipine over a prolonged period of time are in keeping with the results of an unpublished prospective cross-over study using nifedipine. In view of this effect, nifedipine remains our drug of choice in the treatment of CyA-associated hypertension in our heart and lung transplant patients.

* A.J.B. Kirk , I. Omar, D.N. Bateman, J.H Dark.

TRANSPLANTATION 1989, 48;428–430

Prazosin treated pts renal function after 1yr had deteriorated
longer term review 28mths Ccr had deteriorated in Prazosin treated and CyA had improved

Now doing prosp cross over study Nif seem to be doing better in terms of renal function and BP control. Nif has no effect on Cy blood levels



PAPER 6

## LIMITED ABH EXPRESSION IN THE HUMAN HEART

Beverley J. Hunt, [1]Susan J. Thorpe and Magdi Yacoub.

Harefield Hospital & [1]National Institute for Biological Standards and Control, Potters Bar, Herts.

It is unclear from previous work as to the extent of ABH expression in cardiac tissue. Due to the shortage of suitable heart transplant donors it would be ideal not to match donor and recipient for ABO groups. We have therefore assessed ABH expression in cardiac tissue by immunofluorescence and alkaline phosphatase immunostaining using polyclonal and monoclonal antibodies against the A, B, and H antigens and the H antigen specific lectin Ulex Europaeus. Cryostat sections of fresh, frozen samples of ventricles, atria, pulmonary artery and aorta from donors and recipients taken during heart transplantation were studied. The tissues were from eight donors and eight recipients of whom 6 were blood group A, 2 were group AB, 6 were group 0 and 2 were group B.

The endothelial cells lining the endocardium pulmonary artery and aorta, and the mesothelial cells in the surface of the epicardium were intensely stained for blood group antigens in accordance with the patient's blood group, as were the endothelial cells lining blood vessels within the cardiac tissue. In addition histiocytes present in the endomysium were strongly stained. However the cardiac muscle itself was unreactive with all the reagents tested. There were no obvious differences in blood group expression between donor and recipient tissue.

In conclusion ABH expression within the heart is limited. This may partially explain why some heart transplants have occasionally been successful in crossing major ABO barriers.

PAPER 7

## HAMSTER TO RAT XENOGRAFTS ARE NOT REJECTED PRIMARILY BY T CELLS

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Organ transplantation between hamster and rat represents an example of a concordant species difference with vascularised hamster hearts being rejected by untreated rats in 2.7 days. The aim of these experiments was to investigate the mechanisms of rejection in this hamster to rat model.

Monoclonal antibodies against rat T cell subsets demonstrated that CD4 positive cells proliferated in mixed lymphocyte culture of rat responders to allogeneic and xenogeneic stimulators. This rat did not need rat accessory cells present in culture to proliferate to hamster stimulators. Rat responders, needed rat accessory cells in culture to proliferate to human stimulators.

Monoclonal antibodies were used *in vivo* to deplete recipient rats of CD4 positive cells. This therapy given biweekly for 28 days prolonged survival of neonatal heart grafts in the high responder rat combination of DA to Louvain [median survival time = 38.5 days, 1/3 survival beyond 100 days.] This protocol in the hamster to rat xenograft model extended survival beyond control values but unlike the allografts, all xenografts were rejected during antibody therapy [MST=14.9 days]. A series of different protocols including antibodies to rat T cell receptor, the CD8 molecule, or Cyclosporine A therapy did not improve these results.

Lytic antibodies to hamster cells increased from a titre of 1/8 to 1/16,384 by 7 days after vascular heart transplantation. Cobra venom factor, which depletes the C3 component of complement, injected on alternate days at 0.125mg./kg. significantly increased survival of vascularised hearts in rats [MST of control = 2.7, MST of CoF = 5.3]. Injection of either Cyclosporine A or anti CD4 monoclonals to the CoF led to further substantial improvement in survival time.

These data suggest that rejection of hamster hearts by rats is dependent on humoral as well as cellular immune mechanisms.



PAPER 8

## IMMUNOREGULATORY ROLE OF NEWBORN SPLENOCYTES AND THYMOCYTES ON CELL-MEDIATED IMMUNITY AND TOLERANCE INDUCTION

A. Skowron-Cendrzak, B. Mazur-Kolecka, M. Kubera, M. Bubak-Satora, M. Garlicki.
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Splenocytes of newborn syngeneic or allogeneic animals less than two days old significantly prolong skin and heart allograft survival and reduce delayed hypersensitivity in adult mice and rats. This suppressive effect can be abolished by the syngeneic or allogeneic thymocytes of 4 to 6-day-old animals. Indomethacin-sensitive newborn splenocytes also facilitate the induction of either transplantation tolerance in an allogeneic system, or specific hyporeactivity in a xenogeneic one, whereas appropriate immunoregulatory thymocytes abrogate these effects.

There is a functional interdependence between the thymus and the spleen of newborn animals. Early thymectomy prolongs the suppressive activity of splenocytes, while neonatal splenectomy delays the immunoregulatory effect of thymocytes.

