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PAPER 1

PATTERNS OF LYMPHOCYTE INFILTRATION IN HUMAN RENAL ALLOGRAFT BIOPSIES

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Renal allograft biopsies (n=250) were obtained at the time of transplantation (Day 0 controls) and on days 7, 21, 90 and 365 from 83 patients randomly allocated to receive immunosuppression with either cyclosporin (Cy) or azathioprine and low-dose prednisolone (AP). Cryostat sections were stained by an indirect immunoperoxidase technique using monoclonal antibodies to identify T4+, T8+ and total T-lymphocytes. Results, based on a point counting technique, show the percentage area of the tissue occupied by a particular cell type (+SEM).

DAYS AFTER TRANSPLANTATION	0	7	21	90	365	Graft Nephrectomy
<b>STABLE FUNCTION</b>						
T-LYMPHOCYTES	0.3±0.1	4.2±0.7	4.3±0.6	3.1±0.4	2.3±0.4	-
T8+ CELLS	0.1±0.0	2.2±0.4	2.5±0.4	1.9±0.3	1.3±0.3	-
<b>REJECTION</b>						
T-LYMPHOCYTES	-	6.8±0.6	8.2±1.3	-	-	7.3±0.9
T8+ CELLS	-	3.9±0.4	4.7±0.6	-	-	4.2±0.6

In stable grafts T-cell and T8+ cell infiltration was significantly increased on days 7, 21, 90 and 365 compared to day 0 ( $p < 0.0001$  - Mann Whitney U Test). In rejecting grafts at days 7 and 21 the total T-cell infiltrate significantly exceeded that seen in stable function ( $p < 0.01$ ) and was mainly the result of increased T8+ cells ( $p < 0.01$ ). The T4/T8 ratio of the infiltrating cells showed no significant change in relation to rejection. The infiltrate in AP treated patients invariably exceeded that in patients receiving Cy. Significant cellular infiltration occurs within the first week after transplantation, even in grafts with stable function, and is still significant at one year. The T8+ cell infiltrate reflects the importance of this subpopulation as effector cells in graft rejection.

*Infiltrate > in az + pred than cyA.*  
*Thymocytes accounts for 35% infiltrating cells.*  
*Also no correlation with T4/T8 ratios in peripheral blood*

PAPER 2

SERUM FcγR BLOCKING FACTORS CORRELATE WITH RENAL ALLOGRAFT SURVIVAL

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Although Fcγ-receptor (FcγR) blocking antibody has been correlated with renal allograft survival (1), we have been unable to confirm this observation using IgG separated by DEAE cellulose chromatography (2). To assess whether other serum factors were responsible for this apparent correlation we fractionated over sucrose gradients serum taken immediately prior to transplantation from 29 transfused recipients, and from untransfused and normal controls. The resulting serum fractions were tested for FcγR blocking activity with an EA rosette inhibition assay. In agreement with our previous findings FcγR blocking activity in the 7S (IgG) peak did not correlate with graft survival. However 15/16 patients with grafts functioning at one year, and none of the 13 patients with rejected grafts, showed significant FcγR blocking in the fraction containing high molecular weight (>19S) serum factors. The nature of the high molecular weight serum FcγR blocking factors, which appear predictive of allograft survival, is under investigation.

- 1) MacLeod AM et al. Lancet 1982 ii 486.
- 2) Forwell MA et al. Kidney International 1984 26 635.







## PAPER 5

### THE EFFECT OF HYPERGLYCAEMIA ON ISOLATED RODENT ISLETS TRANSPLANTED TO THE KIDNEY CAPSULE SITE

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The effect of hyperglycaemia on transplanted rat islets was studied using a new technique for transplanting a defined number of islets in a blood clot. Normal or streptozotocin-diabetic DA rats were given 400 DA islets under the left kidney capsule (insufficient to reverse diabetes). After 2 weeks the diabetic rats were given a further 1000 islets under the right kidney capsule to reverse diabetes. Kidneys from both groups were examined at 2 weeks and 3 months for gross and histological appearance and for insulin content. After 2 weeks non-diabetic left kidneys showed abundant islet tissue, with an insulin content of  $0.116 (\pm 0.014 \text{ S.E.M.})$  units, compared to  $0.001 \pm 0.0005$  units in the right kidney. Kidneys from diabetic rats showed no islets recognisable grossly and histological examination showed vacuolated tissue, scarcely recognisable as islet tissue. However, 3 months after reversal of diabetes by transplantation of 1000 islets to the right kidney, histologically "normal" islet tissue was again visible on the left kidney, and the insulin content was  $0.16 \pm 0.036$  units. Islets left in normal animals for 3 months contained  $0.195 \pm 0.05$  units.

This quantitative approach to islet transplantation shows that transplant islets survive two weeks hyperglycaemia and suggests that insulin treatment is unnecessary after islet transplantation, even if the blood sugar is initially raised.

1600 islets from 4 donors, hand packed into 4 groups of 400 islets randomly assigned to recipients.

## PAPER 6

### IS THERE A DIFFERENCE IN REJECTION BETWEEN SINGLE AND COMBINED KIDNEY AND PANCREAS GRAFTS? - AN EXPERIMENTAL STUDY

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There is some evidence from those patients who have combined kidney and pancreas grafts that concurrent transplantation of the kidney prolongs pancreas graft survival. Having established a technique for combined grafting in the rat (ref) we studied rejection in both single and combined rat allografts to elucidate the problem.

