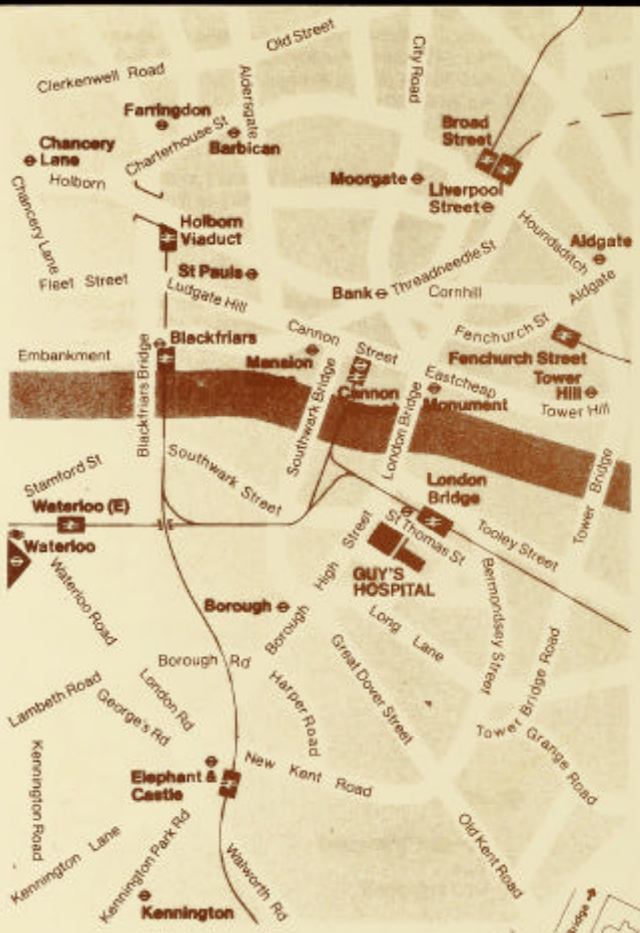

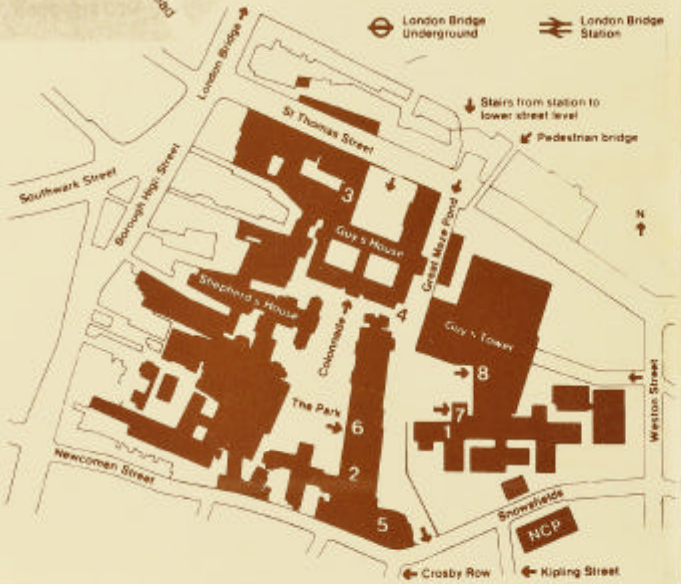

1 ACCIDENT AND EMERGENCY DEPT.
2 ADMISSIONS
3 CHAPEL
4 FLOWER SHOP
5 OUT-PATIENTS
6 HUNT'S HOUSE WARDS
7 NEW GUY'S HOUSE WARDS
8 GUY'S TOWER WARDS

