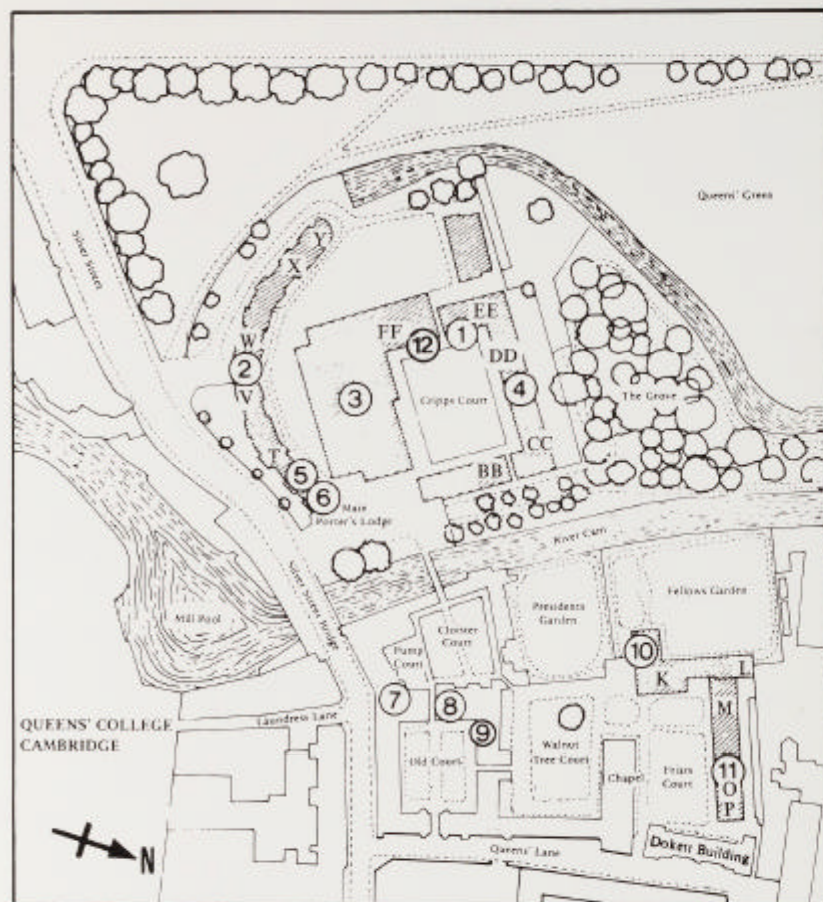


QUEENS' COLLEGE



- | | | | | | |
|----|----------------------|---|--------------------|----|------------------|
| 1 | BAR | 5 | CONFERENCE OFFICES | 9 | MUNRO ROOM |
| 2 | FISHER BUILDING | 6 | PORTERS' LODGE | 10 | ERAMUS BUILDING |
| 3 | HALL | 7 | ERAMUS ROOM | 11 | FRIARS' BUILDING |
| 4 | CRIPPS COURT | 8 | OLD HALL | 12 | LAUNDERETTE |
| BB | X, Y, Z - STAIRCASES | | | | |

The British Transplantation Society

QUEENS COLLEGE, CAMBRIDGE

26th and 27th March, 1991



Major loss is due to relatives refusal - all organs
 For kidneys second cause is failure to ask

PAPER 1

REGIONAL VARIATION IN BARRIERS TO CADAVERIC SOLID ORGAN DONATION

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Six major barriers to solid organ donation from intensive care units (ICUs) are: 1) brain-stem death not a possible diagnosis 2) non-performance of brain-stem death tests when brain-stem death is a possible diagnosis 3) general medical contraindication to organ donation 4) relatives' refusal 5) non-suitability of specific organs and 6) non-procurement of suitable, offered livers.

Using data from the confidential audit of 12133 deaths in ICUs in England from 1 January to 31 December 1989 we show that all but one of these six major barriers to solid organ donation exhibited regional variation ($p < 0.02$) even after adjustment for patient factors (see below). The lack of regional variation in non-performance of brain-stem death tests ($\chi^2_{14} = 9.3$) is important, and as we should expect if ICUs' testing practice were both appropriate and uniform.

Patient factors, notably age at death in ICU (overall mean 57.1 years, sd 21.7) and percentage of deaths due to extracranial cause (overall: 80.6%), varied greatly between regions. Mean age at ICU death in Oxford was 55 years with 71% of deaths due to extracranial cause compared to mean age of 60 years with 88% of deaths due to extracranial cause in South East Thames. Heterogeneity of patient factors substantially inflated the crude comparison between regions of whether brain-stem death was a possible diagnosis: χ^2_{14} was reduced from 84.0 to 35.9 (still highly significant) after allowance for patient factors.