Combined and single kidney and pancreas transplantation was performed in streptozotocin-induced diabetic rats. The animals were compared using biochemical parameters, graft survival times, and histological appearances. Isograft groups were also performed as controls.

No difference in mean graft survival time was seen in the kidney, pancreas, and combined allograft groups. The rise in serum creatinine was similar in both single kidney and combined allografts. However, the eventual rise in serum glucose noted in the single pancreas grafts was not seen in the combined despite destruction of the islets. A rise of serum insulin, to above normal levels, occurred in the single pancreas grafts, but was not observed in the combined, for which the insulin never reached the pre-diabetic values. On rejection the insulin values returned to diabetic levels. No relevant histological differences were seen between the three groups.

This evidence suggests that earlier diagnosis of rejection rather than a lowered susceptibility to rejection is the cause of improved survival in combined transplantation.

1. Nolan M.S. et al. Microsurgery (1984), 5(2), 73-75

Combined allograft transplants & pancreas  
Presence of kidney does not enhance pancreas  
function



## PAPER 7

### THE EFFECT OF CYCLOSPORIN A ON THE INDUCTION OF DONOR CLASS I AND CLASS II MHC ANTIGENS IN CARDIAC AND RENAL ALLOGRAFTS IN THE RAT

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It has previously been demonstrated that there is a 10-fold increase in the expression of Class I MHC antigens and de novo induction of Class II MHC antigens in rejecting cardiac allografts in the rat. This induction of MHC antigens is probably due to soluble mediators, such as interferon  $\gamma$ , released by infiltrating lymphocytes. We have studied the influence of cyclosporin A on MHC induction in grafts because this drug is widely used in clinical transplantation and appears frequently to be associated with moderate cellular infiltration without overt rejection in organ allografts. The model we used was DA strain hearts or kidneys grafted into PVG strain recipients treated with 10mg/kg/d Cyclosporin A given orally in olive oil. Grafted organs were removed for study at days 3,5,7,10 and 14 post-grafting. Immunohistological studies used the peroxidase technique and allospecific mouse monoclonal antibodies reactive with DA but not PVG MHC antigens. Quantitative absorption analyses were performed in graft homogenates.

Cyclosporin A treated heart allografts have a moderate cellular infiltrate, peaking on day 7 post-grafting. There is a 3-4 fold increase in MHC Class I antigen expression at day 7, but no induction of MHC Class II antigens at any stage. This pattern is remarkably similar to heart isografts studied over the same period.

Untreated renal allografts have a fifteen fold increase in Class I expression by day 5 after transplantation, and Class II expression spreads from only the proximal tubules in normal kidney to all the tubules and the vascular endothelium of large vessels. With cyclosporin A, there is a modest increase in Class I antigen expression but the Class II expression remains the same as in the normal kidney. Again the cyclosporin A treated renal allografts behave very similarly to renal isografts.

It is possible, therefore, that the induction of Class II MHC antigens in the graft parenchyma might be a useful parameter to measure for the differential diagnosis of cyclosporin A toxicity, benign cellular infiltrates and active rejection.

## PAPER 8

### TOTAL LYMPHOID IRRADIATION (TLI) AND CYCLOSPORIN A (CYA) USED IN THE PREVENTION OF REJECTION IN A RAT HETEROTOPIC CARDIAC ALLOGRAFT MODEL

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TLI has been used safely in Hodgkins disease for many years, and is now being used, on occasions, in transplantation. The combination of CYA and TLI as immunosuppressive agents may be of advantage by permitting non-nephrotoxic doses of CYA to be effectively employed. The optimal combination, however, of these two drugs is at present unknown.

In this study 41 Wistar rats in 4 groups were used as recipients for PVG heterotopic rat heart transplantation. 10 untreated recipients rejected their allograft in  $8.2 \pm 1.8$  days (group A). In groups B, C and D, recipients received, respectively, CyA 1 mg/kg daily orally post operatively, 5 - 200 rads fractionated TLI pre operatively (1000 rads) and 5 x 200 rads pre operatively + CYA 1 mg/kg daily post operatively. Irradiation was delivered from a 250 kilo volt source, lead shielding being used in the conventional trouser mantle manner. Light sedation using Thalamonal was employed for each fraction used.

## RESULTS

TREATMENT	REJECTION TIMES - DAYS	MEAN
Control - Untreated	6,10,7,5,7,9,9,10,10,9	$8.2 \pm 1.81$
CYA 1 mg/kg/day	12,7,9,9,9,10,11,8,11	$9.0 \pm 1.59$
*5 x 200 rads TLI	20,21,16,16,15,18,17,>100*	$18 \pm 2.3$
5 x 200 rads TLI + CYA 1 mg/kg	>100,>100,28,33,30,32,66, >100,52,>100,52,49,60,64	$52.14 \pm 15$

\* Smaller numbers of fractions of TLI had little effect.

\* Values >100 days were counted as equivalent to the next highest value within the group.

Our data indicates that a small dose of CYA, in itself inefficient, can be combined with a safe dose of pre transplant TLI to produce highly significant prolongation in a cardiac allograft model.