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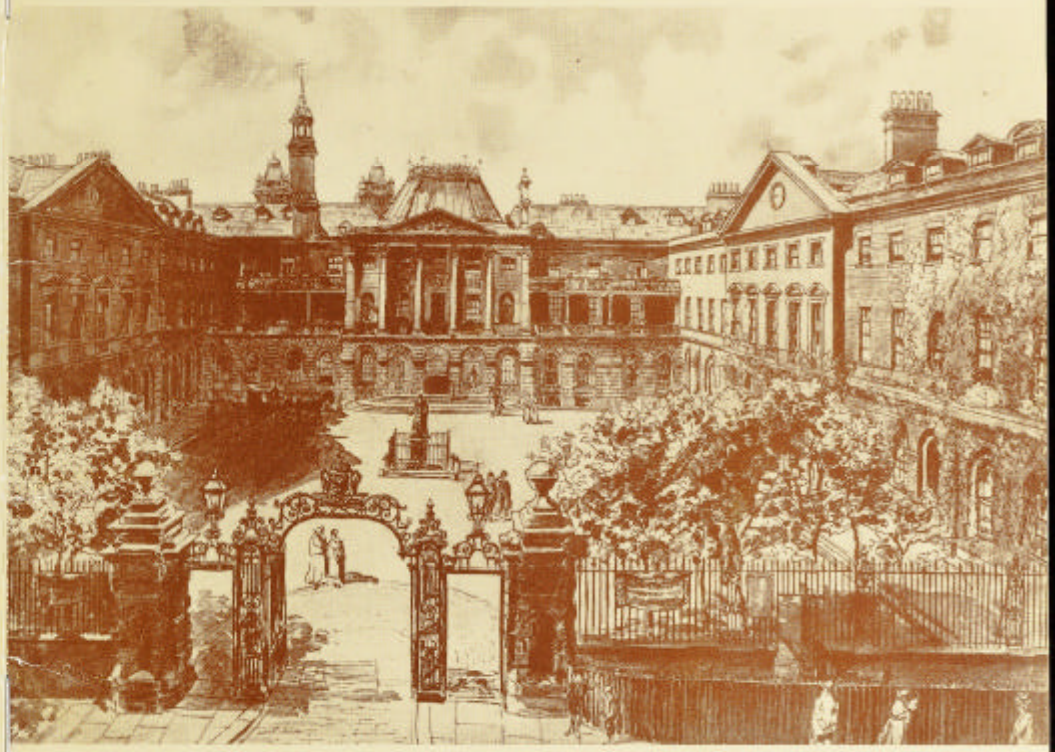

GUY'S HOSPITAL, LONDON

9th November 1983

SMALL ALIQUOT, REPEATED TRANSFUSIONS FROM A SINGLE, THIRD PARTY, HLA-DR DEFINED DONOR

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In a single centre trial of the effects of transfusions, we have continued to pursue a unique method of pre-treating our heterogenous group of graft recipients. Prospective cadaveric recipients, who were not previously transfused, received a single unit of CPDA stored blood in three equal aliquots on Day 0, 14 and 28. The blood donor was selected from a pool of HLA-DR defined donors who had a similar HLA specificity to the prospective recipient, but differed at one or more alleles of the A, B, C or DR locus. Mismatches were minimised to avoid broad sensitization. Twenty-seven patients are included. Twenty kidneys are functioning well between 66 - 870 days, with the majority more than one year. More than 95% of these kidneys are beyond three months which represents the period of maximal transfusion effect. Of the seven kidneys lost within that time frame, two were unrelated to rejection. Rejection crises, in general were mild and easily reversed.

In conclusion, small aliquot transfusions, obtained from a single highly defined donor, is an effective method of producing a beneficial effect, and this suggests that less blood is required than is currently being used.

MINIMAL SENSITIZATION FOLLOWING DELIBERATE UNRELATED TRANSFUSIONS IN RENAL PATIENTS

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The beneficial effect of blood transfusions on graft survival is now generally accepted although one problem is the risk of sensitization. We have investigated sensitization in potential renal transplant patients following deliberate unrelated transfusions (TPT). Fifty patients entered the study. The protocol entailed giving 3 units of blood, each at 4-weekly intervals. We monitored the exact weight, storage time, leucocyte count and viability for each TPT. The cytotoxic antibody status of each patient was assessed against a panel of peripheral blood lymphocytes and CLL cells, prior to and 2 weeks following each TPT. Six patients received one TPT: one produced antibodies to B cells and one to T and B cells, another received a cadaver kidney. Twelve patients received 2 TPT: one then received a cadaver kidney. Thirty-two patients received 3 TPT: 7 produced antibodies to T and/or B cells, another 4 received cadaver kidneys. Of the 8 sensitized patients, 2 produced antibodies to T cells, 4 to B cells, and 3 to both T and B cells. Reactivity was against 5-30% of the panel of PBL and against 3-60% of the panel of CLL cells. The appearance of these antibodies was transient. They became undetectable within one to six weeks. The nature of the transfused blood was found to vary: weight 170-365g; storage time 6-29 days; leucocyte content 0-7 x 10^6/ml; leucocyte viability 0-100%. We conclude that deliberate TPT rarely give rise to high levels of sensitization. Clinical data on those patients who received transplants will be presented.