PAPER 9

## INTRACELLULAR SPECIFIC CYCLOSPORIN A-BINDING PROTEIN(S) IN CsA THERAPY MONITORING

Vera Hasková, Ladislava Rozprimová, Jana Svobodová.
Institute for Clinical and Experimental Medicine, Prague, Prague.

Cyclosporin A (CsA) has significantly improved the success rate of clinical transplantation. It is known that its immunosuppressive activity is not mediated by a membrane-associated mechanism but, by an intracellular process. One or several CsA-binding proteins are assumed to concentrate CsA in the cytosol thus probably mediating its effect.

In our experiments, we isolated and characterised a non-immunoglobulin fraction from calf tissue which was found very active in binding CsA $^{3}H$ tracer of the Sandimmune-RIA-kit. It contains the natural, intracellular receptor, specifically binding to the active site of the CsA molecule, which may also play a part in the immunosuppression of human immunocompetent cells. This material with a high selectivity could be used, instead of specific monoclonal or polyclonal antibodies, to determine CsA levels in patients' blood.



PAPER 10

## CHARACTERISATION OF TISSUE MACROPHAGES AND INTERSTITIAL DENDRITIC CELLS AS TWO FUNCTIONALLY DISTINCT PASSENGER LEUCOCYTES

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Immunohistological studies with a mouse anti-rat macrophage monoclonal antibody (BMAC-5) demonstrated the presence of numerous positive cells in the interstitial connective tissues of many organs. The pattern in heart was virtually identical to that seen with antibodies to class II MHC antigens, and could easily be misinterpreted as staining of interstitial dendritic cells. However, we performed double labelling fluorescence studies in rat heart using the BMAC-5 monoclonal antibody in combination with rabbit antisera to pure rat class II MHC antigens and pure rat leucocyte common (CD45) antigens.

Rhodamine labelled goat anti mouse immunoglobulin and fluorescein labelled goat anti rabbit immunoglobulin were used as the fluorescent probes. The tissue macrophages were identified as cells which were BMAC-5 positive, MHC class II negative and, leucocyte common antigen positive. They could be distinguished from the BMAC-5 negative, MHC Class II positive, leucocyte common antigen positive interstitial dendritic cells. Seven days following lethal irradiation, the class II positive interstitial dendritic cells had completely disappeared from heart, whereas the BMAC-5 positive macrophages were present in undiminished numbers. These studies strongly suggest that the interstitial dendritic cell and the tissue macrophage represnt two distinct populations of leucocyte within the connective tissues of antigenically secluded organs such as the heart. The marked difference in the period of residence in the tissues and the probable functional differences between these two types of passenger leucocyte are likely to have important implications for transplantation.

PAPER 11

## THE PREVALENCE AND NATURE OF CHRONIC LIVER DISEASE IN GLASGOW'S RENAL TRANSPLANTATION RECIPIENTS

E. McGregor, [1]M.C. Allison, [2]A. Mowat, [3]E. McCruden, [1]P.R. Mills, [2]R.N.M. MacSween, B.J.R. Junor, J.D. Briggs.

Renal Unit, [1]Department of Gastroenterology, [3]University Department of Virology, [2]University Department of Pathology, Western Infirmary, Glasgow, G11 6NT.

Renal transplant recipients are at risk of developing chronic liver disease. A review of over 600 transplant recipients has identified 37 patients with persisting or recurrent elevation of serum transaminases. None had hepatitis B surface antigen and testing for antibody to HCV (HCV-Ortho diagnostics) in 29 pre-transplant sera showed 7 (24%) to be positive. Five patients subsequently lost HCV antibody and two, who were initially seronegative, developed anti-HCV after transplantation. Liver histology was available in 25 patients, including 7 with past or present HCV antibody. Hepatitis was histologically present in 12 cases, 4 of whom had past or present anti-HCV. Nodular regenerative hyperplasia was the principal abnormality in 5 patients, one of whom had HCV antibody while iron deposition was frequently observed and was severe enough in 7 cases to warrant venesection. Only one death occurred as the result of liver failure. Thus chronic liver dysfunction in renal transplant recipients has a variety of causes and while an important complication, it carried a low mortality.



PAPER 12

## RENAL ALLOGRAFT REJECTION: FUNCTIONAL IMPAIRMENT OF KIDNEY EPITHELIAL CELL MONOLAYERS MEDIATED BY CYTOTOXIC CELLS

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A line of human kidney epithelial cells was cultured to confluency on filters and the formation of tight junctions was monitored by measuring the trans-monolayer electrical resistance. Typical monolayers developed functioning tight junctions within 4 days and showed an increase in resistance of 1840±440Ω (mean ± sd; n=5).

Conventional $^{51}Cr$ release assays showed that after 4 hours only 21% of suspended kidney cells were specifically lysed by lymphokine activated killer (LAK) cells at an effector to target cell ratio of 50:1. However, when LAK cells were added to functioning kidney cells monolayers at an effector to target cell ratio of 5:1 the monolayers showed a rapid and complete loss of resistance. In the absence of transmonolayer resistance the ion gradients essential for kidney function will not be supported. These results indicate that the ability of cytotoxic effector cells to lyse suspended $^{51}Cr$ labelled kidney cells may not be a sensitive indicator of the ability of effector cells to impair function of structured kidney cell monolayers.