## PAPER 9

### THE USE OF CYCLOSPORIN IN SMALL BOWEL TRANSPLANTATION IN THE RAT

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Heterotopic, vascularized small bowel transplants (SBT) were performed in inbred strains of rats to investigate the effects of Cyclosporine (CyA) on the immunology, structure, and function of intestinal transplants. Lewis x Brown Norway F<sub>1</sub> (LBN) bowel was rejected by untreated Lewis rats (LEW) in 7-10 days (mean±SD=8.3±1.3, n=6). CyA (15mg/kg/day) therapy for 7 days prevented rejection indefinitely (>100 days, n=5). Morphological examination of rejecting LBN mucosa revealed shortening of both crypts and villi, attenuation of the microvillus brush border, and markedly decreased surface area, findings which were largely prevented by CyA. The mucosal transport function of rejecting allografted (LBN into LEW) SBT, isografted (LEW into LEW) SBT, and host intestine were measured electrophysiologically in Ussing chambers. Rejection was associated with impaired epithelial active ion transport as indicated by decreased potential difference and with diminished epithelial barrier function as reflected by decreased trans-epithelial resistance to passive ion flow. CyA therapy maintained potential difference to 80% of control; but the resistance to only 40% of control. LEW into LBN SBT caused fatal graft-versus-host disease (GVHD) in 9-17 days (mean±SD=13.2±3.2, n=5). CyA for 7 days failed to prevent GVHD routinely, but prolonged administration delayed GVHD until CyA was discontinued. In conclusion, CyA will 1) prevent SBT rejection after a short course, 2) preserve a near normal morphology, 3) maintain active ion transport by the mucosa and partially preserve epithelial barriers to passive ion flow, and 4) delay fatal GVHD induced by the large quantity of lymphoid tissue present in the small bowel and its accompanying mesentery.

## PAPER 10

### INDICES OF REJECTION IN SMALL INTESTINAL ALLOGRAFTS

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Cyclosporin (CyA) prolongs canine intestinal allograft survival but few functional studies have been done. We evaluated histology and function in small intestinal grafts after transplantation.

A 100cm ileal segment was auto(24) or allografted(33) as a Thiry Vella fistula. 18 animals receiving cyclosporin-A(CyA), 20ml/kg/day P.O. Daily mucosal biopsies were taken, the intestine was perfused and intraluminal pressures recorded. 14 technical failures were excluded. 18 dogs with autografts survived indefinitely while 14 non immunosuppressed animals survived 8-15 days (11.26±2.6 mean±s.e.m.). In animals receiving CyA survival improved to 19-142 days (70.09±13.34 mean±s.e.m. p=0.0002). In non immunosuppressed animals the earliest histological evidence of rejection occurred at 6.2±0.9 days. From day 6 to day 7 there was a decrease in absorption of water (5.4±1.09 mean±s.e.m. to 1.77±0.26 ml/5min, p=0.0092), alanine (35.18±1.37 to 17.15±4.81%, p=0.0048) and free fatty acids (30.45±2.98 to 5.5±4.75%, p=0.0013) and an increased output of sodium (0.91±0.1 mean±s.e.m. to 1.35±0.02 mmol/5min, p=0.0016) and glucose (0.81±0.12 to 1.29±0.07 mmol/5min, p=0.0062). From day 7 to day 8 intestinal motor activity decreased as shown by a reduced number of intraluminal pressure peaks >50cm/water (79±6.63 mean±s.e.m. to 18.33±11.76, p=0.0012), activity fronts/hr (7±0.73 to 1.5±0.67, p=0.0002) and the duration of activity fronts (141.47±11.34 to 31.7±9.47secs, p<0.0001).

We conclude that CyA prolongs allograft survival. Impaired intestinal absorption is synchronous with the earliest histological evidence of rejection and 24 hours prior to impaired intestinal motor activity.

*Continuous administration of cy very important to prevent GVH*

*Myoelectrical activity persists to just before necrosis & no use to detect rejection*

*- with strain combination*



### THE MEASUREMENT OF CYCLOSPORIN A (CyA) IN WHOLE BLOOD BY H.P.L.C. IN THE DIFFERENTIATION BETWEEN CyA NEPHROTOXICITY AND REJECTION IN RENAL ALLOGRAFT RECIPIENTS

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The assessment of CyA levels in plasma by radioimmunoassay is affected by the temperature at which the blood cells are separated, by metabolites of CyA, and by steroids. To circumvent these difficulties we have measured CyA in whole blood samples using an H.P.L.C. method. Between batch variation was (5.5-12.5%) across a wide concentration range (125-1250ng/ml) and gave a mean recovery from whole blood of 104.9% (range 86-128%). CyA was given 12 hourly and samples taken immediately before, two and four hours after the morning dose. Graft rejection and CyA nephrotoxicity were differentiated retrospectively using clinical, biochemical and histopathological data, and from measurement of interstitial renal pressures, and response to therapy, without knowledge of CyA levels.

**Results:** In patients with no sign of rejection, renal function appeared to worsen as trough CyA levels rose, although the difference did not reach statistical significance, see (table 1)

	PLASMA CREATININE $\mu\text{mol/l}$			
	<200	201-300	301-400	>400
Mean trough CyA levels $\pm$ SEM ng/ml	364 $\pm$ 81	404 $\pm$ 52	404 $\pm$ 89	510 $\pm$ 68
Number of observations	7	23	10	12

However, when plasma creatinine concentration exceeded 300 $\mu\text{mol/l}$ , trough CyA levels were higher (462 $\pm$ 54ng/ml) in 22 patients with nephro-toxicity than 11 patients suffering rejection (220 $\pm$ 55ng/ml,  $p < 0.005$ ). CyA levels at two and four hours were not helpful in differentiating rejection from nephrotoxicity. In conclusion measurement of CyA by H.P.L.C. in whole blood is reproducible, valid, and it appears to be able to differentiate between CyA nephrotoxicity and rejection in patients with moderate to severe renal impairment.