IMPROVED RENAL ALLOGRAFT SURVIVAL IN DOGS FOLLOWING PLATELET TRANSFUSION AND SHORT TERM CYCLOSPORIN A TREATMENT

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The beneficial effects of both third party and donor specific blood transfusions on renal allograft survival are well established, although the mechanisms remain unclear and there is a risk of producing antibodies against donor antigens. A similar beneficial effect using platelets has been achieved in primates.[1] We have carried out a preliminary study in 18 mongrel dogs using platelets from their unrelated kidney donors at the time of renal allografting. Prior to surgery 100 ml donor blood was removed by venepuncture. Bilateral recipient nephrectomy was performed and immediately following revascularisation of the grafted kidney approximately 50×10^8 donor platelets, concentrated from plasma, were administered. Each animal was given Cyclosporin A 25 mg/kg/day for the first 30 days post-operatively. The dose was reduced to 17.5 mg/kg/day on day 31 and again, to 12.5 mg/kg/day on day 61. The drug was discontinued on day 90. Excluding 7 animals dying from technical complications (two from pulmonary emboli), the median survival was 96 days (Table). Six dogs survived after Cyclosporin A treatment was stopped and, of these, two remained healthy and active at 12 and 13 months when they were stolen by antivivisectionists. Previously, we have shown that dogs with renal allografts treated with Cyclosporin A continuously (initially at 50 mg/kg/day, and reducing the dose to 25 mg/kg/day and 10 mg/kg/day on days 28 and 56 respectively), had a median survival of 31 days [2] (Table). There is no previous report of prolonged renal allograft survival in dogs after stopping Cyclosporin A therapy.

Group	N	Survival (days)	Median Survival (days)
Platelets	11	35,45,51,65,75,96,110,114, 133,>360,>390	96
No Platelets	17	10,11,11,13,17,19,20,21,28 33,35,52,62,68,147,>198,>275	31

1. Borleffs, JCC et. al. Lancet 1982; 1; 1117

2. Calne, RY et. al. Transplant. Proc. 1979; 11: 860.

PRELIMINARY RESULTS OF A CLINICAL TRIAL OF AMINOPHYLLINE AS AN ADJUNCTIVE IMMUNOSUPPRESSIVE AGENT

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It has been suggested that the phosphodiesterase inhibitor Theophylline may reverse steroid-resistant rejection episodes after transplantation by stimulating cell activity. In an attempt to determine whether routine aminophylline administration (950 mg/day) would improve graft survival 37 recipients of first cadaveric renal allografts, matched for age, sex, HLA-A and -B typing and transfusion history were prospectively randomised to receive either Azathioprine + steroids (Az+P) or Az+P+Aminophylline (A) from the day of transplantation. Rejection episodes were treated with i.v. boluses of Methylprednisolone (MP). All patients were monitored for changes in circulating T-cell subsets with the OKT monoclonal antibodies. The three-month graft survival rates were 11/18 (61%) in the Az+P group compared with 15/19 (78.9%) in the A group giving mean $\pm$ SD serum creatinines at 3 months of 172 $\pm$ 28 umol/l and 140 $\pm$ 50 umol/l respectively. The Az+P group required more MP for rejection than did the A group (mean Grams MP $\pm$ SD, 6.4 $\pm$ 2.3 v 3.7 $\pm$ 2.7 respectively, $p < 0.001$). During both non-rejecting and rejecting phases OKT4/OKT8 ratios were higher in the Az+P group than in the A group (2.19 $\pm$ 1.5, n = 88 v 1.68 $\pm$ 1.24 n = 119, $p < 0.0027$ during quiescence and 2.92 $\pm$ 1.5, n = 69 v 1.98 $\pm$ 1.64, n = 31, $p < 0.01$ during rejection). In both phases this was due to reduced percentages of OKT4- positive cells in the aminophylline-treated group ($p < 0.0027$). We conclude that aminophylline may be a useful adjunctive agent to conventional immunosuppression and appears to potentiate the reduction in the number of circulating lymphocytes with helper phenotype.