The rapid improvement in renal allograft function which frequently occurs after suitable immunosuppressive therapy of recipients suffering acute rejection suggest that loss of graft function may occur in the absence of widespread kidney cell lysis. Failure of trans-epithelium resistance may be responsible for such reversible kidney dysfunction.

PAPER 13

## CYTOMEGALOVIRUS INFECTION IN PATIENTS TREATED WITH OKT3 FOR STEROID RESISTANT CELLULAR REJECTION IN KIDNEY TRANSPLANT RECIPIENTS

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Cytomegalovirus infection is a well known complication of renal transplantation, mainly related to immunosuppressive therapy. In our transplantation, we have been using monoclonal antibody OKT3 for the treatment of steroid resistant cellular rejection.

Since 1987, 26 patients have received OKT3. All patients were tested by a complement fixation method or ELISA for the presence of anti CMV antibodies preoperatively and at regular intervals post operatively. Serum from all kidney donors was also tested for the presence of anti CMV antibodies.

In all, 26 patients received OKT3. This group can be divided into 4 groups according to recipient/donor CMV status.

| | No. of Patients |
|---|---|
| Donor pos Recipient neg | 6 |
| Donor neg Recipient pos | 4 |
| Donor pos Recipient pos | 4 |
| Donor neg Recipient neg | 9 |
| Incomplete data | 3 |

Of the six patients who were CMV negative pretransplant and received a CMV positive kidney, 5 patients seronconverted with an acute CMV illness. The sixth patient remained well. Of these, 3 patients died, two of whom had overwhelming CMV on autopsy. The third patient had no autopsy. The other two patients developed an acute CMV illness but recovered. This data strongly suggests that OKT3 should not be used in patients who are CMV negative pre-transplant and who receive a CMV positive kidney.



PAPER 14

## ONTOGENY OF MHC ANTIGENS IN THE HUMAN FOETAL PANCREAS AND THE EFFECTS OF TISSUE CULTURE ON THEIR EXPRESSION

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Human foetal pancreas (HFP) is a possible source of transplant material in the treatment of diabetes mellitus. However, its use is restricted as rapid rejection follows allogenic transplantation. To investigate the mechanisms involved, we analysed the HFP to determine the ontogeny of MHC antigens and to assess the effect of tissue culture on their expression.

49 HFPs aged 9–17 weeks were studied by the immunoperoxidase technique using monoclonal antibodies.

In fresh HFP: (1) Class I and Class II positive cells are present from 9 weeks onwards. (2) Class I positive cells include mesenchymal macrophage/dendritic cell series. (4) Ductal and endocrine cells are negative for both Class I and Class II. After one week in culture: (1) Class II positive cells are significantly reduced. (2) Density of Class I expression is increased. (3) Endothelial cells persist and express Class I.

The study indicates that HFP is likely to be immunogenic from an early age; tissue culture leads to loss of Class II positive dendritic cells, but does not abrogate the rejection process, probably because of the persistence of endothelial cells which can act as antigen presenting cells (APCs).

PAPER 15

## IMPORTANCE OF SINGLE AMINO ACID SUBSTITUTION IN THE DRß1 CHAIN FOR T CELL RECOGNITION

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The molecular analysis of the HLA Class II molecules has revealed the presence of highly polymorphic regions which appear to be involved in the formation of epitopes recognised by T cells. We have compared two alloreactive T cell clones reacting specifically with the different Dw subtypes of the DR4 haplotypes. One of these clones (E55) can be stimulated by all the Dw14, Dw4, and Dw13 subtypes whereas the other clone (E38) reacts with all Dw14 and Dw13 stimulator cells with the exception of one Dw13 cell line. Inhibition studies with monoclonal antibodies revealed that the stimulatory epitope is located on the DR and not DP nor DQ molecules. We have correlated this reactivity with the amino acid sequence of the DRß1 chain of these haplotypes. cDNA clones corresponding to the polymorphic first domain of the DRß1 chain of the non-stimulatory Dw13 cell line were isolated and sequenced. The results revealed that this cell differs from the regular Dw13 cell lines by a single amino acid substitution at position 86 and this variation is sufficient to affect T cell recognition. These data indicate that position 86 (together with other amino acids in the third diversity region of the DR molecule) plays an important role in the formation of epitopes recognised by T cells. The relevance of these results in the context of organ transplantation will be discussed.



PAPER 16

## THE EFFECT OF DONOR AND RECIPIENT AGE IN RENAL TRANSPLANTATION: SHOULD AGE-MATCHING BE RECOMMENDED?

[1,2]J. Thorogood, [1]G.G. Persijn, [1]F.A. Zantvoort, [2]J.C. van Houwelingen, and [3]J.J. van Rood.

[1]Eurotransplant Foundation, Leiden, The Netherlands [2]Leiden University Department of Medical Statistics [3]Department of Immunohaematology and Blood Bank, Leiden University Hospital.