### WHOLE BLOOD CYCLOSPORIN LEVELS - CORRELATION WITH CLINICAL EVENTS

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Empirical dosing regimens of Cyclosporin A in transplant recipients tend to result in variable drug levels. A flexible dose schedule, guided by drug monitoring may improve clinical management.

A retrospective analysis comparing whole blood cyclosporin levels with clinical indices was made. Thirty renal transplant recipients receiving 15 mg/Kg CyA for the first 4 post-operative weeks had trough CyA levels measured by radioimmunoassay (Sandoz) at least once weekly, and in some cases, daily. The suggested therapeutic range using this method is approximately 250-1000 ng/ml for the early post-operative period.

Patients were grouped according to their median CyA levels over the 4 week period:-

- those who tended to run at high levels with a median 800 ng/ml (n=7), medium levels
- a median between 400-800 ng/ml (n=13) and low levels with a median less than 400 ng/ml (n=10).

The results were compared with the incidences of rejection, infection and toxicity. A strong correlation between drug levels and acute rejection was found. Rejection episodes were recorded on the basis of clinical treatment with high-dose steroids (all episodes were confirmed histologically). Nine/ten patients who tended to run at low levels suffered rejection - 8 patients requiring more than one pulse therapy. (Two lost their grafts due to irreversible rejection). In the medium and high level groups a total of 7 out of 20 patients suffered from rejection, although 3 of these had low levels prior to rejection. Cyclosporin levels in the week preceeding all rejection episodes (median = 296 ng/ml) were compared with levels in patients who were not treated for rejection (median = 700 ng/ml) and were found to be significantly different. ( $p < 0.001$ ).

No correlation was found between CyA levels and the incidence of infection in the period of study although we have some evidence to suggest that longer-term high levels may be associated with an increased risk of infection, and also with symptoms of drug toxicity. Monitoring of cyclosporin levels in the early post-operative period may be a clinically useful tool in defining effective immunosuppressive regimens.



### RISK FACTORS ASSOCIATED WITH AVASCULAR BONE NECROSIS (ABN) FOLLOWING RENAL TRANSPLANTATION

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The risk factors associated with the development of ABN in 161 consecutive renal transplant recipients were looked for by comparing the 38 patients who developed ABN (24%) with the remaining 123, using a proportional hazards model with step-wise selection of 56 variables. There was a higher incidence of ABN in females than males (37% vs 15%,  $p < 0.004$ ), and patients with ABN had gained significantly more weight during the six months post-transplant ( $9.5 \pm 5.0$  kg vs  $6.3 \pm 4.4$  kg,  $p < 0.006$ ). The probability of patients treated with high and low dose prednisolone developing ABN was 28% and 6% respectively at 3 years ( $p < 0.01$ ). HLA-B8 was significantly associated with the development of ABN in both males and females ( $p < 0.05$ ) (table), whereas an association with HLA-B12 was significant only for males ( $p < 0.05$ ).

Probability of developing ABN at 3 years post-transplant

	High dose prednisolone		Low dose prednisolone	
	Male	Female	Male	Female
HLA-B8 +ve	20%	44%	12%	42%
HLA-B8 -ve	8%	20%	4%	18%

These results suggest that variations in prednisolone pharmacokinetics, possibly HLA-B8, 12 associated, may explain individual susceptibility to the development of steroid induced ABN.

### CLINICAL APPLICATION OF RAPID DEAFF TEST FOR EARLY DIAGNOSIS OF CMV INFECTION IN RENAL TRANSPLANT PATIENTS

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Cytomegalovirus infection is a common and serious complication of renal transplantation. This report surveys 40 renal transplant recipients for active CMV infection for at least 3 months post transplantation. In most cases weekly samples (blood, saliva and urine) were obtained for CMV culture. CMV excretion is detected by early antigen fluorescent foci (DEAFF test).<sup>1</sup> In comparison to conventional culture methods which depend on cytopathic effects apparent at about 2-3 weeks, this test relies on the detection of virus-coded proteins in infected cells by monoclonal antibodies and results are available after 24 hours of culture.

Sixteen patients (42%) showed evidence of active CMV infection. In 14 of the patients, CMV was excreted in the urine, 10 in the saliva, and 6 had CMV viraemia. In 2 patients with pneumonitis bronchial lavage was positive for CMV.

Of the 16 patients, 2 had pneumonitis (one fatal), 5 had a blood culture proven septicaemia and one other presented as a PUO. Thus 8 of the patients (50%) with active CMV infection had episodes of serious infection, as compared to only 2 patients (9%) in the CMV negative group ( $P < 0.05$ ,  $\chi^2 9.686$ ).

In conclusion, CMV infection is common in renal transplant recipients and is closely associated with serious infections. The DEAFF test provides a rapid diagnosis and allows early institution of specific therapy such as anti-CMV immunoglobulins which are now available.

1. Griffiths P et al. Rapid diagnosis of CMV infection in immunosuppressed patients by detection of early antigen fluorescent foci. *Lancet* ii 1984, 1242-1245.



