BRITISH TRANSPLANTATION SOCIETY

PORTSMOUTH 1994

12th & 13th APRIL 1994

BRITISH TRANSPLANTATION SOCIETY

STATEMENT OF ACCOUNTS FOR THE YEAR ENDED 30th SEPTEMBER 1993

Receipts	1992 £	1993 £
Subscriptions Received	7,217	7,692
Immunology Letters Profit	63	–
Bank Interest	2,166	3,198
Net Surplus from Meetings		
Autumn	575	1,227
Spring	2.047	4,640
Audit in Transplantation	111	–
Net Surplus from Bursaries	700	–
Donations to Transplant Information Bureau	–	700
	12,879	17,457
Balance as at 30th September 1992		45,635
Add Excess of Receipts over Payments		6,850
		52,485

Payments	1992 £	1993 £
Postage and Stationery	2,375	686
Bank Charges	50	71
Secretarial Honoraria	1,000	1,000
Audit Fee	35	40
Engraving Medawar Medal	–	15
Summer School Advance	1,000	–
Software for BTS Newsletter	–	600
Net Payment of Bursaries	–	8,195
	4,460	10,607
Excess of Receipts over Payments	8,419	6,850
	12,879	17,457
Represented by:		50,000
Ulster Bank Ltd Business Reserve Current Account		2,485
		52,485

The Statement of Accounts and Balances are in accordance with information and records provided.

HONORARY TREASURER

HONORARY AUDITOR

PAPER 1

LATE REFLUSH IN RENAL TRANSPLANTATION - NO PROTECTION OBSERVED

J Fawcett, DWR Gray, PJ Morris and JA Roake.

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Recent reports have indicated that reflushing cadaver kidneys with preservation solution immediately prior to implantation has a beneficial effect on reducing delayed graft function[1,2]. Therapeutic effects of this manouevre may include washing out metabolic precursors of free oxygen radicals which may reduce reperfusion injury[3].

Recently we have randomly assigned all cadaver kidneys to either reflush with 500 mls of hyperosmolar citrate at 4°C immediately pre-transplantation or no reflush (control group). All kidneys were biopsied before implantation and immediately prior to wound closure. Subsequent managementof all patients was a sper our standard protocol. The trial was halted after recruitment of 9 patients to each arm because of suspicion regarding harmful effects of treatment. Demographic and immunological features and ischaemia times were similar in both groups. In the control group 6/9 achieved primary graft function but only 3/9 in the reflush group did so. Per-operative graft biopsies taken at a median of 30 minutes after reperfusion were abnormal in 6/9 reflushed kidneys but in none of the control group. Other parameters show a similar trend and will be presented. Our data have not supported a beneficial effect of reflushing; indeed it has possibly been harmful.

References.

1.Parrott NR, Forsythe JLR, Matthews JNS et al,(1990).Late perfusion: a simple remedy for renal allograft primary nonfunction. Transplantation **49**,913.

2.Lodge JPA, Kashi SH, Lam FT, Lord A and Giles GR(1993). The reflush effect - a prospective analysis of late perfusion. Transplantation **55**,567.

3.Bulkley GB(1987). Free radical-mediated reperfusion injury: a selective review. British Journal of Cancer **55**(supplement VIII),66.

PAPER 2

REDUCED FIRST DOSE EFFECT OF MONOVALENT ANTI-CD3 (M-antiCD3) USED IN ACUTE REJECTION IS ASSOCIATED WITH DIMINISHED PRO-INFLAMMATORY CYTOKINE RELEASE.

Abbs IC, Clark M^, Waldmann H^, Chatenoud L*, Koffman CG, Sacks SH.

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The antiCD3 antibody OKT3 used in the prevention and treatment of allograft rejection has a number of unwanted side effects, thought to result from release of pro-inflammatory cytokines. T cell activation and cytokine release result from simultaneous CD3 cross-linking and OKT3 Fc domain binding to Fc receptors on other immune cells. Monovalent antiCD3 (M-antiCD3) antibodies do not cause cross-linking and should not cause cell activation and cytokine release. In this pilot study, M-antiCD3 (2 mg/day for 8 days) was used to treat acute cellular rejection in 5 renal transplant recipients. TNF, IFN gamma and IL6 release were measured and patient tolerance of the drug monitored. M-antiCD3 caused an initial rapid fall in circulating lymphocyte count (to 22+/- 2.2% of baseline) and was successful in reversing rejection. The drug was well tolerated, without an appreciable first dose effect, and cytokine release was diminished compared to published data for OKT3. [First dose TNF peak 64.3+/- 19.6 pg/ml cf. 644+/-153 pg/ml. First dose IFN gamma peak just above baseline compared to 8-10 fold increases following OKT3. First dose IL6 peak elevated in 1 patient only]. The lack of first dose effect following M-antiCD3 is possibly the result of diminished release of pro-inflammatory cytokines. This drug may be a useful addition to the reagents available to treat allograft rejection.

PAPER 3

DO WE GIVE ENOUGH CYCLOSPORIN?

MJ Bowles and G Williams

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The aim of this study was to assess the value of Cyclosporin (CyA) profiles in CyA dosage management. 162 profiles were performed over 16 months in 40 patients and analysed retrospectively. Profiles were performed from 6 to 707 days (median 61 days) after transplantation (Tx). Blood samples were taken at 0, 2, 4, 6 and 8 hours after the morning CyA dose. Rejection episodes were diagnosed by renal biopsy, and CyA nephrotoxicity by a fall in serum Creatinine one week after a cut in CyA dose.

The mean area under the CyA vs time curve (AUC) was significantly lower at the time of rejection (3821 hr.ng/ml; n=13) than that of a time-from-Tx matched group of uncomplicated non-rejecting profiles (5479 hr.ng/ml; n=43) ($p<0.02$; Wilcoxon rank sum test). An AUC above 6400 hr.ng/ml discriminated rejection from non-rejection (0/13 vs 13/43 profiles respectively; $p<0.05$; Fisher's exact probability test). Pre-dose and peak CyA concentrations did not have such discriminating cut-off values. The mean oral CyA clearance (=dose/AUC) during rejection episodes was significantly greater than during non-rejection (20.4 vs 12.4 ml/min/kg respectively; $p<0.05$).

A comparison of profiles in patients in whom dose reduction was followed by a fall in Creatinine (n=29) with those in whom Creatinine rose (n=25) showed that there were no significant differences between the mean pre-dose CyA concentration (363 vs 312 ng/ml), peak concentration (1238 vs 1171 ng/ml) nor AUC (6005 vs 5506 hr.ng/ml). However, the mean Creatinine levels were significantly different (200 vs 134 μmol/l; $p<0.01$). Of the two groups of profiles, 6/29 and 0/25 respectively had Creatinine levels above 220 μmol/l ($p<0.05$).

In conclusion, rejection is associated with high CyA clearance and low CyA concentrations. Creatinine level may be a better guide than CyA level to CyA dose reduction.

PAPER 4

COMPARISON OF ATG AND CYCLOSPORINE IN RENAL TRANSPLANT RECIPIENTS WITH POOR INITIAL GRAFT FUNCTION

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Renal Transplant Unit, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne

The use of prophylactic ATG in renal allografts with poor initial graft function has been reported by many centres to improve graft survival and avoid the nephrotoxic effects of cyclosporine.

62 patients from a single centre were entered into a prospective randomised study comparing cyclosporine with prophylactic ATG. Poor initial graft function was defined at 24 hours post transplant as a four hour creatinine clearance of less than 15 ml/min. Patients were randomised by computer allowing for HLA match, sex and parity, cytotoxic antibodies and age. 31 patients received cyclosporine (group 1) commenced at a dose of 10 mg/kg per day and 25 mg of prednisolone daily. 31 patients received rabbit ATG (Merieux, Lyon) for 10 days according to flow cytometric monitoring of CD3+ lymphocytes (group 2). The aim in these patients was to keep the absolute T cell count less than 50 cells/µl. Group 2 patients also received 25mg of prednisolone daily and cyclosporine was commenced on day 7 at 7mg/kg per day. There was no difference between the two groups for the major variables that affect graft outcome.