REMOVAL AND PREVENTION OF RESYNTHESIS OF ANTI-HLA ANTIBODIES IN PATIENTS AWAITING RENAL TRANSPLANTATION

D. Taube, M. Thick, K. Welsh, L. Kennedy, M. Bewick, J.S. Cameron, C.S. Ogg, C.J. Rudge, D.G. Williams, Guy's Hospital, London.

One third of our patients awaiting renal transplantation have circulating anti-HLA Class 1 antibodies which, when directed against donor cell antigens, make transplantation impossible. In order to remove and prevent the resynthesis of these antibodies, we have plasma exchanged and treated five high priority anti-HLA antibody-rich patients with cyclophosphamide and prednisolone. Dilution and absorption experiments indicated that these antibodies were predominantly directed against single HLA Class 1 antigens which, in high titre, cross reacted with multiple HLA Class 1 antigens. Before treatment, the patients' anti-HLA antibody titres persistently ranged between 1/16 and 1/128 and their sera reacted with over 85% of our lymphocyte donor panel. Following treatment, their anti-HLA antibody titre fell to 1/8 or less and panel reactivity was reduced to less than 43%. Three of the five patients have subsequently been successfully transplanted and the other two are awaiting transplantation. The pre-treatment sera of two of the three transplanted patients gave positive cross matches with their donor's cells, whereas the cross matches with their post treatment sera were negative.

ABSORPTION OF AUTOANTIBODIES FROM THE SERA OF RENAL PATIENTS USING AUTOLOGOUS LYMPHOBLASTOID CELL LINES

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It has been demonstrated by several groups that autoantibodies may be responsible for positive pretransplant crossmatches but are not damaging to renal allografts, and may in fact enhance graft survival. If a patient has only autoantibodies then a positive crossmatch may be disregarded in planning for a transplant. Unfortunately, some patients appear to have both auto and alloantibodies and it may be impossible to determine which is responsible for a positive crossmatch. We have recently performed absorption studies using autologous Epstein-Barr virus transformed B-lymphoblastoid cell lines from a number of patients on our transplant waiting list in an attempt to differentiate autoantibodies from alloantibodies. Three groups of patients were studied and two to four sera were tested from each patient. A two stage absorption procedure was employed using 50×10^6 cells to absorb 100 ul of each serum sample studied. Group A consisted of four patients who were non-transfused and awaiting a first transplant and had only autoantibodies. Group B included three patients who were highly sensitized but had no detectable autoantibodies. Group C was made up of seven highly sensitized patients who had autoantibodies detected in autologous crossmatch. Unabsorbed and absorbed sera were crossmatched against autologous T and B cells, a panel of random normal T and B cells, and a panel of B cells from patients with chronic lymphocytic leukemia, which are known not to react with autoantibodies. In Group A all activity against autologous and panel cells was removed by cell line absorption. In Group B, where no autoantibodies were detected, no effect of absorption was seen on reactivity to either the CLL or normal panel cells. The results in Group C demonstrated removal of all activity toward autologous cells, no effect on CLL activity, and removal of 10 - 50% of activity against the normal panel. We conclude from this data that lymphoblastoid cell lines can be used to absorb autoantibodies but will not affect alloantibodies. Using cell line absorptions, it is possible to differentiate autoantibodies from alloantibodies when they occur together in the sera of patients awaiting transplantation.

DOES CLASS 1 MISMATCHING INFLUENCE RETRANSPLANTING AFTER GRAFT FAILURE

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The proportion of sensitised patients awaiting retransplantation is increasing, and requires a disproportionately large share of the total resources. We examined anti-HLA antibodies produced by 100 patients who had lost their first renal allograft, and related this to the Class 1 mismatch of the failed graft. None of these patients had been previously sensitised. 66% of these patients produced anti Class 1 antibodies. Of these, 26% were poly specific, and 74% were narrowly specific. 81% of the narrowly specific antibodies were directed against a mismatched determinant on the failed graft. Specificity was determined using a computerised 2 x 2 statistical test, and cross-reactivity was tested by dilution and specific absorption followed by backtesting. The degree of mismatch did not affect the number or specificities of the antibodies produced. However, some mismatched determinants were more likely to induce antibody production. Since these determinants are common, they not only induce sensitisation more frequently, but the antibody will also be directed against significant numbers of donor kidneys at retransplantation. Therefore, mismatch of these determinants will significantly affect the probability of being able to receive a second graft.