## PAPER 15

### INTERFERON (IFN) AND INTERLEUKIN-2 (IL-2) PRODUCTION IN CONVENTIONALLY-SUPPRESSED AND CYCLOSPORINE (Cy-S)-TREATED RENAL ALLOGRAFT RECIPIENTS

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The cytotoxicity of natural killer (NK) cells is regulated by the lymphokines IFN and IL-2, these three components being implicated in host defences against viruses, tumours and allografts. To determine the effects of immunosuppression on these inter-relationships, NK activity, IL-2 production and IFN $\alpha$  generation were measured simultaneously in the peripheral mononuclear cells of 25 healthy control subjects, 25 conventionally immunosuppressed (Az + P) and 35 Cyclosporine (CyS)-treated renal allograft recipients. Mean ( $\pm$  S.D.) results were as follows:-

	Controls <sup>a</sup>	Az+P group <sup>b</sup>	CyS group <sup>c</sup>
IFN $\alpha$ production (units/ml)	278 $\pm$ 129(n=25)	107 $\pm$ 75.5(n=25)	127 $\pm$ 96.4(n=35)
IL-2 generation (units/ml)	83.5 $\pm$ 39(n=25)	46.7 $\pm$ 51.6(n=18)	26.9 $\pm$ 40.1(n=22)

(a v b,  $p < 0.02$ , a v c,  $p < 0.02$ , sum of Ranks test). Thus IL-2 and IFN generation are impaired during both regimens. However, the two immunosuppressed groups may be divided into those with "normal" and "low" NK activity when compared with NK activity in controls. In the Az + P group both IL-2 and IFN $\alpha$  responses were similar in the normal and low NK reactors. In the CyS group both IFN $\alpha$  and IL-2 producing capacity were significantly lower in the patients with low NK activity than in those with normal NK activity ( $p < 0.002$  and  $p < 0.02$  for IFN and IL-2 respectively).

Low NK activity is related to impaired lymphokine production in CyS-treated but not conventionally-treated patients. Furthermore, since IFN $\alpha$  is produced by activated monocytes, these data provide evidence for an in vivo effect of Cyclosporine on monocytic as well as T-cell function.

## PAPER 16

### COMPARISON OF MONOCLONAL ANTIBODIES IN IMMUNOLOGIC MONITORING OF RENAL TRANSPLANT PATIENTS

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Sequential monitoring of peripheral blood T cell subpopulations was performed by flow cytometry in 360 blood samples obtained from 35 renal allograft recipients using OKT and Leu monoclonal antibodies (MoAb). Cells which were labelled with the Leu antibodies were double labelled with an anti HLA-DR MoAb to identify activated cells in the different subpopulations. The percentage of T cells expressing the "cytotoxic-suppressor" phenotype stained with the Leu2a MoAb was significantly lower than the percentage of OKT8<sup>+</sup> cells (14.5 $\pm$ 9.1% and 18.3 $\pm$ 8.0%; respectively; mean $\pm$ SD;  $p < 0.0001$ ). The percentage of T cells expressing the "helper-inducer" phenotype stained with the Leu3A antibody was also lower than the percentage of OKT4<sup>+</sup> cells (47.6 $\pm$ 12.8 and 50.5 $\pm$ 11.2 respectively). Since the discrepancy between the Leu2a and OKT8<sup>+</sup> cells was relatively higher (21%) than that between the Leu3a<sup>+</sup> and OKT4<sup>+</sup> cells (6%), the mean Leu3a/2a ratio was significantly higher than the mean OKT4/8 ratio (6.64 $\pm$ 9.7 versus 3.60 $\pm$ 2.82;  $p < 0.0001$ ). A high discrepancy between the Leu2a<sup>+</sup> and OKT8<sup>+</sup> cells, indicated by an OKT8/Leu2a ratio  $\geq 1.5$ , correlated with a high percentage of DR<sup>+</sup> cells within the Leu2a population ( $>6\%$ ), whereas an OKT8/Leu2a ratio  $< 1.5$ , correlated with a low percentage DR<sup>+</sup> cells ( $\leq 6\%$ ;  $p < 0.0001$ ). These observations suggest that activation of some T cells with the "cytotoxic-suppressor" phenotype leads to antigen modulation in which the OKT8 epitope remains unaffected but the Leu2a epitope has disappeared or become undetectable with the present method.



Pre-op Cyfl 5-9 mg/kg oral.  
Methylpred 500 mg pre.  
500 mg iv. post-perfusion.

PAPER 17

Horze ATG

Cyfl 5-10 mg/kg  
Pred 0-3 mg/kg

CLINICAL HEART-LUNG TRANSPLANTATION

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78 primary lung  
24 primary vascular

Between November 1982 and February 1985 87 patients have been referred for consideration of heart-lung transplantation at Papworth. Sixty-three had primary lung disease and 24 primary cardiac defects. Many early referrals did not meet our criteria mainly because of age and medical unsuitability. Twenty-seven patients have been assessed, eight await assessment. Sixteen patients have been accepted as recipients, four have died waiting and nine currently await transplantation.