Results

	Group 1 (CyA) n=31	Group 2 (ATG) n=31	Significance
1 year patient survival	28 (90%)	30 (96%)	NS
1 year graft survival	27 (87%0	24 (77%)	NS
Mean no of rejection episodes	0.9	0.4	p<0.04
Mean no of days to function	8.1	7.4	NS

Graft losses due to non-immunological reasons accounted for one graft in group 1 and four grafts in group 2. There was no difference between the groups for the incidence of infections.

We conclude that the use of prophylactic ATG does decrease the incidence of acute allograft rejection when compared with cyclosporine but it does not improve renal allograft survival and it does not decrease the length of time before the graft functions.

PAPER 5

THE USE OF NON HEART-BEATING KIDNEY DONORS IN A SINGLE CENTRE

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Shortage of organs has demanded a renaissance of interest in the use of non-heart-beating (NHB) and elderly donors in renal transplantation. This retrospective analysis compares results using non-hospice, non-in-situ perfused NHB donors with those from multi-organ and single-organ (kidneys only) donors in a single centre over a four year period between 1988 and 1991.

	NHB	MULTI-ORGAN	SINGLE-ORGAN
Number	34	123	87
Mean Donor Age	33	34	50
Mean Recipient Age	42	43	48
2 Year Patient Survival %	94	94	89
2 Year Graft Survival %	66	81	62
Duration Of Primary Non-Function (Days)	22	8	11
Mean 2 Year Creatinine	156	121	140

There was no significant difference in either the degree of HLA matching or incidence of rejection between the groups. The single organ donors were significantly older.

Results using multi-organ donors were significantly better than the other 2 groups but graft survival in the NHB group was very similar to the single organ group. In both the latter groups there was a high incidence of grafts which never functioned. Expansion of procurement in this area is desirable but must include assessment of viability and pathology pre-transplantation.

PAPER 6

DOPPLER ULTRASOUND ASSESSMENT OF TRANSPLANT RENAL ARTERY STENOSIS

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Doppler ultrasound examinations were performed on 95 renal transplant recipients with resistant hypertension, polycythaemia or unexplained renal impairment. 64 male and 31 female patients with a mean age of 43 (18-73) years were screened from a total transplant population of 643. The studies were performed by a single observer, and increased peak systolic velocity (PSV) in the transplant artery above 1.5 m/sec used as the indication for proceeding to arteriography. 28/95 patients had an increased PSV (> 1.5 m/sec), and all were investigated by arteriography. Renal artery stenosis (RAS) was demonstrated in 7/8 cases with PSV > 2.5 m/sec and 0/20 cases with PSV 1.5-2.5 m/sec (sensitivity 100%, specificity 95%).

Previous surveys have shown Doppler ultrasound to be a sensitive test for transplant RAS but to lack specificity. This study has shown that using the criterion of a PSV of > 2.5 m/sec, Doppler ultrasound is also highly specific and therefore it can reduce the number of unnecessary arteriograms to a very small number.

PAPER 7

TREATMENT OF INVASIVE ASPERGILLOSIS IN CARDIAC TRANSPLANT RECIPIENTS WITH LIPOSOMAL AMPHOTERICIN

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Invasive aspergillosis in the immunocompromised host is one of the most difficult therapeutic problems and is associated with a high mortality. We report our experience with liposomal amphotericin B therapy of 8 patients with invasive pulmonary aspergillosis following heart transplantation. Six of these cases appear to be related to building work on the hospital site. The diagnosis was made in 6 of the 8 cases by isolation of apsergillus from bronchoalveolar lavage fluid. The fungus was also seen by cytological staining of the lavage fluid in 5 of these cases. In the other 2 cases where bronchoalveolar lavage was not performed the fungus was isolated from sputum culture. In all 8 cases the diagnosis was clinically suspected due to a moribund state, fever, productive cough and appearance of new opacities on chest x-ray. All 8 patients had been treated for rejection with augmented steroid therapy and 6 of the 8 had received OKT3. One further case had received plasmapharesis for humoral rejection. Dosage of liposomal amphotericin was initially 1 mg/kg intravenously building up to 3mg/kg on day 5. Total cumulative dose given was 3g in each case. There were 2 deaths due to disseminated aspergillosis and 6 survivors. One patient complained of severe low back pain during the first dose and one patient developed a skin rash, there was no other drug related morbidity. Mean creatinine at the commencement of therapy was 183.5 mcmol/l and 203.5 mcmmol/l at the end of treatment in the 6 survivors. One of the patients who died was receiving haemodialysis at the time liposomal amphotericin was commenced and the mode of death in the remaining case was multiorgan failure. Intravenous liposomal amphotericin treatment for invasive pulmonary aspergillosis following cardiac transplantation was successful in 6 of the 8 patients and was associated with minimal toxic effects.

PAPER 8

Rapid and early detection of CMV infection in renal transplant recipients using pp65 direct antigenaemia test.

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Conventional cell culture (CCC) and detection of early antigen fluorescent foci (DEAFF) are commonly used for diagnosis of cytomegalovirus (CMV) infection. The direct antigenaemia test (DAT) is a new rapid method for direct detection of CMV in leucocytes using a monoclonal antibody directed against the viral matrix protein pp65.

47 heparinised blood samples from 21 renal transplant recipients were tested for CMV infection using CCC, DEAFF and DAT. 17 samples from 9 patients were positive by DAT. Two samples were positive by DAT and DEAFF but not by CCC and 1 sample was positive by all three methods. Eight of the 9 patients with positive DAT had further evidence of CMV infection from culture of blood and/or urine and by serology at a later time. One patient had severe CMV pneumonitis and died. No patient with negative DAT had evidence of CMV infection by culture, DEAFF or serology. The number of positive cells per 10^5 leucocytes detected by DAT ranged from 2 to > 5000. There appeared to be an association between high levels of antigenaemia and disease severity. Inoculum toxicity occurred with both DEAFF and CCC. Two of the DEAFF positive specimens were positive at 72 hours and one at day 7 after inoculation; the CCC positive specimen was positive at day 10 after inoculation.

DAT does not depend on cell culture and therefore has no toxicity problem. It is a direct method with results available within 4 hours. DAT appears to be very promising in the early and rapid diagnosis of CMV infection in transplant recipients.

PAPER 9

FIRST LINE CYCLOSPORIN A MONOTHERAPY IN CADAVERIC RENAL TRANSPLANTATION

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Since 1983, we have used Cyclosporin monotherapy as the preferred prophylaxis against renal transplant rejection in the belief that avoiding induction and triple therapy (where possible) reduces risk of infection and cancer, without jeopardizing successful immunosuppression.
204 patients received first, second or third cadaveric renal transplants followed by "intention to treat" Cyclosporin (CyA) monotherapy, and are available for follow-up at least 5 years post transplant.
Treatment Groups: 100 patients received maintenance CyA only during follow-up, (Gp I), in 29 long-term Prednisolone was added (Gp II), 67 were converted to triple therapy (CyA, Pred and Imuran), (Gp III) and 8 received Imuran and Prednisolone for systemic toxicity or nephro-toxicity due to CyA (Gp IV).
Results: Overall 5 year patient and graft survival (Gp I II III and IV) was 81% and 69% respectively and in CyA monotherapy (Gp I) was 93% and 80% respectively. Significant CMV infections occurred in 4% of patients overall.
42 of 100 monotherapy patients (Gp I) suffered no rejection, with a 5 year graft survival of 92% whereas 58 with reversible rejection had a 5-year survival of 71%. Graft loss was increased in Group I, (and in Groups II and III combined) in those patients whose average trough CyA levels fell below 200 ng/ml (whole blood HPLC) ($p = 0.00007$, and 0.011 respectively).
Conclusions: First-line CyA monotherapy yields acceptable graft and patient survival in first and subsequent renal transplant recipients, provided that CyA blood levels are kept above 200 ng/ml. This policy results in a strikingly low incidence of CMV infection, and offers an advantageous alternative to first-line induction or triple therapy.