Regional variation as shown raises questions about

- 1) differential provision of ICU facilities;
- 3 and 5) a need for greater harmonization of practice in deciding on organ suitability;
- 4) review of what made Yorkshire and Wessex most successful in 1989 in achieving high consent rates (48/53 consented (91%) and 56/67 in Wessex, 84%); and
- 6) logistical solution to the variation in non-procurement of offered, suitable livers.

FK 506 is safe better graft & pt survival
 incidence of hypertension is low

PAPER 2

Neurotoxicity doesn't seem more problem than CyA
 Headache & insomnia greater

PRELIMINARY RESULT OF PROSPECTIVE RANDOMIZED TRIAL OF FK 506 VERSUS CYCLOSPORIN (CyA) IN ORTHOTOPIC LIVER TRANSPLANTATION

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Blind trial until after op finished & randomized, not blind afterwards.

FK 506 has been found to be a potent immunosuppression in clinical transplantation. The aim of the study is to compare the episodes of rejection, patients and graft survival following primary orthotopic liver transplantation in two treatment groups. FK 506 versus CyA. Patients between 16 to 60 years of age were included in the trial. Patients with neoplastic disease, fulminant acute liver failure, pre-existing renal, pulmonary, cardiac diseases or technically unsatisfactory grafts were not randomized. Between February 17, 1990 to August 31, 1990 81 (41 FK 506 and 40 CyA) patients entered the study. FK 506 was given 0.15 mg/kg/day IV and CyA mg/kg/day IV. Subsequent adjustment in the doses were made with daily trough levels and the side effects. Both groups of patients received methyl prednisone 20 mg per day in the beginning. Data were analyzed by intent to treat. The median follow up for both the CyA and FK 506 was 180 day range (91 to 281 days). Results during the follow up period are summarized in the table below.

	FK 506 (n=41)	CyA (n=40)	
Patient survival	95.1% (n=39)	85.0% (n=34)	not sig
Graft survival	92.7% (n=38)	77.5% (n=31)	
Rejection free	53.7% (n=22)	15.0% (n=6)	sig
First episode of rejection	19.2 days	8.4 days	
Retransplant	4.9% (n=2)	15.0% (n=6)	

Results suggest better patients and graft survival with lower incidence of rejection in FK 506 treated patients.

Bact infection of higher in CyA group, due to more steroid which was shown on CyA group sig
 no diff in viral or fungal infection

PAPER 3

PREDICTORS OF SURVIVAL WHILST AWAITING HEART-LUNG TRANSPLANTATION IN CYSTIC FIBROSIS PATIENTS, AND ASSESSMENT OF POST-OP SURVIVAL AND QUALITY OF LIFE.

Linda
LD Sharples, N Caine, T Hathaway, C Dennis, T Higenbottam, J Wallwork.

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At this institution we have used heart-lung transplantation (HLT) as a treatment for end stage Cystic Fibrosis (CF) over five years from October 1985 to October 1990. Of the 30 patients for whom donor organs have become available, one year actuarial survival post-op is 79% (95% confidence interval 63% to 95%). We have studied all 64 CF patients accepted for transplantation in this period to identify those indices of health status which best predict which patients should be given priority for HLT, and to assess the procedure in terms of improved survival and quality of life.

Using Cox regression exercise tolerance, lung function, blood gases and body mass variables were considered as predictors of survival to HLT. Low forced expiratory volume in one second (FEV₁) (relative risk (RR) 0.14, p=0.01), low minimum arterial oxygen during a 12 minute walk (RR=0.95, p=0.001) and high PaCO₂ (RR=1.37, p=0.03) were predictive of poor survival. Treating transplant as a time dependent variable in Cox regression, and adjusting for these indices of health status at assessment, transplant to non-transplant risk of death was 0.27 (p=0.12). Although this does not reach statistical significance at traditional levels it is, nonetheless, an encouraging reduction in risk.

In comparing quality of life for patients before and after transplant, as measured by the Nottingham Health Profile, patients reported significant improvements in physical mobility and energy indices (p=0.01), and significantly fewer restrictions in home and leisure activities (p=0.05).

In conclusion CF patients who are recipients of HLT can expect improved survival and quality of life. FEV₁, minimum arterial oxygen saturation on a 12 minute walk and PaCO₂ are the best predictors of survival on the waiting list and so the best guides for deciding priority for early surgery.