It has recently been suggested that donor-recipient "age-matching" in renal transplantation could significantly improve graft survival, particularly when using kidneys from older donors [1]. We examined the Eurotransplant database for 6397 patients receiving their first kidney transplants from unrelated non-living donors, transplanted between 1st January 1984 and 31st December 1987 and followed up to October 1988. A significant effect of age-difference was observed over the 4.5 year period analysed ($p=0.0001$, logrank test). One-year graft survival was 85.8%, 84.4% and 82.1% for donors >5 years younger, donors within 5 years of age and donors >5 years older than recipient, respectively. The age-difference remained independently significant when other prognostic factors were allowed for in a Cox multivariate regression model. However, when we included donor and recipient age in the model as individual ages (not age-difference) we observed that for the very young and older donors the age-difference method would not have identified those at highest risk of failure. We concluded that the relationship between donor and recipient age is probably quadratic, rather than the linear effect suggested by using age-difference. Certain combinations are at high risk of failure even when the donors are of similar age or younger than the recipient and this should be borne in mind when selecting recipients.

Reference

[1]Donnelly, P.K. and Henderson, R. "Age-matching" for Renal Transplantation. New England Journal of Medicine: In Press.

PAPER 17

## A SIMPLE CYTOTOXICITY ASSAY TO DETECT ANTI-EPITHELIAL CELL ANTIBODIES PRODUCED IN ASSOCIATION WITH RENAL TRANSPLANT LOSS

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N.W. Regional Tissue Typing and Immunology Laboratories and Renal Transplant Unit, Manchester, U.K.

It has been shown that antibody directed against epithelial cells (ECA) can be associated with graft loss in renal transplant recipients. We have therefore designed a micro-cytotoxicity assay allowing sera to be screened for antibodies cytotoxic to the epithelial cell line A549. A549 cells were cultured in Terasaki trays for 24–48 hr. Culture supernatants were removed from the cell monolayers by 'flicking', 1ul of test serum added and incubated for 60 min at 22°C. Rabbit complement (3ul) was then added for a further 60 min when the tests were stained with a cocktail of acridine orange and ethidium bromide.

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Thirty two patients whose renal transplants failed in the absence of lymphocytotoxic antibodies were screened in this novel assay against A549. Five of the 32 patients had ECA present at the time of transplant nephrectomy. Treatment of the sera with dithiothreitol showed the antibodies to be IgM and since we failed to block the reactivity with a monomorphic anti-HLA class I antibody, they were not directed against HLA class I antigens.

These results suggest that when transplant failure is not related to anti-HLA antibody production, the investigation of ECA could be of value when assessing a patient for a subsequent transplant.

[1]AW Harmer et al British Transplantation Society November 1989 (presentation).

2 pts followed longit. Two course of 2 grafts to rapid failure second graft



PAPER 18

## MONITORING OF RENAL GRAFT STATUS BY SEQUENTIAL ASSAY OF PLASMA AND URINARY TNF LEVELS

[1]P.J. McLaughlin, [2]A. Aikawa, [1]H. Davis, [2]R.A. Sells, and [1]P.M. Johnson.

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Tumour necrosis factor-alpha (TNF) is a cytokine product of activated macrophages which has an important role in the initiation of inflammatory and immune responses. Many types of immune response are associated with TNF release into the circulation, including immune rejection following renal transplantation.

We have developed a sensitive and specific enzyme-linked immunosorbant assay (ELISA) capable of detecting the low circulating levels of TNF (>20 pg/ml). Plasma and urinary TNF levels have been sequentially monitored in fourteen renal transplant recipients. There were eleven acute rejection episodes in the weeks following grafting. In 7/11 cases, plasma, and/or urine TNF was detectable 1-4 days before diagnosis of the rejection episode. In 1/11 cases, urine and plasma TNF levels were raised 1 day after diagnosis of acute rejection. In the remaining three cases, no TNF was detectable despite evidence of acute rejection. In 4 patients who maintained normal post-operative graft function, plasma TNF was detected in only one case; this was persistently detected for several weeks post-transplantation. In 5 cases of acute tubular necrosis, no elevated levels of TNF were detected. Thus, serial monitoring of TNF may represent a potential adjunct to more invasive techniques for differential diagnosis associated with poor graft function.

Cytotoxic
Prof y diff.
TNF present in absence of rejection
& converse
TNF not in infection
Seq : pt had early ATN not ↑ TNF
fell after success treatment rejection CMV
infection ↑↑

PAPER 19

## IMAGING AND QUANTITATION OF ALLOGENEIC KIDNEY TRANSPLANT REJECTION IN THE RAT BY IN VIVO USE OF $^{111}$IN LABELLED ANTI-LYMPHOCYTE AND ANTI-CLASS I AND II MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) MONOCLONAL ANTIBODIES

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PVG recipient rats were transplanted with DA donor kidneys and the animals were injected intravenously with indium–$^{111}$ labelled monoclonal antibodies (McAb) of the following reactivities: 1) MRC OX–19, anti-CD5 like, rat Pan T cell. 2) MRC OX–39, anti-rat IL–2 receptor, 3) MN4–91-6 anti-Class I MHC donor specific, 4) F17–23–2 anti-Class II MHC donor specific. At different times after injection, the rats were imaged using a scintillation camera then they were killed and different tissues sampled for radioactivity counting. Animals injected with each of the three antibodies (2,3,4) showed uptake in the rejecting transplants on the scans. The ratio of uptake transplant/kidney using the MN4 McAb was about 20:1 in some animals. Imaging of PVG isografts showed no uptake in the transplant and the ratio Tx/kid did not differ from unity. Cyclosporine treated animals did not show alteration of MN4 uptake seen in non-modified allografts. The use of the other McAbs in cyclosporine treated animals is still underway.