PAPER 10

MECHANISMS OF CORNEAL XENOGRAFT REJECTION
IN THE FISCHER RAT

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PURPOSE To investigate the host humoral response and mechanisms of rejection in rat xenograft recipients of guinea pig and chicken donor corneas.
PROCEDURES Inbred F344 rats received unilateral full thickness 3mm diameter orthotopic corneal xenografts or isografts. Recipient eyes were examined daily for up to 14 days. Recipients were killed at 12h, 24h, 2d, 4d, 7d and 14d (total 6 at each timepoint). Nine athymic rats and six F344 rats with prevascularised corneas also received guinea pig donor cornea. Ocular sections were examined by H&E and immunoperoxidase staining. Integrity of graft endothelium was examined silver staining of corneas. Recipient serum, pre- and post-graft, was examined by flow cytometric analysis for presence of antibody binding to donor leucocytes.
RESULTS Rejection was diagnosed by loss of transparency. Median survival time for guinea pig donor was 3d (n=25), and chicken 2d (n=16). MST in athymic rats was 3d (n=9) and in rats with pre-vascularised corneas 2d (n=6). All isografts survived. Histopathology, immunohistochemistry and endothelial cell studies showed that xenograft failure was characterized by early epithelial and endothelial cell damage, granulocyte infiltration, immunoglobulin deposition and at 7-14 days, a cell-mediated and conspicuous eosinophil response. Pre-formed rat antibody was detected for leucocytes of both donor animals: post-graft sera demonstrated increased binding.
CONCLUSIONS Rejection of xenograft epithelium and endothelium is probably mediated by antibody and / or complement in tear and aqueous fluids. A cell-mediated response is evident from d7.

PAPER 11

EXPRESSION AND REGULATION OF MHC AND ADHESION MOLECULES BY HUMAN ALVEOLAR EPITHELIUM.

Anne Cunningham & John Kirby

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The expression of MHC by respiratory epithelium has been reported to increase during pulmonary allograft rejection (1,2). In order to extend this observation, alveolar epithelial cells (type II pneumocytes) were isolated from human lung (unused transplant donor or lobectomy specimens) and characterised by the presence of lamellar bodies at the TEM level, and intracellular alkaline phosphatase. Isolates, cultured and cytokine stimulated cultured pneumocytes were examined for the expression of MHC and adhesion molecules by flow cytometry. All cultures were demonstrated to be epithelial by virtue of the expression of the HEA-125 glycoprotein. CD45 was not expressed by isolated or cultured cells. Antigen expression was quantified by comparison to quantum beads and results expressed as molecules of soluble fluorochrome (MESF).

antigen	primary isolate	cultured cells	γ-IFN stimulated cells (100U/ml)
class I	$1.6x10^6 \pm 7x10^5$	$2.6x10^6 \pm 6x10^5$	$1.4x10^7 \pm 2x10^6$
HLA-DR	$6.2x10^5 \pm 2x10^5$	$1.6x10^4 \pm 8x10^3$	$1.6x10^5 \pm 9x10^5$
HLA-DP	$8.6x10^5 \pm 4x10^5$	$8.6x10^3 \pm 5x10^3$	$1.2x10^5 \pm 4x10^5$
HLA-DQ	$2.7x10^4 \pm 2x10^4$	$1.2x10^3 \pm 1x10^3$	$1x10^4 \pm 5x10^3$
LFA-3	$7.7x10^3 \pm 6x10^3$	$8x10^4 \pm 3x10^4$	$1x10^5 \pm 5x10^3$
ICAM-1	$2.2x10^4 \pm 7x10^3$	$4.9x10^6 \pm 3x10^6$	$6x10^6 \pm 7x10^5$*

*key: values represent MESF above control ± SEM (n = 3-7 replicates). *these cultures were stimulated by IL-1β (10U/ml) and not γ-IFN.*

Isolated type II pneumocytes express class II MHC (HLA DR and DP; not DQ) which was lost in culture ($p = 0.035$). HLA DR expression was significantly increased by γ-IFN ($p = 0.0001$). γ-IFN stimulation also significantly increased class I MHC expression ($p = 0.019$). ICAM-1 expression was increased in culture ($p = 0.011$) and LFA-3 was induced ($p = 0.002$). VCAM-1 and B7 were not expressed by isolated or γ-IFN stimulated cultures. Changes in adhesion molecule expression by cultured cells may reflect the differentiation of type II into type I pneumocytes in vitro. The expression of class II and adhesion molecules by alveolar epithelium may potentiate the rejection response, targeting damage to respiratory epithelium, and raises interesting questions concerning immunoregulation by epithelium in the lung.
References(1) Milne et al., 1992. Transplantation **54**, 748, (2) Rose et al., 1989. ibid **48**,506.

PAPER 12

MONITORING OF POST TRANSPLANT ANTIBODY USING EBV CELL POOLS

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The antibody panel reactivity of 40 renal transplant patients has been monitored on a daily basis for up to 2 months following transplantation, using flow cytometry.

The method uses Epstein-Barr virus (EBV) transformed B-lymphocytes of different HLA types grown up separately and then pooled. An estimate of panel reactivity of neat serum samples can then be determined using single colour flow cytometric analysis, the presence of antibody being determined by percentage of fluorescein conjugated anti-human IgG binding. Two pools containing a total of 15 EBV lines were selected to cover a wide range of HLA class I and class II types.

Of the 40 patients, 32 received first transplants and 8 second or subsequent grafts. Three patients died with functioning grafts in place, minimum follow up for all other recipients is 6 months.

All of the 12 patients with treated severe rejection episodes (biopsy proven) were seen to have increased antibody levels post-transplant. Of these, 5 suffered immunological graft loss accompanied by *de novo* antibody production. 6 out of 7 recipients with functioning grafts were sensitised pre-transplant.

Two patients with primary transplants, and without severe rejection episodes, were observed with markedly increased levels of antibody post-transplant. This antibody was demonstrated to be directed at donor leukocytes.

A transient antibody production was noted in 11 patients with little detectable antibody pre-transplant, 4 of these having slight rejection episodes which responded immediately to treatment. In the majority of cases this transient production was observed at the same time as initial ALG immuno-suppression was withdrawn.

No change in antibody production was observed in the remaining 15 recipients and none of these had treated rejection episodes.

In summary all patients who rejected or had severe rejection episodes were seen to have increased antibody levels at the time of rejection, and none of the patients for whom no increase in antibody level was observed suffered major rejection episodes. This method provides an easy and quick way of monitoring antibody post-transplant without interference from ALG, ATG or monoclonal therapies such as OKT3.

PAPER 13

XENOTRANSPLANTATION: INHIBITION OF CYTOLYSIS IN A PORCINE XENOGRAFT MODEL

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Activated human lymphocytes have the potential to form specific adhesive bonds with porcine renal epithelial cells. These interactions include:

CD11a + CD18 --> Porcine ligand

CD29 + CD49d --> Porcine ligand

It is known that blockade of adhesive interactions between allogeneic cells can ameliorate experimental allograft rejection.

It is not known whether this strategy would have any beneficial effect following porcine xenotransplantation.

Porcine renal epithelial cells were cultured and characterised by immunocytochemical staining. They were then radiolabelled with ^{51}Cr and seeded into the wells of U-bottomed 96 well plates. Human lymphocytes were prepared and activated using optimum concentrations of PHA or IL-2. After activation they were added to the porcine cells and incubated for 4 hours at 37°. The ^{51}Cr containing supernatant was examined by γ-spectrometry to assess cytolysis.

It was found that PHA activated lymphoblasts produced no significant lysis of the porcine renal cells during the 4 hour incubation. However when lymphokine activated killer (LAK) cells were used, significant lysis was produced. In the presence of monoclonal antibodies specific for the adhesion molecules CD11a, CD18, CD2 and CD49d, significant inhibition of lysis was seen. ($P<0.005$, $P<0.005$, $P<0.05$, $P<0.05$ respectively.)