18 accepted, 3 transplanted, alive & well 5-12 mths afterwards

Between April 1984 and February 1985 three patients have been transplanted. The reasons for transplantation were; fibrosing alveolitis; Eisenmenger complex with ASD and primary pulmonary hypertension after two years continuous Prostacyclin therapy. The availability of suitable donor organs has been the main restricting factor; size compatibility, good lung function and clear chest x-ray are essential. We have introduced a new method of preservation of the donor lungs, colloid rather than crystalloid based, that has given excellent early postoperative function. This may lead to distant organ procurement.

Immunosuppression therapy has been Cyclosporin A with low dose steroids and a five-day perioperative course of Equine ATG. All patients are alive and well with good lung function at eleven, seven and two months after transplantation. Heart-lung transplantation is a viable clinical procedure for selected young people with disabling pulmonary or cardiopulmonary disease.

Endstage per or submory vas. disease

Less than 45 yrs.

No previous cardiac surgery

Stable social conditions

Donor

< 35 yrs.

ABO compat

Normal gas exchange + compliance

Clear X-ray, no infection

No major chest trauma

No major chest trauma

Must fit the recipient

Post-transpl pulmar

oedema: Why?

Fluid fingers 750

20% salts poor

album 200

20% mantel 50

Temp 4°

osmolal 32

PAPER 18

THE ROLE OF MAJOR AND MINOR TRANSPLANTATION ANTIGENS IN THE BLOOD TRANSFUSION EFFECT

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Pretransplant blood transfusion can enhance kidney allograft survival in both clinical and experimental situations. However, in the case of rat allografts the blood transfusion effect in rats is strictly strain specific. By contrast, the clinical effect is very broad although it is still possible that the donors of the transfusions and the donors of the kidneys may have to share some antigens. We have investigated the requirements for sharing of MHC and minor alloantigens by the blood and kidney donors in the rat renal allograft model. A beneficial effect is observed when the blood and kidney share some of the MHC antigens or minor (background) alloantigens. For instance, a DA (RT1<sup>a</sup>) rat given a PVG.RT1<sup>b</sup> blood transfusion will accept a LEW (RT1<sup>b</sup>) kidney because of sharing of the RT1<sup>b</sup> MHC antigens and will accept a PVG.RT1<sup>c</sup> kidney, despite no previous exposure to RT1<sup>c</sup> MHC antigens, because blood and kidney both have PVG minor antigens. However, activation of suppression to minor antigens requires that the blood transfusion differs from the recipient at the MHC. Hence, DA (RT1<sup>a</sup>) rats given PVG.RT1<sup>a</sup> blood will not accept PVG.RT1<sup>c</sup> kidneys, in contrast to the earlier example. We attribute this failure to activate anti-minor suppression to a "lack of help" provided by an allo-MHC stimulus. There are some constraints on activation of suppression to minor alloantigens but, in general, re-exposure to either major or minor antigens on the kidney leads to allograft acceptance even across MHC barriers. This effect could account for the broad reactivity of the clinical effect.



### SUPPRESSION OF ALLOGRAFT REJECTION IN THE RAT BY NORMAL BUT NOT HEAT INACTIVATED OR IRRADIATED DONOR SPLEEN CELLS

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Donor specific suppression of graft rejection by antigen pretreatment, is well recognised in experimental tissue transplantation and is extremely relevant to the observation that blood transfusions significantly improve survival of human kidney allografts.

In the rat, suppression of allograft rejection by pretreatment with viable spleen lymphocytes has been reported (1,2), however the mechanism remains uncertain. In the Lewis (RT1<sup>b</sup>) to Dark agouti (RT1<sup>a</sup>) strain combination, indefinite renal allograft survival (median survival time, MST, >100 days) was induced by pretreating recipients intravenously with  $10^6 \cdot 10^8$  viable spleen lymphocytes, seven days before transplantation. Pretreatment with  $10^6$  or  $10^5$  cells was ineffective (MST 10 days). However  $10^7$  viable, but heat inactivated (55°C for 10 minutes) or irradiated (1000 rads) lymphocytes resulted in unmodified allograft rejection in all animals (MST 10 and 11 days respectively versus 10 days for third party treated controls).

Cell surface expression of major histocompatibility antigens was quantitated for normal, heat inactivated and irradiated lymphocyte preparations by cytofluorograph and scatchard analysis, and found to be identical for all three preparations.

Studies to monitor trafficking of these lymphocytes (<sup>51</sup>chromium labelling) in recipient animals suggest that the length of time donor cells remain in the recipient circulation, may play a role in the induction of specific immunosuppression by spleen lymphocytes.

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2. Silber SJ, Miller JFAP, Morris PJ. Graft-versus-host reactivity and renal allograft survival in rats given allogeneic spleen cells or spleen allografts. Transplantation 1976; 22:160-166.

### THE ROLE OF Ia<sup>+</sup> BONE MARROW DERIVED CELLS IN THE INDUCTION OF INTESTINAL GRAFT-VERSUS-HOST REACTION IN MICE

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The small intestine is one of the major target organs in acute graft-versus-host disease (GvHD) after allogeneic bone marrow transplantation, but the pathogenic mechanisms are not yet understood. In addition, the nature of the allogeneic target structures are not known.