1 yr actual survival 76%

PAPER 4

EARLY RESULTS OF THE DOMINO PROCEDURE

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The "Domino" procedure provides an increased supply of donor hearts. This procedure requires a change in the technique of heart-lung transplantation, ie SVC and IVC anastomoses, in order to preserve the SA node for the heart recipient. Twenty-eight "Domino" procedures were performed at Papworth Hospital between November 1988 and November 1990. The results are compared to non-domino heart and heart-lung transplantation during the same period.

DOMINO PROCEDURES

	Heart-lung Live donors 28	Domino Heart Recipients 28
Diagnosis	Cystic Fibrosis 17 Emphysema 6 PPH 4 Eisenmenger 1	IHD 13 Dilated CM 13 Other 2
Age	17 - 51 (31) y	10 - 59 (41) y
Ischaemic time	106-226 (176)	90-213 (150)
Hospital stay	median - 29 days	median - 22 days
Early mortality (3 mths)	2/28	6/28
Cumulative survival (3 mths)	91%	80%

NON-DOMINO PATIENT SURVIVAL

	Heart-Lung	Heart
3 mth cumulative survival	75%	77%

We conclude that the early results of the "Domino" procedure are acceptable and compare well with non-domino heart and heart-lung recipients during the same period.

PAPER 5

THE ROLE OF NATURAL ANTIBODIES IN THE HYPERACUTE REJECTION OF SWINE TO PRIMATE XENOGRAFTS

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Hyperacute rejection of organ xenografts is thought to be mediated by the reaction of serum components of the recipient with blood vessels in the donor organ. Based on the immunopathology of rejected organs, we have suggested that the pathogenesis might involve the activation of endothelial cells triggered by natural antibodies (NA) and complement (C). To test this hypothesis we compared various human sera for variation in the ability to activate porcine endothelial cells as manifested by the release of heparan sulfate (HS) from the cells and asked to which extent such variation reflected differences in NA titer and/or C activity. The sera mediated release of 3.5% to 57% of endothelial cell-associated HS. HS release correlated significantly with the titer of IgM antibodies that bound to cultured endothelial cells or to a triad of glycoproteins believed to represent the major targets of NA in porcine to primate xenografts; correlation was also observed with the total concentration of IgM. The release of HS did not correlate with corresponding properties of serum IgG, with anti-swine hemagglutination or with iso-hemagglutination titers. HS release correlated with deposition on endothelial cells of iC3b, but not with serum complement activity. These findings suggest that in the reaction between human serum and xenogeneic endothelial cells, it is the concentration of xenoreactive IgM and not differences in complement activity, within a normal range that limits the ensuing pathophysiological events.

Taffy: is it the circulating antibody? or is it complement?
A complement activation alone is not sufficient, but
may be some situations in which antibody is not acting
Have carried out their exp'ts for a few mins to 12 hrs
Rh monkey recognizes IgM in human. In monkeys
allowed to survive for some time there is an IgG antibody

PAPER 6

XENOGENEIC LIVER PERFUSION FOR FULMINANT HEPATIC FAILURE - RESEARCH ASPECTS

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This study addresses the possibility of using extra corporeal perfusion through a pig liver for the treatment of fulminant hepatic failure, and investigates the immunobiology of xenogeneic responses induced.

The biomechanics of perfusion was established using autologous pig blood perfused through pig liver ($n = 11$). It was found that cannulation of both hepatic artery and portal vein were required for inflow and only a single vena cava outflow sufficed. Various biochemical changes such as pH, blood gas profiles and electrolytes were observed. Changes in these parameters were easily corrected to physiological levels by the addition of bicarbonate and potassium chloride and the adjustment of oxygen partial pressures when required. It was also found that perfusion pressure between 10-20 mmHg, and haematocrit between 10-20% were optimal to prevent excessive ascites and facilitate good bile production.

There were no instances of hyperacute rejection ($n = 21$), when the liver was perfused with human blood under the same conditions as controls regardless of ABO compatibility. This was confirmed by the histological studies of the perfused livers.

Using this circuit, based on the passage of human blood through pig liver, the liver perfusion experiments showed excellent preservation of liver function, morphologically, biochemically and histologically with a mean perfusion time of 4.88 ± 0.82 hours (human - pig, $n = 22$) and 6 ± 1.93 hours (pig - pig, $n = 15$). These early results demonstrate that viability of a pig liver can be maintained in a circuit perfused with human blood.