These results indicate that therapeutic blockade of specific adhesive interactions may be a useful tool against cellular rejection of xenografted porcine organs.

PAPER 14

INTRINSIC RENAL SYNTHESIS OF C3: AN IMPORTANT PARTICIPANT IN ALLOGRAFT REJECTION

P Andrews, A Pani, W Zhou, J Finn*, P Mathieson*, S Sacks (J S Cameron)

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Recent evidence suggests that C3 is constitutively synthesised by renal tissue, and that local C3 synthesis is increased in immune complex-mediated nephritis. The role of local C3 synthesis in transplant rejection is, however, unknown. Using a semi-quantitative polymerase chain reaction, we compared local C3 mRNA production in 30 human allograft biopsies to that of normal renal cortex. C3 mRNA was produced by all normal and transplanted renal cortex. Expression was higher in transplant biopsies (mean 0.40 ± 0.1 v 0.038 ± 0.009, $p<0.005$), and 24/30 transplant biopsies expressed more C3 mRNA than the maximum control. 13/14 biopsies with pure cellular rejection had increased C3 expression (mean 0.51 ± 0.085, $p<0.005$); in contrast, 4/5 biopsies with cyclosporin toxicity had normal C3 expression. Increased expression was correlated with immunoperoxidase staining of the tubular basement membrane for C3, and there was a trend towards increased graft loss.

One interpretation of the above would be that mononuclear cell infiltrates (of recipient origin) are responsible for the increased C3 mRNA seen. There was, however, no correlation with the degree of mononuclear cell infiltration, assessed histologically, suggesting that intrinsic renal cells might also be contributing to the pool of local mRNA. We investigated this using amplification refractory mutation system PCR to determine the C3 allotype of mRNA expressed by the transplanted kidney. We examined 29 donor/recipient pairs, of which 9 were suitably mismatched at the F/S locus. Donor-specific allotype was detected in 6/9 biopsies at up to 61 days post-transplantation, a time when donor lymphocytes are reported to be absent from the graft. We conclude that at least some of the increased C3 mRNA present is produced by intrinsic renal cells, with important implications for mechanisms of transplant rejection.

PAPER 15

HLA-DR HETERODUPLEX, HLA-DP SSCP ANALYSES AND THE MLR IN BONE MARROW DONOR SELECTION.

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The recently described techniques of DNA *Heteroduplex* and *Single Strand Conformational Polymorphism (SSCP)* analyses have the advantage of being both technically simple and rapid. They allow for the matching of two or more individuals using PCR amplification followed by non-denaturing polyacrylamide gel electrophoresis.

In these systems, matching is achieved by visual comparison of the specific pattern of polymorphic amplification products (PCR-fingerprints). Unique banding patterns are seen for all combinations of HLA class II haplotypes so far examined. In addition, so called "DNA crossmatching" can be performed whereby donor and recipient DNA is mixed prior to PCR amplification. If donor and recipients are fully matched at the HLA-DRB locus, the crossmatch banding pattern will be identical in all three cases. Any mismatches will exhibit a novel banding pattern. This latter technique serves as confirmation of compatibility.

The most obvious clinical application of these techniques is the rapid screening of Class I (HLA-A, B and Cw) matched individuals for HLA-DR, DQ and DP compatibility in the selection of matched related and unrelated donors for bone marrow transplantation.

We have analysed recipients (n=32) and their potential donors (n=91) using conventional serological typing, HLA-DRB SSOP and SSP and donor/recipient MLR analyses, and compared these data to those derived from the HLA-DR and DP analyses described above.

Of the 47 donors for the recipient group requiring matched unrelated donors (n=8), 9 were HLA-DR *Heteroduplex* identical and 12 were both HLA-DR *Heteroduplex* and HLA-DP *SSCP* identical with 8 of this group MLR negative. In our 24 patients with potential family donors (n=44), 13 were HLA-DR *Heteroduplex* identical, 5 were HLA-DP *SSCP* identical and 7 were identical for both HLA-DR and DP. Of those, 21 were MLC negative.

This data shows that established compatibility for both HLA-DR and HLA-DP correlates with a negative MLR between recipient and potential donor. Fingerprinting analyses predict the MLR and hence negate the requirement for MLR testing between matched related bone marrow donors and their recipients.

PAPER 16

Th1 and Th2 CYTOKINE GENE EXPRESSION IN HUMAN RENAL ALLOGRAFTS

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Allograft rejection in experimental models depends on the production of interleukin-2 and interferon-γ by the Th1 subset of T helper cells. It has recently been shown that other lymphokines, particularly interleukin-4 and interleukin-10, produced by Th2 subset cells have actions antagonistic to the Th1 cytokines and may be associated with graft acceptance. This study was designed to examine the balance of Th1 and Th2 cytokines in human renal allografts and its association with graft rejection and acceptance. Fifty recipients of human cadaveric grafts were randomly allocated to three treatment groups of Cyclosporin A monotherapy, Cyclosporin A + Prednisolone, and Cyclosporin A + Prednisolone + Azathioprine. Renal graft biopsy was taken at the time of transplant, on day 10, on day 100 post transplant, and for suspected rejection. The biopsies were analyzed for interleukin-2, interferon-γ, tumour necrosis factor-α, interleukin-6, interleukin-4, and interleukin-10 mRNA expression by reverse transcription/ polymerase chain reaction. In addition, daily blood and urine samples were analyzed for circulating levels of cytokines by ELISA. In all 113 biopsies were analyzed by RT/PCR.

The biopsies (in %) showing cytokines on PCR

	IL-2	IFN-γ	TNF-α	IL-4	IL-6	IL-10
Rejecting	22	0	9	0	30	39
Non-rejecting	9	0	0	5	32	27

There was no significant difference among the three treatment groups in the pattern of cytokine expression. Serum cytokine levels do not correlate with gene expression, and only occasionally were increases in serum levels coincident with rejection. The biopsies showed generally low expression of mRNA, with predominance of Th2 type (IL-10 and IL-6) cytokines. This clinical picture of Th2 type cytokine expression differs from the experimental models, modified, no doubt, by the immunosuppressive drugs.

PAPER 17

mRNA EXRESSION FOR IL-2, AND p55 AND p70 SUBUNITS FOR ITS RECEPTOR THROUGH THE EVOLUTION OF RENAL ALLOGRAFT REJECTION

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It has been difficult to correlate the expression of Interleukin-2 (IL-2) with histological rejection. However there is consensus that infiltrating mononuclear cells responding to IL-2 are pivotal. In this study, amplification of mRNA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for IL-2 and the p55 alpha (IL-2Rα) and p70 beta (IL-2Rb) chains of the IL-2 Receptor have been linked to the time course of rejection from day 1 to day 5 in acute unmodified Lewis to Da rat renal allografts.

Histology on tissue from day 1 to 5 post-transplant showed a critical point at day 4 when the pattern of infiltrate changes from a mild focal to severe diffuse distribution with rejection rapidly following; concommitant RT-PCR of mRNA for IL-2 showed peak amplification at day 3. To determine the differential mRNA expression of the IL-2Rα and IL-2Rb chains, primers were designed to target message for these proteins in the rat. Low expression detected in normal kidney was upregulated through rejection with message for IL-2Rb peaking on day 2 and remaining high until day 5, whilst IL-2Rα peaked at the day 4 point.

If translated to functional protein this pattern of mRNA expression may be significant with increased IL-2Rb, as an early indicator of infiltration whilst IL-2 mRNA is maximally amplified prior to, and IL-2Rα mRNA on, the time point of most marked infiltration. The correlation of all three parameters to histology may be useful in the clinical monitoring of rejection.