We have shown previously that the intestinal phase of graft-versus-host reaction (GvHR) in unirradiated mice is characterised by increased numbers of intraepithelial lymphocytes (IEL) and increased crypt cell production rate (CCPR). These alterations occur in the absence of cytotoxic T lymphocytes and are dependent on Lyt 1<sup>+</sup> T cells recognising Class II MHC alloantigens, and we have now examined whether tissue or bone marrow (BM) cells provide the stimulus for intestinal GvHR. Characteristic mucosal alterations occurred in grafts of parental gut grafted under the kidney capsule of F<sub>1</sub> hosts and in the intestine of F<sub>1</sub>→P bone marrow chimeras after induction of GvHR with parental spleen cells. In contrast, no intestinal alterations were found in P→F<sub>1</sub> chimeric mice after injection of parental cells. Thus, the intestinal phase of GvHR does not depend on the presence of allogeneic intestinal epithelium but can be induced by allogeneic BM cells which we show to be Ia<sup>+</sup>.

We conclude that the intestinal phase of GvHR in unirradiated mice is due to delayed type hypersensitivity induced by Class II MHC antigens on recirculating BM derived cells.



### INHIBITION OF SKIN GRAFTS REJECTION IN RATS BY A PROTEIN (IRG) EXTRACTED FROM THE SERUM OF A PREGNANT WOMEN OR FEMALE ANIMALS

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Since long ago immunologists tried to understand why in Vivipares the embryo is not rejected because it is 50% foreign to the mother. Now we know it. Few years ago Mrs. Chateaufreynaud-Duprat with different collaborators has established that the serum of pregnant Salamandra, and later also of gestating women inhibits the classical cytotoxic activity of lymphocytes. Now the active component of the serum has been purified; it is a glycoprotein of the  $\alpha_2$  heavy globulin fraction or a smaller molecule absorbed by a heavy globulin. It has an inhibition activity on one of the components of the complement, probably C<sub>3</sub>. Therefore the embryo is not killed and rejected. This glycoprotein is completely absent in serum of men of not pregnant women and of pregnant women who make undesirable abortions. A simple diagnostic method permits to detect such cases. The factor appears very soon after fecondation and disappears after delivery. Unexpectedly it appears also in animals subjected to a non specific inflammatory reaction.

Skin grafts in rats of two incompatible strains which in controls are rejected in around 12-13 days are maintained for 30 days after one injection of the purified protein, and 40-50 days after a second injection. We call this protein IRG (inhibitor of rejection of grafts). It has the advantage over chemical immunosuppressors because it is a natural protein and it does not suppress antibody formation.

### CORTICAL PROSTAGLANDIN SYNTHESIS IN RAT RENAL ALLOGRAFT REJECTION

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Acute renal allograft rejection is accompanied by increased platelet deposition<sup>1</sup> and reduced vascular perfusion<sup>2</sup> within the graft. Such changes may be related to alterations in renal prostaglandin synthesis. 6 keto prostaglandin F<sub>1 $\alpha$</sub>  (6 keto PGF<sub>1 $\alpha$</sub> ) and thromboxane B<sub>2</sub> (TxB<sub>2</sub>) were measured by radioimmunoassay in incubates of cortical slices taken from rat renal allografts (DA to F<sub>1 $\alpha$</sub>  Agus/WAG) or isografts (DA to DA) 1 to 7 days after transplantation into nephrectomised recipients. Histological changes of cellular rejection appeared at 3 days with areas of haemorrhage and infarction appearing at 7 days. The results are shown in the table.

		Unoperated	Day 1	Day 3	Day 5	Day 7
Number of Animals	Allograft	5	5	5	6	6
	Isograft	5	5	5	5	6
Serum Creatinine (umol/l)	Allograft	57.0±6.0	81.6±3.7	81.2±7.3	162.0±25.2	385.5±81.1
	Isograft	42.4±2.2	75.4±5.6	83.0±6.3	69.8± 7.4	69.3± 8.2
Mean±SEM		(NS)	(NS)	(NS)	(p<0.02)	(p<0.01)
Cortical TxB <sub>2</sub> (pmol/g)	Allograft	24.5±2.6	24.5±2.4	61.3±9.6	300.3±53.1	2610.3±664.7
	Isograft	24.5±2.6	26.3±2.5	37.8±2.9	39.1± 5.0	59.2± 10.0
Mean±SEM			(NS)	(p<0.05)	(p<0.002)	(p<0.001)
Cortical 6 keto PGF <sub>1<math>\alpha</math></sub> (pmol/g)	Allograft	50.7±7.3	47.2±3.3	56.4±3.7	92.6± 8.3	357.8± 66.6
	Isograft	50.7±7.3	51.7±3.6	69.1±5.0	91.6± 6.7	76.0± 15.2
Mean±SEM			(NS)	(NS)	(NS)	(p<0.001)

It is concluded that an increase in cortical thromboxane synthesis is an early feature of acute renal allograft rejection in the rat and may play a role in reducing vascular perfusion or increasing platelet deposition in rejecting kidneys.

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### INCREASED PROSTAGLANDIN E PRODUCTION FOLLOWING BLOOD TRANSFUSION

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The association between pre-transplant blood transfusion and improved graft survival has been clearly established, though the mechanism by which blood transfusion confers allograft protection remains to be defined. The immunoregulatory role of some prostaglandins has also received increasing attention, in particular prostaglandin E (PGE) has been reported to suppress a variety of immune responses. *In vitro* production of PGE by mononuclear cells was studied following blood transfusion in 15 previously non-transfused dialysis patients, prior to, and 14 days following each blood transfusion. The dialysis patients received up to 10 units of whole blood, one unit every 14 days. Twelve non-transfused control subjects were also studied. PGE levels in 24 hour cell culture supernates were assayed by radioimmunoassay and found to increase with successive blood transfusions in the dialysis patients (correlation coefficient of 0.727,  $p < 0.05$ ). The PGE concentration was  $13,354 \pm 6,379$  pg/ml following the tenth blood transfusion, compared to  $5308 \pm 1216$  pg/ml in patients prior to transfusion ( $p < 0.05$ ) and  $3534 \pm 762$  pg/ml in controls ( $p < 0.01$ ). The ways in which PGE may be involved in immunosuppression remain to be defined.