PAPER 7

EX-VIVO HYPERACUTE XENOREJECTION OF DISCORDANT HEART GRAFTS; DEMONSTRATION OF A ROLE FOR COMPLEMENT.

J. FORTY, R. HASAN, M. METCALFE, N. CAREY, D.G.J. WHITE & J. WALLWORK.

PAPWORTH HOSPITAL, PAPWORTH EVERARD, CAMBS.

*Rabbit to pig
Rabbit to human*

An isolated working heart preparation has been developed for use with blood derived perfusates. This sensitive model has permitted investigation of hyperacute discordant xenograft rejection.

Rabbit hearts may be maintained with an artificial perfusate for a mean time of 353 minutes (n=4). Hearts may be maintained with rabbit blood for 215 minutes (n=4).

Perfusion of rabbit hearts with pig blood results in rejection at 56 minutes (n=4); $p < 0.001$. Perfusing a second heart with the xenogeneic blood results in rejection at a mean time not significantly different.

Complement consumption with cobra venom factor (CoF) prevents xenorejection, mean survival then being 206 minutes (n=4); $p < 0.002$.

Examination of hearts after perfusion revealed myocardial haemorrhage in all hearts to a degree proportional to perfusion time. Infiltrates of lymphoid cells and neutrophils in xenoperfused hearts were present even when xenorejection was prevented with CoF.

Immunohistochemical staining of the xenoperfused hearts demonstrated deposition of pig IgG and IgM, and C3 to a variable extent. In the presence of CoF immunoglobulins were deposited without C3.

Perfusion of rabbit hearts with human blood results in a different pattern of rejection again, however, complement-dependent. Additionally in these perfusions it is possible to demonstrate C4 and C9 deposition.

Xenorejection in the two discordant combinations studied is complement dependent.

PAPER 8

XENOGRAFTING: ESTIMATION OF HUMAN ANTI-PIG PRECURSOR CTL FREQUENCIES.

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The limiting dilution frequency assay (LDA) has been used widely to measure the frequency of antigen-specific precursor cytotoxic T lymphocytes (CTL) in peripheral blood samples. In both experimental and clinical transplantation it has been shown that the frequency of donor-reactive CTL increases during periods of acute allograft rejection. It is reasonable to assume that a high pre-transplant frequency of donor-reactive CTL is indicative of a high potential for cytotoxic cell mediated graft damage.

In this study we compared human allogeneic precursor CTL frequencies with those for the human-pig xenogeneic combination. The limiting dilution titrations for both combinations produced data which conformed to single-hit kinetics thereby indicating that human precursor CTL can respond directly to alloantigens presented by human splenic cells and to xenoantigens presented by porcine cells. It was found that the frequency of human precursor CTL which were specific for porcine spleen cells (range: 1/6,792 - 1/18,391) was generally between three and five times lower than the frequency of CTL which were specific for randomly selected allogeneic spleen cells (range: 1/1,340 - 1/4,400).

The results of this study suggest that the graft-reactive CTL response may be less vigorous in the human-pig xenogeneic combination than in conventional allogeneic human transplantation.

*After get over the initial effects will have bfr
with cellular rejection*

*Two phases immediate graft thrombosis can be separated
by this model from classical rejection*

PAPER 9

DELAYED CARDIAC XENOGRAFT REJECTION
IN COMPLEMENT DEFICIENT GUINEA-PIGS

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Cambridge, U.K.

Complement (C) is believed to play an important role in the rejection of discordant xenografts. We report here a study of the rejection of discordant rat to guinea-pig heterotopic heart grafts in normal or genetically C deficient guinea-pigs. DA rat hearts were transplanted into the anterior neck of C2, C3 or C4 deficient guinea-pigs. No immunosuppressor was used.

Normal guinea pig rejected rat hearts hyperacutely in approximately 30 minutes. Rat heart in complement deficient guinea-pigs survived for a mean of 4.5 days (N=17). These studies did not show a difference between the complement deficiencies suggesting that the hyperacute rejection was mediated by the classical pathway in this model.

Group	number	Survival time	Mean survival time
Normal guinea-pig	13	<30 min. x13	30 min.
C4 deficiency	10	7,6,6,6,5,5,5,4,3, (day)	5.2 days
C3 deficiency	2	5,3, (day)	4 days
C2 deficiency	5	4,4,4,4,2, (day)	3.6 days

In these experiments 24 rat hearts grafted into complement deficient guinea-pigs failed within the first 24 hours due to inadequate pressure in the guinea pig carotid artery. Mean pressure in the guinea-pig was between 80/60 - 70/50 mmHg. Mean pressure in the rat was 120/95 - 110/90 mmHg. This low blood pressure makes this potentially valuable model for studying xenograft immunology technically very difficult with a high failure rate. Despite this these studies show that complement deficient guinea-pigs do not hyperacutely reject a discordant rat heart xenograft confirming the importance of complement in this process.