PAPER 18

GENERATION OF MEMORY T-CELLS FOLLOWING ANTI-CD3 ACTIVATION: OPPOSING EFFECTS OF IL-2 AND IL-4

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The role of CD4 + ve T-cells in graft rejection is now well-established in a number of systems. Therefore, an understanding of how the transition of CD4+ve T-cells from a naive to memory state can be manipulated is of great interest and may be therapeutically important. We examined the effects of IL-2 and IL-4 on the development of T-cell subsets following activation with anti-CD3 in the presence of these cytokines, singly or in combination. There were no gross differences in the generation of CD4 or CD8 subsets but when the subpopulations of the CD4 cells were examined, clear differences were apparent that could result in significant functional changes. Two complementary markers, CD29 and CD45RA, have been associated with memory and naive T-cells respectively. Anti-CD3 supported the presence of a large population of CD29-bright CD4+ve cells and this was enhanced following activation in the presence of IL-4. When cells were stimulated in the presence of IL-2, CD29-bright cells were not generated and furthermore, the presence of IL-4 could not reverse this effect of IL-2. Corresponding, complementary changes were observed in the CD45RA population of the CD4+ve cells. There were few CD45RA cells remaining after activation with anti-CD3 alone or in the presence of IL-4, but a significant proportion of the CD4+ve cells bore CD45RA when activation was carried out in the presence of IL-2 or IL-2+IL-4. Our observations show that activation with anti-CD3 initiates the transition from "naive" to "memory" cells, that IL-4 supports and augments this transition, and that IL-2 antagonises it; the effect of IL-2 is dominant over that of IL-4. We suggest that activation of T-cells in the presence of IL-4 can lead to the generation of a population of CD4+ve cells able to help the development of antibody and cytotoxic responses, while the presence of IL-2 may direct the cells towards a suppressor-inducer population.

PAPER 19

CD4 AND CD8 MONOCLONAL ANTIBODY THERAPY PROLONGS RENAL ALLOGRAFT SURVIVAL IN THE DOG.

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Rat anti-dog monoclonal antibodies (mabs) to CD4, CD8, and Thy1 antigens were given to mongrel dogs in receipt of double haplotype mismatched renal allografts which, untreated, are rejected by day 6 (see table).
Mab therapy comprised 2 days pretreatment with CD4, CD8 and Thy1 mabs, with the depleting Thy1 mab given to reduce the circulating T cell mass, and thereafter only the CD4 and CD8 mabs were continued to either day 10 or 28.
The mabs were also evaluated as 'induction therapy' in dogs receiving alternate day cyclosporin (cyA, 25mg/kg) and azathioprine (aza, 5mg/kg), with doses halved every 14 days and stopped on day 56.

Mab therapy	CyA & Aza Therapy	Day CD4 & CD8 mabs stopped	No of dogs	Survival (creatinine >300μmol/l)	Median survival
-	-	-	6	[2], 5, 5, 5, 6, 6	5
-	+	-	3	12, 16, 25	16
+	-	10	10	[1, 2, 3, 4, 6], 7, 10, 15, 31, 59	15
+	+	10	10	[1, 4], 32, 35, 35, 36, 41, 41, 47, 52	39
+	+	28	4	[5], 53, 57, >118	57

[Figures in brackets are deaths due to causes other than rejection.]

Results (see table)

1. CD4 +CD8 + Thy1 mab therapy prolongs canine renal allograft survival.
2. Combined alternate day cyA/aza therapy with10 day mab induction therapy is synergistic.
3. 28 day mab-induction therapy further extends survival, with one dog having good graft function >62 days off all treatment.
4. Mab therapy alone results in an antiglobulin response manifesting clinically as anaphylaxis. Adjuvant immunosuppression inhibits this response permitting prolonged mab therapy.

PAPER 20

TUMOUR NECROSIS FACTOR MICROSATELLITE MARKERS AND *IN VITRO* TNF LEVELS IN HEART TRANSPLANT RECIPIENTS

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Human TNF genes are situated 225 kb centromeric to HLA-B and 375 kb telomeric to C2/BF. The TNF A and TNF B gene products are recognised as essential mediators of the immune response. TNF levels in transplant recipients have been shown to vary from patient to patient, and may be associated with rejection severity. We have studied three linked, polymorphic microsatellite markers (TNFa, TNFb and TNFd) which flank the TNF genes to investigate microsatellite alleles and *in vitro* levels of TNF in twenty three heart transplant recipients.

The microsatellite sequences were PCR amplified and differentiated on an acrylamide gel. TNFa is the most polymorphic marker with ten alleles detected; TNFb has four and TNFd five. TNF levels were those observed after *in vitro* sample stimulation with endotoxin.

This initial study shows a significant increase in TNF-α level in patients with the TNFd allele, d3 compared with patients lacking d3. A mean TNF-α level of 529 pg/ml calculated from seventeen d3 positive patients was significantly different from a mean level of 190 pg/ml in six d3 negative patients ($p<0.001$). The TNF level did not correlate significantly with rejection grade.

This apparent variability of TNF expression could be explained by a possible association between TNFd alleles and differences in the TNF-α promoter region. These results imply that Mhc coded TNF gene polymorphisms may be used to predict the level of heart transplant recipients immune response.

PAPER 21

VASCULARIZED MOUSE-TO-RAT HEART GRAFTS: AN UNEXPECTEDLY DIFFICULT MODEL OF XENOTRANSPLANTATION

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We were interested to study vascularized xenografts between closely related species, with a view to analysing xenograft rejection in the absence of hyperacute rejection. We chose the mouse to rat system, and have developed a model where the mouse heart is anastomosed to the left renal vessels of the rat (aorta to renal artery, pulmonary artery to renal vein). Survival times of BALB/c hearts transplanted to Lew rats was short (3 days x 13, 4 days), and the pathology at the time of rejection suggested antibody mediated rejection. Athymic nude rats also rejected the mouse heart xenografts in 2-3 days. This was somewhat surprising in view of the phylogenetic closeness of the two species. High doses (20 mg/kg/day) of cyclosporin A orally did not prolong survival times, but splenectomy prolonged survival by 1-2 days. In view of the remarkable effectiveness of the CTLA4Ig (a fusion protein between the human CTLA-4 T cell activation molecule and human IgG1) for suppressing human to rat pancreatic islet xenografts (1) and rat cardiac allografts (2), we were interested to test this reagent in our model. In spite of the remarkable effects of CTLA4Ig on the human to mouse system, high doses (200 ug/day) had no effect on our mouse to rat heart grafts. Moreover cyclosporin A plus CTLA4Ig had no effect at all. Splenectomy plus cyclosporin A (10 mg/kg/day orally for 14 days) gave some prolongation of survival (5, 6, 7, 7, 21 days). The combination of CTLA4Ig (200 ug/day intravenously for 10 days) plus splenectomy plus cyclosporin A (10 mg/kg/day orally for 14 days) has given good prolongation of survival in the animals so far tested (20, 20, 24 days). In the last group all xenografts survived while therapy was maintained.

Our results demonstrate that xenografts between closely related species can be vigorously rejected, and that CTLA4Ig is much less effective with vascularized grafts than with islet xenografts.