Conclusion: In vivo use of anti-IL2 receptor, anti-Class I or Class II MHC can be useful in the early diagnosis of rejection, however, its value in the follow-up of the grafts needs still to be established.



PAPER 20

## THE ROLE OF HAEMOPOIETIC STEM CELLS IN TOLERANCE INDUCTION

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Immunological tolerance can be established in immature neonatal mice by the intravenous injection of adult haemopoietic $F_1$ hybrid cells. It is not clear, however, whether the unresponsiveness results from the introduction of allogeneic stem cells or from other cells in the inoculum that either contribute a specific cellular function or add to the antigenic load.

To examine this we used the drug 5–Fluorouracil (5—FU) which, when inoculated in vivo, selectively eliminates proliferating cells (eg lymphocytes) whilst sparing non-cycling cells (eg stem cells). (CBA x A/J) $F_1$ adult mice were injected intravenously (i.v.) with 150 mg/Kg 5–FU. 7 days later bone marrow was harvested from these and from normal mice and injected i.v., in varying doses, into newborn (less than 24 hours old) CBA mice. All cell recipients were tested with A/J strain skin graft 8–10 weeks later.

Cells from 5–FU treated mice did not yield an increased proportion of tolerant mice. To explore the possibility that 5–FU treatment had depleted a minor lymphoid population essential in tolerance induction, a mixture of 2 million bone marrow cells from 5–FU treated mice and spleen cells from normal mice (at a ratio 9:1) was injected into neonates, but likewise failed to increase the proportion of tolerant mice.

This result indicates that antigenic load is vital in the induction of tolerance, and that cell losses during the preparation of haematopoietic cells need to be minimized in any attempts to establish tolerance and cellular chimerism by injection of bone marrow cells directly into the fetus.

PAPER 21

## CORNEAL GRAFT SURVIVAL IN THE RAT IS NOT DUE TO ITS "PRIVILEGED SITE"

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We have demonstrated that the fate of rat orthotopic corneal grafts (3 mm diameter) in avascular beds is dependent upon the strain combination of inbred rats used. These fully allogeneic strain combinations can produce high, moderate, and low responder states.

Using one of (high) rejector combinations (DA to AO; median survival time; 11 days, n=15), some factors affecting orthotopic corneal graft rejection have been investigated. The size of donor corneal button was shown to be of a critical importance for the fate of corneal grafts (4 mm; 10.5 days, 3 mm; 11 days, 2 mm and and 1.5 mm; >100 days). Eccentric grafting where a 2 mm (non-rejecting size) corneal button was taken from the centre and grafted to the periphery or vice versa. Centre to periphery showed chronic rejection, while periphery to centre produced acute rejection.

Two different pretreatments with hypothermic preservation or UV-B irradiation to the donor corneal buttons produced a significant prolongation of 3 mm (rejecting size) corneal grafts of 25 days and >100 days respectively.

We suggest that a low density of class II positive dendritic cells and its uneven distribution in the cornea are responsible for the unique character of corneal graft survival.

Small corneal grafts 2 or 1.5 less susp. to rejection
Was this due to distance from limbus did grafts from
various sites on the graft.
Centre to centre no rej.
Periph to centre some rejected
Took 3 corneal grafts (rej. size) + found keeping
hypothermia
them for 4 days (dendritic cells due) and these
removed after UV



PAPER 22

## AN EVALUATION OF THE EFFECTS OF PRIMARY NON FUNCTION ON THE PROGNOSIS IN CADAVERIC RENAL TRANSPLANT RECIPIENTS TREATED ELECTIVELY WITH CYCLOSPORIN A MONOTHERAPY

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Cyclosporin A monotherapy is first line immunosuppression following cadaveric renal transplantation on our unit. Primary non function (PNF) was treated by reduction of the dose of Cyclosporin A and supplementation with prednisolone. We have studied 157 consecutive cadaveric renal transplant recipients to assess the effect of PNF on graft function one year after transplantation.

Of 157 recipients, 40 (25%) had PNF (20 out of 113 locally harvested and 20 out of 44 imported kidneys ($p < 0.001$)). However there was no significant decrease in graft or patient survival at one year attributable to PNF. The mean serum creatinine in primary functioning grafts at one year was 189 umol/l (±SD 77.4) versus 207 umol/l (±SD 86.1) in PNF grafts (NS).

In primary functioning kidneys, graft survival at one year was 84.6%, and in PNF it was 75% ($0.5 > p > 0.1$ ($x^2$)), and patient survival at one year was 91.5% in primary function compared to 87.5% in PNF ($p > 0.5$).

We conclude that in PNF, successful outcome was usually achieved by reducing the Cyclosporin dosage. In this relatively small series there seems to be no significant reduction in patient or graft survival at one year as a result of PNF. Although imported kidneys suffer PNF more frequently than local, the prognosis is no different.