SYNERGY BETWEEN T CELL SUBSETS AND LYMPHOKINE IN ACUTE ALLOGRAFT REJECTION

P.A. Lear, C.D. Heidecke, J.W. Kupiec-Weglinski, T.B. Strom, N.L. Tilney, (R.A. Sells). Department of Surgical Research, Harvard Medical School, Boston, Massachusetts.

Interrelationships between lymphoid populations and lymphokine have been investigated in B rats, produced by lethal X-radiation and bone marrow reconstitution of adolescent thymectomized LEW. Heterotopic (LEW xBN)F_1 cardiac allografts are rejected acutely in unmodified LEW (c.7 days) yet survive indefinitely in B hosts. Adoptive transfer of 10^8 alloimmune splenic T cells (Tsl) into B recipients results in rejection of long standing allografts within 18 days. However, concomitant administration of Interleukin 2 rich conditioned medium (IL-2CM) with 10^8 Tsl results in graft rejection in 9 days. Tsl were divided into T helper (Th) and T cytotoxic/suppressor (Tc/s) populations using monoclonal antibody techniques. Transfer of Th (W3/25+ OX8-) resulted in graft destruction in a time frame inversely related to the number of cells transferred (2×10^7 - 35 days, 10^8 - 13 days), and this was independent of the concomitant IL-2 administration. The transfer of Tc/s (OX8+ W3/25-) never resulted in rejection; all grafts survived indefinitely. However, the addition of IL-2CM to the inoculum initiated a palpable deterioration in graft function between 5 - 8 days, which resolved despite continuing IL-2CM therapy. These findings were supported histologically. Recombining 6×10^7 Th and 4×10^7 Tc/s ($=10^8$ Tsl) + IL-2CM, and transfer, resulted in acute rejection. These data suggest synergy between Th, Tc/s and IL-2CM in the acute allograft response, with Th playing a pivotal role.

CYCLOSPORIN A INDUCES HIGH LEVELS OF CIRCULATING T SUPPRESSOR DERIVED IL-2 INHIBITOR IN VIVO

P.A. Lear, N.L. Tilney, J.W. Kupiec-Weglinski (R.A. Sells), Surgical Research Laboratory, Harvard Medical School, Boston, Massachusetts.

(Lew x BN)F_1 cardiac allografts survive 1 week in unmodified Lew rats, indefinitely in recipients treated with Cyclosporin A (CyA, 15 mg/kg IM for 7 days). Acute rejection of long standing grafts in CyA modified hosts could be recreated through the abrogation of T suppressor lymphocytes (Ts) by Cyclophosphamide (CY, 50 mg/kg IP), followed by the administration of syngeneic alloimmune lymphocytes. To monitor the activity of a putative Ts derived IL-2 inhibitor, sera from CyA and CyA + CY treated heart grafted recipients were tested for their ability to inhibit IL-2 induced proliferation of an IL-2 dependent cytolytic T cell line (CTLL-2). Data are presented from a representative experiment with the activity of IL-2 inhibitor expressed as the %age of ^{3}H-thymidine incorporation by CTLL-2 cultured with IL-2 (2 units) + graded concentrations of experimental sera/cultures containing IL-2 alone.

Serum concentration	IL-2 inhibitor in experimental animals		
	CyA (7 days post Tx)	CyA (30 days)	CyA (30 days) + CY
50%	58%	88%	66%
25%	33%	42%	36%
12.5%	0%	27%	5%
5%	0%	3%	0%

Increasing the IL-2 concentrations (10u vs 2u) exceeded the suppressive activity of IL-2 inhibitor in sera of CyA + CY treated hosts, but was less effective in CyA only treated animals. This report is first to describe elevated endogenous levels of IL-2 inhibitor in CyA modified graft recipients, which is significantly diminished following CY therapy. CY-sensitive Ts may contribute to the acquisition and maintenance of CyA mediated immunosuppression in vivo.