1. Lenschow et al. *Science* vol. 257, p. 789, 1992
2. Turka et al. *Proc. Natl. Acad. Sci. USA* vol. 89, p. 11102, 1992

PAPER 22

PROLIFERATIVE RESPONSES OF HIGHLY PURIFIED HUMAN CD4$^+$ T CELLS TO PORCINE ENDOTHELIAL CELLS

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In order to investigate whether human T cells can directly recognise pig endothelial cells we have adopted a xenogeneic mixed lymphocyte endothelial reaction in which highly purified human CD4$^+$ and CD8$^+$ T cells are incubated with pig aortic endothelial cells (PAEC). The PAEC, although negative for vWF, were identified as being endothelial by their morphology, uptake of AcLDL, and formation of tubes on collagen gels. PAEC were shown by flow cytometry to be SLA class I positive but SLA class II was undetectable. Human T cells were purified by positive selection using the Dynabead/Detachabead system and shown to be functionally free of monocytes. Proliferation was measured by ^{3}H-thymidine uptake after 6 days. In a series of five experiments strong proliferation by human peripheral blood mononuclear cells (PBMC) and CD4$^+$ T cells was observed. Human CD8$^+$ T cells also proliferated to PAEC, but, weakly. Further experiments directly compared the responses to the human endothelial cell line EAhy.926 with and without prior treatment with human recombinant interferon gamma (rhuIFNg). Flow cytometry showed upregulation of HLA class I, and induction of HLA class II in EAhy.926 following rhuIFNg treatment, however, no measurable effect was observed on PAEC SLA expression. Untreated EAhy.926 (HLA class II negative), in contrast to SLA class II negative PAEC, was unable to stimulate CD4$^+$ T cell proliferation. The response to EAhy.926 was dependent on treatment with rhuIFNg but there was no effect of rhuIFNg on the CD4$^+$ T cell response to PAEC. Recombinant porcine interferon gamma (rpoIFNg), in contrast, was found to induce SLA class II on PAEC and this altered the kinetics of CD4$^+$ T cell recognition, the response beginning three days earlier.

In conclusion, the CD4$^+$ T cell proliferative response induced by SLA class II negative PAEC is at least as strong as that induced by HLA class II positive human endothelial cells. The possibility that humans have T cells primed to pig SLA or other antigens is being investigated.

PAPER 23

THE IMPORTANCE OF PERSISTING ALLOANTIGEN IN MAINTAINING UNRESPONSIVENESS IN CARDIAC ALLOGRAFTED BLOOD TRANSFUSED RATS

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We have investigated the mechanism of tolerance induced by pre-operative donor specific blood transfusion (DST) in DA cardiac allografted PVG rats. Previous work showed that unresponsive DST PVG rats contained CD4 T cells which on transfer to DA cardiac allografted athymic nude recipients induced rejection. The present study explores this observation further. We asked whether CD8 T cells played a (suppressive?) role. Using nude recipients reconstituted two months before with CD4 T cells alone the results showed that giving such recipients a DST two weeks before DA heart grafts, with or without anti-CD8 (mAb OX8) treatment, prevented rejection. Thus, tolerance induced by DST does not involve or require CD8+ cells. Analysis of CD4 T cell subsets defined by mAb OX22 (anti-CD45RC) showed that following DST the CD45RC- subset (a subpopulation normally poor at evoking rejection) was now enriched in allorejection potential as shown by transfer to cardiac allografted nude recipients. The graft destruction induced by the CD45RC- subset was, however, entirely prevented if nude recipients received a specific (but not a third party) blood transfusion two weeks before heart grafting and cell transfer. The results suggest that unresponsiveness following DST involves 2 stages: 1) the induction stage which converts alloresponsive CD4 T cells from a resting (CD45RC+) to an antigen-experienced (CD45RC-) non-rejecting state and 2) the maintenance stage (T cell independent) which depends on the persistence of an antigen-specific component.

PAPER 24

ABROGATION OF THE HUMAN ANTI-PIG XENOGRAFT REACTION WITH SOLUBLE OLIGOSACCHARIDES

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The binding of human natural antibodies to pig endothelium is the main barrier to pig to human xenotransplantation. The major endothelial antigens are oligosaccharides. The dominant antigen is galα1-3 gal β1-4 glcNAc. We investigated the ability of this antigen's terminal disaccharide, gal α1-3 gal, to block the human anti-pig xenograft response.

In cytotoxic and FACS assays using pig lymphocytes, gal α1-3 gal significantly inhibited human anti-pig antibodies (up to 80%). Similarly, the binding of human IgG and IgM to the whole antigen (galα1-3 gal β1-4 glcNAc) in an ELISA assay was inhibited by increasing concentrations of soluble gal α1-3 gal.

We then perfused pig kidneys *ex vivo* with autologous pig blood (negative control), human AB blood (positive control), or human AB blood pre-incubated with either 5 mg/ml or 25 mg/ml gal α1-3 gal. Biopsies from the positive control kidneys showed arterial and arteriolar thrombosis and extensive glomerular congestion and endothelial damage. The kidney perfused with blood pre-incubated with 25mg/ml, but not 5mg/ml, of gal α1-3 gal showed marked but not complete abrogation of these changes.

These results suggest a possible therapeutic role for soluble oligosaccharides in xenotransplantation.

PAPER 25

DEFINITION OF HISTOCOMPATABILITY GENES IN PIG SMALL BOWEL TRANSPLANTATION

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Pigs have been widely used as a large animal model of organ transplantation because of physiological and immunological similarities to humans. The swine Mhc has homology with that found in humans, with the HLA-DRB1 sequence showing a 71% homology with SLA class II genes.

We have established a small bowel transplant model utilising randomly obtained, "free range" pigs. Whilst studying the pathophysiology of allografted transplants in this model it became apparent that some non immunosuppressed animals were surviving without clinical or histological evidence of rejection.

The high level of homology between the human and swine genome has enabled us to analyse the latter by restriction length polymorphism (RFLP). Using the human HLA-DRB probe pRTV1 in conjunction with a range of restriction enzymes we have detected SLA Class II polymorphisms. Furthermore, we have developed an alternative strategy using swine SLA-DRB sequence data to produce generic SLA-DRB PCR primers for use in single strand conformational polymorphism (SSCP) analysis. Using these techniques in a random panel (n = 22) of Large White / Duroc crosses we have successfully demonstrated extensive SLA-DRB polymorphism and have retrospectively typed donor-recipient small bowel transplant pairs.

Non immunosuppressed small bowel allografts were performed in 8 animals who survived for 0, 8,10,10,11,>35,>35,>35 days. Retrospective analysis using molecular biological techniques demonstrated that the long survivors showed marked homology for SLA class II with their respective donors ($p = 0.03$). This would indicate that long survival times obtained from large animal experimentation should be treated cautiously in the absence of histocompatability matched grades. To establish true allogeneic models in large animals requires a programme of prospective "trotterprinting" which can easily and quickly be facilitated using the molecular biological techniques described.

PAPER 26

RENAL ALLOGRAFT REJECTION: TUBULAR EPITHELIAL CELLS PRESENT ALLOANTIGEN IN THE PRESENCE OF CO-STIMULATORY CD28 ANTIBODY

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Human tubular epithelial cells (TEC) express class II MHC antigens during acute allograft rejection. In this study the ability of cytokine-stimulated renal epithelial cells to present alloantigen was examined by Mixed Leucocyte Kidney Culture (MLKC) in the presence of co-stimulatory antibodies.

TEC were cultured from disaggregated cortical tissue and characterised by immunofluorescence staining for cytokeratin. The cells were treated with interferon-γ (100U/ml) and induction of class II MHC antigens was observed by semi-quantitative flow cytometry. After 4 days allogeneic peripheral blood lymphocytes (PBL) were added to irradiated TEC in 96 well plates at a range of stimulator:responder ratios. Intact anti-CD28 antibody or an isotype-matched control was titrated into the assays. Lymphoproliferation was assessed by measuring ^{3}H-thymidine incorporation and the concentration of IL-2 in each assay supernatant was measured by CTLL-2 bioassay.

Time	Concentration of iL-2 (Units/ml)			
	MLKC		PBL	
	CD28 (10μg/ml)	IgG	CD28	IgG
3 Days	$0.1 \pm 0.01^{**}$	$3x10^{-3} \pm 6x10^{-5}$	$5x10^{-3} \pm 2x10^{-3}$	$5x10^{-3} \pm 4x10^{-3}$
5 Days	$0.02 \pm 5x10^{-3*}$	$2x10^{-3} \pm 5x10^{-5}$	$7x10^{-3} \pm 2x10^{-3}$	$6x10^{-3} \pm 2x10^{-3}$

Significance of CD28-mediated augmentation of IL-2 production in MLKC: * $p<0.01$, ** $p<0.001$

Concentrations of between 3μg/ml and 12.5μg/ml of anti-CD28 antibody significantly ($p < 0.001$) increased lymphocyte proliferation and IL-2 production during MLKC; concentrations above this were inhibitory. Maximal IL-2 concentrations were observed after 3 days. The optimal stimulator:responder cell ratio was 1:8. Cultures with control antibody did not show significantly increased proliferation or IL-2 production. Anti-CD28 antibody had no effect on resting PBL.