Non sign.

Primary non function did not lead to diff
of graft or pt function at 1yr

PAPER 23

## THE SHORT TERM IMPACT OF THE BENEFICIAL MATCHING SCHEME

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We have taken our Unit's cold ischaemic times (CIT), immediate non function (INF) rate and matching achievements from 1988 when 29 of 37 kidneys transplanted were locally harvested, and compared these with 1989 data during which we participated in the Beneficial Matching exchange scheme, and 21 of 31 cadaveric transplants were performed with organs "imported" via UKTS.

The CIT of UKTS kidneys has remained similar – mean (SD) 26.7 (8.7) hours in 1988 and 25.7 (9.5) hours in 1989. This is significantly longer than the CIT of locally retrieved kidneys which fell slightly from a mean of 15.8 to 13.8 hours ($p < 0.001$).

The INF rate of imported organs was 12/21 (57%) in 1989, compared to 20% for local kidneys in both years ($p < 0.05$). Since more organs have been imported this has resulted in a significant overall increase in INF from 8/37 (21%) to 14/31 (45%) in 1989 ($p<0.01$). The HLA beneficial matching facilitated by organ exchange has greatly improved – from 8/37 (22%) to 18/31 (58%), although the number of transplants performed has fallen, despite maintained donor numbers. We are consequently concerned that the Scheme has not returned as many kidneys as we have exported (21 versus 33), resulting in an end of year positive balance of 12.



PAPER 24

## RENAL TRANSPLANTATION IN THE ELDERLY

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Current pessimism over the prospects for increasing the supply of cadaver kidneys (Gore et al BMJ Nov 1989) led us to review the results of cadaveric renal transplantation in the elderly to establish whether the prognosis in these patients justified using this precious resource on them.

A retrospective review was performed of cadaveric renal transplants into recipients aged 60 years or more between 1982 and 1989 inclusive. Forty-four such patients were identified, mean age 64.5 years, range 60 to 72 years. All but one received their first transplant. Cyclosporin monotherapy was the elective post-operative immunosuppression.

The actuarial graft survival using the Peto method was 90% at one year, 83% at five years. Five of the forty-four kidneys have failed, all within two years of transplantation. Four of these recipients died.

Overall patient mortality was 16/44 (36%) of which 10/44 (23%) occurred in the first year after transplantation. Cardiovascular disease accounted for 10 deaths, all in patients with functioning transplants.

Thus, excellent graft survival can be achieved in cadaveric renal transplantation in patients aged over 60 years, albeit with considerable mortality. Age alone should not therefore be considered a contraindication to renal transplantation. Careful cardiovascular screening may improve patient survival.

Peto method shows 80% donor graft survival @ 5 yrs but actual pt ~~graft~~ survival was 53%

PAPER 25

## PREGNANCY IN RENAL TRANSPLANT PATIENTS

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Twenty-five pregnancies occurring in 21 renal transplant patients are reported. The patients were aged 21–35 (mean 29) and the interval from transplantation to delivery was 6 months to 17 years. Thirteen patients received azathioprine and prednisolone as immunosuppression, 1 prednisolone alone and 7 cyclosporin and prednisolone. There was 1 termination of pregnancy. Two pregnancies resulted in spontaneous abortion, 1 resulted in stillbirth due to a prolapsed cord at term and the remaining 21 in live births. There was a triplet pregnancy in which 1 child is mentally retarded. There was a high incidence of premature delivery and obstetric intervention, but all the babies survived and are developing normally to date. The babies tended to be of low birthweight for gestation period, 68% being below the 50th centile, regardless of immunosuppressive drugs utilised. During pregnancy, 8 patients required antihypertensive drugs and 9 patients developed significant proteinuria resolving after delivery. In only 1 of the pregnancies was there deterioration in renal function requiring early delivery. In the remaining patients there was no deleterious effect on renal function either during or after pregnancy. Pregnancy in renal transplant patients is a relatively safe occurrence for both mother and foetus, but requires careful monitoring.

8 m 6 LR 'all live births

17 m 15 Kab.

7 on Cy

13 on Aza



PAPER 26

## ANTIBODY CLASS, HLA SPECIFICITY, AND RELATIONSHIP WITH PATERNAL ANTIGENS OF SERUM LYMPHOCYTOTOXIC ANTIBODIES FROM HIGHLY SENSITISED MULTIPAROUS DIALYSIS PATIENTS

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Multiparous dialysis patients are at particular risk of transfusion induced broad sensitisation. Hence paternal antigens encountered during pregnancy may be the target antigens for cytotoxic activity in such highly sensitised patients (HSP). However, parous females may also form cytotoxic antibodies to non-HLA linked paternal lymphocyte antigens. We wished to determine for antibodies in the sera of multiparous HSPs: (1) whether they were directed to paternal HLA antigens, (2) their immunoglobulin class, and (3) the contribution of antibodies to paternal non-HLA antigens and autolymphocytotoxins to the breadth of panel reactivity (PRA).