### INTRA-RENAL PRESSURE (IRP) MEASUREMENT IS USEFUL IN DETERMINING THE CAUSE OF DETERIORATING EARLY GRAFT FUNCTION

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The differentiation between Cyclosporin (CyA) nephrotoxicity and rejection in renal transplant recipients remains a difficult problem. Treatment is based upon multifactorial analysis of physical signs, biochemical data, measurement of CyA levels, and transplant biopsy. In our centre, CyA assay results and biopsy data may not be available immediately when the therapeutic decision needs to be made.

We have adopted the technique of Salaman et al, to measure intra-renal pressure (IRP) and have evaluated its usefulness in clinical decision making. Since January 1984 we have performed 90 IRPs in 48 renal transplant recipients. In 12 patients with stable renal function in the early post-transplant period the mean pressure was 31 mm.Hg. (range 25-45). On all further occasions the IRP was measured in patients with a rising serum creatinine. Evaluation of the correctness of the prospective decision following IRP measurement was made by retrospective analysis of the biochemical, clinical, and histopathological data. In 15 rejecting grafts the mean pressure was 46 mm.Hg. (range 36-90); this included patients on Imuran and prednisolone, in whom we were assessing progress during ATN post transplant. In 36 patients suffering from CyA nephrotoxicity, IRP was low and averaged 27mm.Hg (range 20-45,  $p < 0.05$ ). The IRP was not helpful in differentiating chronic rejection from other pathology in patients presenting with a late rise in serum creatinine. However in one patient with transplant renal artery stenosis, the IRP was unrecordable. No complications attributable to IRP measurement occurred.

Overall the correct clinical decision was made in over 90% of patients. We consider IRP measurement makes a valuable contribution in the differential diagnosis of early renal transplant failure.

Ref. Salaman JR et al. Fine needle intra renal manography. A U test for rejection in Cy treated recipients of kidney transplants. Lancet volume 2 pg 709-711 1983



### AN ASSESSMENT OF INTRARENAL HYDROSTATIC PRESSURE MEASUREMENTS IN THE DIAGNOSIS OF ACUTE RENAL ALLOGRAFT REJECTION

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Postoperative intrarenal pressure measurements may be an aid to the diagnosis of acute renal transplant rejection, especially in patients treated with cyclosporin A<sup>1,2</sup>. Serial measurements of intrarenal pressure were made in 38 recipients using a fine needle technique. 32 intraoperative and 207 postoperative measurements were made and 39 clinical rejection episodes (23 confirmed by biopsy) monitored. Intraoperative pressures in grafts with immediate function ( $37.4 \pm 4.0$  mmHg, Mean  $\pm$  SEM) were not significantly different from those with delayed function ( $30.9 \pm 4.8$  mmHg) whereas postoperative pressures were greater ( $p < 0.01$ ) in kidneys with acute tubular necrosis ( $29.4 \pm 1.9$  mmHg) than in functioning grafts ( $20.4 \pm 0.9$  mmHg). Pressures recorded during clinical rejection episodes ( $44.3 \pm 2.3$  mmHg) exceeded ( $p < 0.001$ ) those during quiescent periods ( $23.6 \pm 1.0$  mmHg). During rejection episodes, higher pressures ( $p < 0.01$ ) were recorded from tender or palpably enlarged grafts ( $52.3 \pm 3.0$  mmHg) than in the absence of these signs ( $36.3 \pm 3.1$  mmHg) and patients whose transplant biopsies showed cellular rejection tended to have greater pressures ( $50.1 \pm 4.1$  mmHg) than those with concomitant vasculopathy ( $36.4 \pm 3.9$  mmHg) but the latter did not reach statistical significance. In 7 cases of cyclosporin toxicity the intrarenal pressure was  $17.8 \pm 4.2$  mmHg. Using a diagnostic cut off point of 40 mmHg, the investigation failed to recognise 26% of acute rejection episodes and wrongly categorised 21% of non rejectors in the presence of acute tubular necrosis. It was therefore of limited predictive value.

1. Salaman J.R., Griffin P.J.A. Fine needle intrarenal manometry: a new test for rejection in cyclosporin-treated recipients of kidney transplants. *Lancet* 1983, 2, 709-711.
2. Wagner E., Pichlmayr R., Wonigeit K., Klempnauer J., Bungendahl H., Lauchart W. Differentiation between rejection and cyclosporin A nephrotoxicity by monitoring interstitial pressure in human renal allografts. *Transplantation Proceedings* 1983, 15, 489-492.

### FORTHCOMING MEETINGS

27th - 29th November, 1985

2nd CONGRESS OF THE EUROPEAN SOCIETY OF ORGAN TRANSPLANTATION, Munich, Germany.

3rd - 8th August, 1986

XI INTERNATIONAL CONGRESS OF THE TRANSPLANTATION SOCIETY, Helsinki, Finland.

### Halls of Residence