Cytokine-stimulated renal epithelial cells do not express the B7 ligand for CD28. Reported failure to stimulate primary lymphoproliferation in allogeneic MLKC may be accounted for by the lack of appropriate lymphocyte co-stimulation.

PAPER 27

THE VALUE OF FLOW CYTOMETRIC CROSSMATCHING IN LUNG TRANSPLANTATION: RELEVANCE OF PRETRANSPLANT ANTIBODIES TO LUNG EPITHELIAL CELLS.

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Whilst there is increasing evidence for accelerated graft rejection in the presence of pre-existing antibody against donor antigens in clinical renal and cardiac transplantation, the importance of such antibodies in lung transplantation is uncertain. We have therefore examined the relationship between the preoperative antibody status of single and double lung transplant recipients and the presence of rejection over a six month period after transplantation. Rejectors and non rejectors were defined by the presence or absence of one or more episodes of treated A_2 rejection over the first six months.

The flow cytometric test system was used to determine the presence of pre-existing antibodies in 43 transplants to donor T and B cells and a number of cell lines.(EAHy926,A549,U937,Fibroblasts,and HUVEC).Although patients exhibited a wide range of both cytotoxic and binding antibodies, a highly significant association was only found between the presence of IgM antibodies to the lung epithelial cell line (A549) and rejection. Only 1/7(14.3%) of patients in the non rejection group showed antibodies to A549 cells whereas 15/23(66.7%) with antibodies showed problems with clinical rejection. (P=0.024).

This study suggests the importance of preformed antibodies in the sera of lung transplant patients. With greater understanding of the precise specificity of such important antibodies improvement in graft survival figures may occur. The development of new immunosuppressive regimes to reduce antibody levels before transplantation may be critically important in the future.

PAPER 28

HEPATIC ALLOGRAFT REJECTION: REGULATION OF THE POTENTIAL IMMUNOGENICITY OF HUMAN BILIARY EPITHELIAL CELLS

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Biliary epithelial cells (BEC) are a major immunological target after liver transplantation. In this study expression of class I and class II MHC antigens and of the T cell adhesion receptors ICAM-1 (CD54), LFA-3 (CD58) and VCAM-1 was quantified after treatment of cultured BEC with IFN-γ and TNF-α.

BEC were prepared from disaggregated liver tissue by immunomagnetic separation HEA-125 +ve cells and were propagated. The cells were characterised by identification of intracellular cytokeratin 19; in all cases epithelial cells constituted >85% of the population. The BEC were stimulated for 4 days with either IFN-γ (100U/ml), TNF-α (100U/ml) or with both of these cytokines. The cells were then labelled with antibodies specific for class I and class II MHC antigens, ICAM-1, LFA-3 and VCAM-1 and were counterstained with FITC-conjugated secondary antibodies prior to flow micro-fluorimetry. Each fluorescence intensity was converted to FITC equivalents.

The constitutive expression of LFA-3 ($6x10^4 \pm 1x10^4$ FITC equivalents) was not augmented by cytokine treatment. None of the cell samples expressed VCAM-1. Changes in expression of the other antigens are tabulated below:

Antigen	Fluorescence After Cytokine Treatment (FITC Equivalent ± SEM)			
	Medium Control	TNF-α	IFN-γ	TNF-α + IFN-γ
Class I	$4x10^5 \pm 5x10^4$	$4x10^5 \pm 3x10^4$	$3x10^6 \pm 5x10^{5}$''	$3x10^6 \pm 4x10^{5}$''
Class II	$1x10^3 \pm 300$	$1x10^3 \pm 600$	$2x10^5 \pm 7x10^{4}$''	$3x10^5 \pm 1x10^{5}$''
ICAM-1	$3x10^5 \pm 1x10^5$	$1x10^6 \pm 1x10^{5}$'	$2x10^6 \pm 5x10^{5}$'	$3x10^6 \pm 1x10^{5}$'

'$p<0.01$ ''$p<0.001$

It is likely that both IFN-γ and TNF-α are produced during rejection. These cytokines increase the expression by BEC of both classes of MHC antigen and of ICAM-1. Upregulated expression of these molecules may increase immunological damage of intrahepatic bile ducts during liver allograft rejection.

PAPER 29

EVIDENCE FOR CHRONIC PROGRESSIVE CYCLOSPORIN (CYA) NEPHROTOXICITY IN EXPERIMENTAL ANIMALS

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Chronic administration of cyclosporin (CyA) results in deterioration of renal function due to the development of arteriolopathy and interstitial fibrosis. The aim of this study was to define the fibrogenic effect of CyA in the rat. Adult male Wistar rats (375-400g) were injected with CyA (12.5 mg/kg, I.P.) daily. Control rats received an equivalent volume of the olive oil vehicle. Groups of animals (n=6) were sacrificed after 2, 4, 8 and 12 weeks. At the start and at sacrifice body weight, serum creatinine and urea concentrations, whole blood CyA levels and blood pressure (tail cuff method) were measured. At sacrifice kidneys were fixed with formol calcium and embedded in paraffin wax. Sections were stained with Haematoxylin and Eosin, Periodic Acid Schiff and Masson's trichrome for morphological evaluation. Sections were also stained by the avidin-biotin peroxidase method for immunohistochemical detection of fibronectin, Collagen I, III and IV. After 4 weeks of treatment, serum creatinine increased (47±3 vs 35±2, P>0.005) and creatinine clearance rate decreased (0.29±0.03 vs 0.53±0.04, P>0.001). Systolic blood pressure was higher in rats treated for 12 weeks (165±2 vs 139±4, P>0.05). There was a progressive increase in the wet weight of CyA treated kidneys per 100g body weight (0.74±0.02 vs 0.63±0.02, P>0.05 after 8 weeks and 0.68±0.02 vs 0.59±0.02, P>0.005 after 12 weeks). After 2 weeks of treatment irregular notching of the capsule was evident and the underlying tubules appeared basophilic but there was no evidence of interstitial fibrosis. By 4 weeks however areas of striped interstitial fibrosis were evident arising from the notched capsule and extending around the basophilic tubules and became progressively more marked at 8 and 12 weeks. Immunohistochemical staining revealed progressively increased abnormal deposits of fibronectin, collagen III and IV but not collagen I in these areas after 4, 8 and 12 weeks but not in those rats treated for only 2 weeks. The finding that kidney weight increases with the duration of CyA injections suggests that this CyA induced fibrosis is different from other forms of renal scarring, which normally result in a loss of kidney mass. Our results clearly indicate that in this model fibrosis is progressive. This is at variance with recent suggestions that chronic CyA nephrotoxicity is intermittent and occurs in transplanted patients only after episodes of acute rejection.

PAPER 30

THE ROLE OF CYTOKINES AND CD4 T CELLS IN THE GENERATION OF CD4 AND CD8 CYTOTOXIC T CELLS

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Cytotoxic T lymphocytes (CTLs) are a potential effector mechanism in graft rejection by virtue of being able to kill cellular components of the transplant. Both CD8 and CD4 T cells can acquire cytotoxic potential. Furthermore CD4 T cells are required for the generation of CD8 CTLs. However there is controversy from data on clones on which of the Th1 and Th2 subsets of CD4 T cells are cytotoxic and which ones help in the generation of CD8 CTLs. We have therefore investigated the role of Th1 and Th2 CD4 cells and cytokines secreted by them in the generation of primary allospecific CD4 and CD8 CTLs in the mixed lymphocyte reaction. The results show that primary generated cells with Th1 cytokine secreting profiles are cytotoxic but those with Th2 characteristics are not. However inhibition of IFN-γ or IL-12, cytokines associated with the generation of Th1 cells, has little or no inhibitory effect on the generation of CD4 CTLs. By contrast both Th1 and Th2 cells can help in the production of CD8 CTLs. The results indicate that although switching allograft responses to Th2 could prevent the generation of an antigraft CD4 cytotoxic response this would not prevent the generation of CD8 CTLs.