No sera from the 11 previously untransplanted HSPs studied contained autolymphocytotoxins. Cytotoxicity in all 11 sera was blocked by pre-incubation of paternal lymphocytes with a monoclonal antibody to a monomorphic class 1 determinant. Similarly 4/11 sera were active against paternal class 11 antigens. Absorption of sera with paternal lymphocytes reduced PRA by > 50% in 8 and by over 80% in 3 of these. DTT digestion had no significant effect on PRA in any of the sera.

The results imply that cytotoxic antibodies in the sera of multiparous HSP are of IgG class, are directed to class 1 HLA antigens, and that paternal antigens account for a major proportion of sensitisation. When such patients require transfusion, sensitisation rates might be reduced by avoiding blood from donors sharing paternal class 1 determinants.

PAPER 27

## THE USE OF FLOW CYTOMETRIC CROSSMATCHING (FCXM) IN CARDIAC TRANSPLANTATION

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One of the most important pre-transplant tests is that of the cytotoxic crossmatch between the donor cells and the serum of the recipient. However, as the sensitivity of this crossmatch test has been questioned, we have developed as assay using flow cytometry to demonstrate antibodies (IgG) in the sera of renal graft recipients before transplantation. We have performed retrospective FCXM in 35 cardiac transplants and compared the results with the incidence of rejection. Patients with rejection were classified as having "moderate" (Billingham) histological grade rejection in the first three months after transplant. No correlations between either any of the donor (age, sex, blood group, cause of death) or recipient parameters (age, sex, blood group, tissue typing match, panel reactivity, anastomosis time or hospital stay) with rejection were shown. Results of FCXM analysis are shown below.

| | FCXM Negative | FCXM Positive |
|---|---|---|
| NON REJECTORS | 21 | 5 |
| REJECTORS (moderate) | 3 | 6 |

A highly significant association (P>0.02 using Fisher's exact test) between FCXM result and rejection was found. In patients with a negative FCXM only 19.2% showed moderate rejection whereas patients with positive FCXM showed 67% incidence of rejection.

This study shows data that may help to identify a group of heart transplant patients more likely to reject and perhaps allow a modified immunosuppressive regime to be used.



PAPER 28

## THE EFFECT OF CAPSULE COMPOSITION ON THE INSULIN RELEASE CHARACTERISTICS OF CULTURED ALGINATE/POLY-L-LYSINE ENCAPSULATED ISLETS

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Encapsulation of transplanted islets in alginate/poly-l-lysine has been proposed as a method to prevent rejection and autoimmune damage. The effect of capsule composition on islet function has not previously been studied. Rat islets were encapsulated in high mannuronic acid alginate and then further encapsulated in: 1) poly-l-lysine alone, 2) poly-l-lysine + high guluronic acid alginate, 3) poly-l-lysine + high mannuronic acid alginate. Encapsulated and non-encapsulated islets were then placed in tissue culture and at weekly intervals for 4 weeks groups of 50 islets removed and their glucose-stimulated insulin release studied in a perifusion system. Results were corrected for islets DNA content.

The response times and stimulation indices (ratio of stimulated to basal insulin secretion) were similar for the encapsulated and non-encapsulated islets and were unaffected by capsule composition. The stimulated insulin release (uU/ng DNA/min) of the encapsulated islets was less than that of the free islets but remained constant throughout the 4 week period of tissue culture.

We conclude that alginate/poly-l-lysine encapsulated islets (regardless of capsule composition) have similar response times and stimulation indices to free islets but relatively reduced glucose-stimulated insulin production. This reduction is evident after 1 week in tissue culture.

PAPER 29

## RATS DEVELOP CYCLOSPORIN NEPHROTOXICITY AT LOW DOSES: RESULTS FROM A NEW MODEL

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Methods of measuring glomerular filtration rate (GFR) in rats that require surgical preparation make the experiments unphysiological and are unrepeatable. This has hindered the investigation of Cyclosporin nephrotoxicity in rats.

Sprague-Dawley rats (10 per group) received: Cyclosporin 5, 10, 15 and 25 mg/kg body weight or vehicle (cremaphor) by daily intraperitoneal injection for 2 weeks. We measured GFR (chromium[51] labelled EDTA bolus intravenous injection and 60 minute blood sample) and lithium clearance at 1, 7 and 14 in each animal.

| | Mean GFR mls/minute | | | | | Mean Fractional Excretion Lithium (Lithium clearance/GFR) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CyAmg/kg/day | 0 | 5 | 10 | 15 | 25 | 0 | 5 | 10 | 15 | 25 |
| Day 1 | 2.39 | 2.59 | 2.94 | 3.11 | 2.69 | .51 | .35 | .26 | .26 | .45 |
| Day 7 | 2.61 | 2.59 | 1.81+ | 2.16+ | 1.16* | .45 | .40 | .3 | .29 | .42 |
| Day 14 | 2.22 | 1.96+ | 1.3* | 1.41* | 0.69* | .52 | .57ø | .4ø | .34 | .9ø |

$p < 0.0005$*, $p < 0.005$+ and $p < 0.05$ø (paired t test) compared with day 1.