DOES ATN INFLUENCE GRAFT SURVIVAL IN PATIENTS RECEIVING AZATHIOPRINE (AZA) OR CYCLOSPORIN A (CYA)

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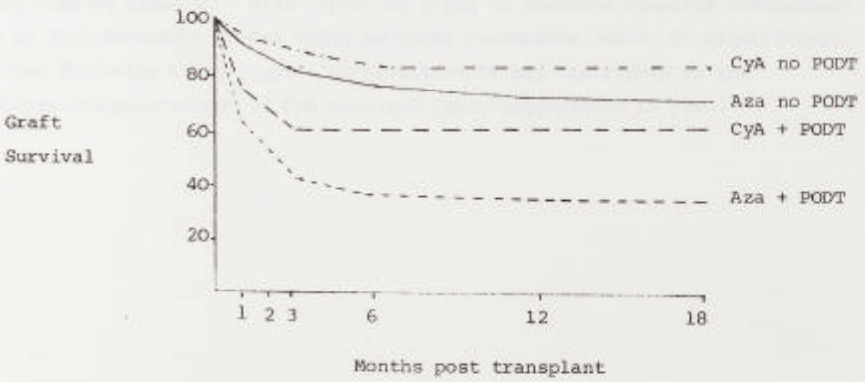
208 patients were divided retrospectively into 4 groups on the basis of their immunosuppressive therapy (Aza or CyA) and their requirement or not for post operative dialysis treatment (PODT). Of 125 patients on Aza, 55 of them (44%) required PODT while 39 of 83 (46%) on CyA required PODT. N.S. Actuarial graft survivals, HLA, A,B or DR mismatches, creatinine at 4 weeks and 6 months and the proportion of heart beating (HB) and non heart beating (non HB) donors were examined for each group.

Results. There were no significant differences in HLA, A,B or DR mismatches for patients in any of the groups nor in the creatinine levels at 4 weeks or 6 months although there was a trend for creatinine levels on CyA to be higher. There were differences in the relative proportions of HB and non HB donors between the 4 groups in that the CyA groups had a higher proportion of HB donors (46% CyA with no PODT and 33% CyA and PODT) than did the Aza groups (22% Aza with no PODT and 16% Aza with PODT), the difference between CyA and no PODT and both Aza groups being significant ($p<0.02$). Actuarial graft survivals for the 4 groups are shown below and it can be seen that either on Aza or on CyA a requirement for PODT produces a poorer long term prognosis. Ischaemic damage of the kidneys as evidenced by the need for haemodialysis would seem to be a detremental factor to long term graft function.



DIFFERENTIATION BETWEEN REJECTION AND CYCLOSPORIN NEPHROTOXICITY USING FINE NEEDLE INTRA-RENAL MANOMETRY

J.R. Salaman, P.J.A. Griffin, Renal Transplant Unit, The Royal Infirmary, Cardiff.

Because of renal toxicity, rejection episodes can be difficult to diagnose in patients with kidney transplants receiving Cyclosporin. Wagner et al (Transplant. Proc. 15 489, 1983) have left a catheter under the kidney capsule and shown that sub-capsular pressure rises with rejection but not with toxicity. We have measured intra-renal pressure by inserting a fine needle (25 G) directly into the kidney and connecting it to a manometer with a long length of fine plastic tubing filled with saline. The pressure was measured by observing the movement of a bubble in the tubing as pressure was applied and then released. Blood Cyclosporin levels were monitored regularly and conventional renal biopsies obtained whenever renal function declined. 32 recipients of renal allografts were studied between 1 and 270 days post transplant. 129 pressure readings were obtained (each an average of 2.3 measurements) during episodes of normal function, rejection, nephrotoxicity and acute tubular necrosis (ATN).

Function Status	Episodes Studied	Biopsies Taken	Pressure Tests	Pressure mm/Hg (S.D.)		
Normal	38	1	78	27.1	(10)	
Rejection	19	23	30	51.3	(16)	$p < 0.001$
Toxicity	8	9	11	26.6	(6)	
ATN	7	7	10	28.7	(10)	

Rejection but not toxicity or ATN cause a highly significant increase in intra-renal pressure. This very simple test therefore might be of value in monitoring patients receiving Cyclosporin A.

"HEART TRANSPLANTATION - THE FIRST 52 PATIENTS"

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Fifty two patients underwent cardiac transplantation between January, 1979 and July, 1st, 1983. Ages ranged between 16 and 52 (mean 42) years and all but four were men. 20 patients had cardiomyopathies and 29 ischaemic heart disease. During this period 353 patients were referred for transplantation, of whom 183 were assessed in hospital. 106 were accepted as potential recipients and of these 40 subsequently died while awaiting transplantation.