PAPER 31

DYNAMICS OF IL2 RECEPTOR EXPRESSION IN RAT ALLOGRAFTS: ANALYSIS BY AUTORADIOGRAPHY AND NUCLEAR IMAGING USING RADIO-IODINATED IL2 PROBES

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The high affinity receptor for the cytokine interleukin 2 (IL2) is up-regulated on the surface of activated immune cells. It is a key signal transducing element in the activation of the immune system during the allograft response. To study the dynamics of interleukin 2 receptor (IL2R) expression in renal allograft rejection we have utilised radiolabelled IL2 as radioligand probes. Allogeneically transplanted rats (LEW to DA) were injected at 3 and 5 days post transplantation with ^{125}iodine-IL2 (^{125}I-IL2) and tissue was examined using a combination of autoradiography, to determine the tissue distribution of labelled probe, and immunohistochemistry, to identify IL2R expression in the same section. Between days 3 and 5 post transplant there was an increase in tissue binding of radiolabelled IL2 and an increase in the expression of the IL2R in corresponding areas of the interstitial cellular infiltrate. Parallel studies using a more penetrating isotope of iodine to enable measurement of whole organ radioactivity by external image analysis, showed a corresponding increase in whole organ radiolabelled IL2 binding between 3-5 days post transplantation ($p<0.05$). Syngeneic controls (DA to DA) showed no increase in tissue binding of radio-labelled IL2, either by autoradiographic analysis or external imaging, and no increase in receptor expression. These date indicate that intravenous radiolabelled IL2 binds to rejecting renal allografts via the high affinity IL2 receptor and that the increase in tissue binding between days 3-5 after transplantation reflects an increase in high affinity IL2 receptor expression by the cellular infiltrate. These results demonstrate the potential of radiolabelled IL2 imaging in the sequential analysis of the allograft response.

PAPER 32

HPV 16 AND CIN IN THE IMMUNE COMPROMISED WOMAN.

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The renal transplant woman is at least ten times more likely to develop abnormal cervical cytology and cervical cancer than her immunocompetent counterpart and is more commonly infected with the oncogenic HPV type 16. This study was designed to investigate the relationship between HPV type 16 infection, CIN and immune suppression and thereby provide information on the pathogenesis of cervical disease and advise on the timing and type of screening required.

Fifty transplant patients were screened by cervical cytology, colposcopy, directed biopsy and semi-quantitative PCR analysis of HPV 16. The incidence of CIN and the prevalence of infection with HPV 16 was compared with a GP control population. Fourteen (28%) of the transplant women had CIN, which was six times that of the GP screened population ($p=<0.01$). Six (12%) of the transplant patients had high grade cervical disease which has a malignant potential ($p=<0.01$). The prevalence of infection with HPV 16 was twice that of the normal population (p=NS). However, semi-quantitative analysis demonstrated a significant association of medium/high copy numbers of the HPV 16 genome and high grade and multifocal disease, 6/7 had CIN 2 or more ($p=<0.01$). Fourteen patients had low copy numbers of HPV, 4 of these had minor grade disease which was similar to the GP screened population (p=NS).

CIN and cervical cancer has a complex multifactorial aetiology. Although HPV 16 in medium/high copy numbers has an association with high grade cervical disease, the case for minor grade disease is unclear. A *causal* rather than a *casual* association has yet to be demonstrated. It appears likely that loss of immune surveillance via CD_4 and interleukin-2 suppression is more important than infection with HPV 16.

PAPER 33

COMPLEMENT ACTIVATION PRODUCTS IN ORTHOTOPIC LIVER TRANSPLANTATION

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Plasma levels of three complement activation products (C3d, C5b-9 & C3bBbP) were measured in serial plasma samples taken from 21 patients undergoing orthotopic liver transplantation. Marked increases in all three parameters were found during the perioperative period and there was a particular association between elevation of C3d levels and the early reperfusion period and severity of graft damage as assessed by histology. Within 24 hours of operation all three parameters generally returned to baseline levels.

During the postoperative period a total of 24 graft rejection episodes were confirmed by histology. Elevation of C5b-9 & C3bBbP levels to more than 150% baseline levels was found in all 24 episodes and in 21 episodes 'predicted' the biopsy findings by 1-3 days. In a total of 304 patient sample days, levels of C5b-9 & C3bBbP accurately indicated a status of 'no rejection' for 192 days; active rejection episodes for 73 days and resolving rejection episodes for 39 days. Immunohistology of liver biopsies showed deposition of activated C9 component of complement in the grafts during rejection episodes.

In conclusion measurement of complement activation products may be useful in monitoring for allograft rejection. Complement may have a significant role in the pathogenesis of hepatic allograft rejection.

PAPER 34

HEART TRANSPLANT REJECTION STRONGLY CORRELATES WITH HLA MISMATCH.

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The effect of HLA incompatibility on heart transplant (HTPX) survivals has proved difficult to evaluate due to the strong bias towards transplants with a high degree of HLA mismatch (MM). This bias is a result of the relatively short cold ischaemia time which has traditionally deterred HTPX centres from pursuing prospective HLA matching policies. With accurate rapid prospective HLA matching now feasible we examined the role of HLA mismatching in HTPX on endomyocardial biopsies (EMB) from 157 consecutive orthotopic HTPX performed from April 1987 to August 1993. EMB grade $>=2$ was used as the cut off point for definition of clinically significant rejection. We find that HTPX with least HLA-DR MM have a highly significantly reduced frequency of EMB grades $>=2$. This HLA-DR mismatching effect is observed with analysis of EMB results from the first three months post transplant, the first year post transplant and with the total data.

HLA-DR MM	HTPX No.	EMB No.	%Grades $>=2$
0	13	148	18
1	53	797	29
2	91	1624	34

$p<0.00005$

This study clearly shows that HTPX matched at the HLA-DR locus have a significantly reduced incidence of EMB MM grades indicative of maintenance immunosuppression being insufficient for management of rejection. Given the results of this study and that high quality HLA typing can now be achieved from donor peripheral blood we identify an urgent need for the widespread application of prospective HLA matching in HTPX.

PAPER 35

THE IMPACT OF GENETIC MISMATCHING ON BMT OUTCOME

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Strict HLA matching severely limits the numbers of unrelated donor BMT (UD-BMT) performed. But some mismatched grafts are successful for example paediatric and TCD transplants. The effect of mismatching was analysed in a prospective multicentre study involving transplants performed between 1989-1993 in 42 centres worldwide. 305 UD-BMT were compared with 628 HLA genetically identical BMT (ID-BMT) collected as controls, 33 (11%) and 4 (1%) of UD-BMT were mismatched (MM) for one or two HLA-A, B DR antigens respectively (37 patients in total) and 268 were matched (M). Kaplan Meier life table analysis showed the following % probabilities (95% CI):

Outcome:	**ID-BMT (628)**	**M-UD-BMT (268)**	**MM-UD-BMT (37)**
Survival (12m)	63 (61-65)	45 (38-51)	24 (7-41)
AGVHD (100d)	38 (35-42)	45 (39-51)	50 (33-66)
Engraftment (100d)	98 (97-99)	87 (83-91)	84 (71-98)

In multifactorial analysis HLA-A, B DR mismatching failed to reach significance as an independent risk factor, but donor type (ID versus UD) and interestingly sex mismatch reached significance after correction for all major clinical and therapeutic variables suggesting an influence of unidentified HLA and non HLA genes. **In summary, results of BMT are universally poorer with UD than with ID due to genetic mismatches that cannot be identified by routine HLA-A, B, DR typing and a prospective study is underway to elucidate this vital difference.**