100 control measurements provided normally distributed data. GFR fell significantly with dose and time. Fractional excretion of lithium tended to increase suggesting reduced proximal tubular reabsorption.

This model allows sequential accurate measurement of renal function in intact rats. It has shown that this species, hitherto thought to be resistant to Cyclosporin nephrotoxicity, is in fact sensitive to doses as low as 5mg/kg/day.



PAPER 30

## HAEMODYNAMICS AND REGULATION OF VASOACTIVITY IN CYCLOSPORIN NEPHROTOXICITY OF THE NORMAL HUMAN KIDNEY

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Cyclosporin (CyA) induced dysfunction in the innervated normal kidney may result from disturbance of the microvascular circulation but its regulation, reversibility and means of abrogation are poorly defined. In 9 patients with stable cardiovascular state receiving low dose CyA for 3 months (2.5 mg/kg/day) for psoriasis, RBF, filtration fraction (FF) and GFR were serially assessed using a novel Tc-99m DTPA dynamic technique (1), together with the vasoactive hormones: PRA, angiotensin II (AII), aldosterone and atrial natriuretic peptide (ANP).

| Time (months) | (0) | CyA | (3) | No CyA | (6) |
|---|---|---|---|---|---|
| GFR (ml/min/1.73m$^2$) | 96±4 | | 82±5* | | 90±3 |
| RBF (%C.O.) | 17.3±1.2 | 14.3±1.5 | 15.1±1.2 | | |
| Individual RBF (% C.O.) | 9.0±0.6 | 7.6±0.7* | 7.6±0.7* | | |
| FF (%) | 19.9±1.6 | 21.1±1.9 | | 23.7±2.8 | |
| PRA (pmoles/hr/ml) | 4.3±0.6 | 3.2±0.4 | | 3.7±0.5 | |
| Aldosterone (pmoles/l) | 527±61 | | 385±48 | | 429±49 |
| AII ambulant (pg/ml) | 10.3±0.9 | | 13.7±1.4 | | 10.7±1.4 |
| ANP (pmoles/l) | 3.3±0.8 | 4.2±0.3* | 2.9±0.3 | | |

Means ± SEM *p= < 0.05 student's t test (0) vs. (3) and (6)

CyA nephrotoxicity was associated with a fall in GFR and a significant and variable reduction in RBF but was reversible. This change was inversely correlated with PRA ($r_s$=0.74, $p<0.05$) and aldosterone ($r_s$=0.7, $p<0.05$); ambulant AII and FF were correlated ($r_s$=0.49, $p<0.01$). Alteration of glomerular microcirculation by CyA in the intact normal human kidney, in contrast to the transplanted kidney, may reflect AII dependent efferent arteriolar vasoconstriction.

1. Nucl. Med. Commun. 8: 823-37, 1987.

PAPER 31

## EARLY BACTERIURIA – ITS SIGNIFICANCE IN CURRENT PRACTICE OF RENAL TRANSPLANTATION

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In a prospective study of 200 consecutive renal transplants from April 1988, patients were screened regularly for significant bacteriuria. All patients were transplanted with preoperative cephalosporin prophylaxis. They received one gram of steroid with diuretics peroperatively, and Cyclosporin postoperatively. The results of the first three months follow-up were analysed.

One-hundred and twenty patients (60%) had no urinary tract infections, eighty patients (40%) had 131 episodes of bacteriuria (range 1–5). Eight episodes were associated with bacteraemia. Forty of the episodes were related to indwelling catheters. Over half of the bacteriurias were asymptomatic. The presence of symptoms equated with the severity of the infection.

There were 69 episodes of bacteriuria alone, i.e. excluding concurrent rejection and cyclosporin toxicity as alternative causes of renal dysfunction. In 19 of these there was a rise in serum creatinine of greater than 10%, which in 15 returned to pre-infective levels after treatment. The bacteriuric group was comparable with the noninfected group in incidence of acute tubular necrosis, cyclosporin toxicity and renal function at three months, but rejection episodes per patient were more common (1.08 c.f. 0.77, p<0.0001, chi-squared). Bacteriuria is common after renal transplantation, often asymptomatic. In 30% of cases it causes significant but usually reversible renal dysfunction.



PAPER 32

## RENAL TRANSPLANTATION IN CHILDREN WITH NEUROGENIC BLADDER AND OUTLET PROBLEMS

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Children in end stage renal failure secondary to neurogenic bladder or lower urinary tract obstruction had been considered as high risk patients for renal transplantation.

Since January 1975 till October 1989, one hundred and seventy children received renal grafts at our institutions. Among these, seven children aged 1 to 14 years had such original diseases of ESRD. There were neurogenic bladder in three girls, anterior urethral valves in a boy, posterior urethral valves in two boys and fibrous contracture of bladder neck in a boy. Kidney grafts were all donated by either of the parents in the seven cases. In three recipients with neurogenic bladder, intermittent self catheterization was used in two recipients and creation of colonic conduit was performed in one recipient. Transurethral resection of urethral valves or bladder neck and bilateral nephroureterectomy were performed as pretransplant preparation in four outlet obstructive boys.

At the end of October 1989, all seven children are alive with well functioning grafts 10 months to 9 years 4 months after transplantation surgery.