Donor ages ranged from 16 to 37 (mean 23) years and donor heart ischaemic time from 96 to 252 (mean 165) minutes. There was one operative death and 6 other deaths within 30 days of operation (early mortality 13%). Two immunosuppressive regimes have been used: anti-thymocyte globulin, azathioprine and steroids for the first 29 patients and cyclosporin A and steroids for the next 23 patients. All surviving patients have had right and left heart catheterisation at annual intervals.

Thirty of the 52 patients are surviving (3 year actuarial survival 51%). Of the 16 late deaths, 6 have been from acute rejection, 4 from accelerated coronary artery disease, 3 from infection and one each from dysrhythmia, graft failure and brain damage. Those patients who have survived beyond 6 months have had a substantial improvement in health status and in measured exercise capacity.

STRAIN SPECIFIC CALCIFICATION OF THE HEART IN THE CYCLOSPORIN TREATED MOUSE

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During toxicological studies on Cyclosporin A (CyA), it was found that CyA therapy (80 mg/kg orally for 14 days) in CBA/Ca mice induced weight loss, and changes in calcium metabolism which resulted in the deposition of calcium in the heart. Histological examination showed either multiple discrete foci of calcification or a diffuse calcifying process present chiefly in the interstitial fibrous tissue; changes which were accompanied by an increased serum urate and creatinine with hypocalcaemia (mean 2.12 $\pm$ 0.07 mmol/l (treated) v. 2.34 $\pm$ 0.09 mmol/l (control) $P < 0.01$). Subsequent metabolic studies performed in the Lewis rat, treated with CyA, revealed hypercalcuria (mean 0.016 $\pm$ 0.0007 mmol/24 hr. (treated) v. mean 0.003 $\pm$ 0.001 mmol/24 hr. (control) $P < 0.01$) and hypocalcaemia. These data may indicated CyA toxicity at the renal tubule with impaired calcium reabsorption. Studies in other strains demonstrated similar findings in C3H/He and DBA/2 mice. However, Balb/c, C57lB/10 and 'A' strain were not susceptible. Furthermore, the evident non-susceptibility of F_1 hybrids of susceptible and non-susceptible strains suggests that CyA toxicity in the mouse is an autosomal recessive trait.

PHENOTYPIC CHARACTERIZATION OF NATURAL KILLER CELLS AFTER RENAL TRANSPLANTATION

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A monoclonal antibody HNK-1 has been reported to identify natural killer (NK) and antibody dependent killer (K) cells. Using simultaneous two colour immunofluorescence analysis on a FACS IV, we have demonstrated an appreciable overlap in the expression of HNK-1 and Leu-2a (expressed on the 'suppressor/ cytotoxic' T cell subset). Further, we have shown that the HNK-1(+) Leu-2a(-) subset is located within B cell areas of normal lymphoid tissue - suggesting a physiological role of these cells in B cell regulation. (1) Functional analysis has revealed that the NK activity in fact resides within the HNK-1(+) Leu-2a(-) subset.

Monitoring of HNK-1(+) cells, in 20 patients given conventional immunosuppression, revealed a significant decline in the total numbers after transplantation but no correlation with rejection episodes.

In a smaller number of these patients, overlap studies of HNK-1 and Leu-2a expression were compared with similar analyses in long term graft recipients and healthy controls. The results (see table) show that while patients may have normal/high percentage numbers of HNK-1(+) cells, the percentage numbers (and therefore total numbers in these lymphopaenic patients) of the HNK-1(+) Leu-2a(-) subset were lower than controls. This deficit was still present in patients transplanted over a year previously.

	A % Leu-2a(+)	B % HNK-1(+)	C % HNK-1(+) 2a(-)	%C/B
Normal n=9	20.9*	13.9	7.9	56.8
Early post transplant n=4	20.4	19.3	6.5	33.7
Long term recipients n=5	36.0	25.7	4.0	15.6

These findings, in conjunction with our previous location of this subset to B cell areas, may account for the increased incidence of B cell malignancy and infection following transplantation.

(1) Ritchie et at., Clin. Exp. Immunol. (1983) 51, 439