PAPER 10

MECHANISMS OF CONCORDANT XENOGRAFT REJECTION

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In spite of documenting similarities in the phenotype and nature of *in vitro* cellular responses of rat cells to allogeneic and hamster stimulators, depletion or inhibition of T-cells in rat recipients does not result in improved survival of hamster heart xenografts. Rejection in this concordant model is mediated by antibody/complement mechanisms, since long term survival could only be achieved by depletion of complement in concert with cellular immunosuppression. The primacy of antibody/complement mediated rejection was confirmed by the following: (i) no cellular infiltration in rejected grafts from untreated animals, (ii) extremely high induced anti-hamster antibody titres at the time of rejection, (iii) binding of both IgM and IgG antibodies to xenografts (iv) no C3 binding in grafts from surviving hearts in complement depleted recipients, in spite of strongly positive antibody binding. Additionally, anti-hamster antibody was purified into IgM and IgG fractions. Most of the measurable lytic activity resided in the IgM fractions, but both fractions were capable of inducing hyperacute rejection of freshly transplanted hamster hearts in rat recipients. These data prompted the reclassification of concordant grafts into "easy-concordant" and "difficult-concordant" grafts. The former are represented by very closely related species (chimpanzee to man) where rejection is allograft-like and can be conventionally suppressed, while the latter grafts (baboon to man, hamster to rat) are rejected by humoral mechanisms. It has also been shown that graft accommodation occurs during treatment, since cessation of deplementation results in graft survival in the face of anti-graft antibody challenge.

More had cobra venom factor.

PAPER 11

Prolonged survival of Hamster to rat heart xenografts with Cyclophosphamide therapy.

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We have previously demonstrated that hamster to rat cardiac xenograft survival is not prolonged in athymic recipients or by T cell immunosuppressive regimes which are highly effective in prolonging allografts. Complement depletion of the recipient with cobra venom factor (COF) significantly prolonged graft survival from 3 days in untreated controls to 5.5 days. A combination of COF and cyclosporin A (Cy A) produced prolonged hamster heart xenograft survival (>100 days) in approximately 20% of the rats. All recipients produced high titres of lytic anti-hamster antibody.

To investigate the possibility of developing an immunosuppressive regime which would inhibit the production of this antibody the effect of cyclophosphamide was studied. In total 125 hamster-to-rat cardiac xenografts were performed in 20 different dose schedules. This revealed that the timing of the cyclophosphamide dose was important in preventing antibody production, that cyclophosphamide alone was insufficient to prevent graft rejection, combination with Cy A could produce consistent long term graft survival and cessation of cyclophosphamide therapy did not result in the subsequent appearance of anti-hamster antibodies despite the continued functional presence of the heart graft. Of 17 rat recipients treated with Cyclophosphamide (20 mg/kg) during the critical time period and continuous Cy A (10 mg/kg/day) 12 (70%) still have beating hearts at > 50 days. The remaining 5 died with beating hearts (MST 22.4 days).

These data demonstrate that the alternative pathway of complement is not involved in the rejection of these concordant xenografts and that antibody is a critical component of the process. Cyclophosphamide therapy may be of value in inhibiting rejection of other concordant xenografts.

gradually reduced cyp to 2 wks, 1 wk, and eventually long term survival of 60% after 4 days only. Dramatic reduction of antibody²⁴ production in Cyp groups. Tried to stop Cyp at 20 days, lost all the grafts. Gradual animal loss thought to be ~~due to~~ due to Cyp, now a group in which Cy A is reduced to 5mg

PAPER 12

M Katami, DJG White and PG Watson

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Experiments were performed to investigate the nature of corneal xenograft rejection. Closely related species grafts were performed by transplanting corneas of young Dunkin Hartley guinea-pigs orthotopically into PVG rats. To perform grafts between distantly related species, corneas of young Dutch rabbits were transplanted into BN rats orthotopically. All donors were female and all recipients were male. None of the corneal xenografts showed hyperacute rejection. Paradoxically, the rejection time of corneal grafts from the closely related guinea-pig to rat (MST: 7.8 days, median: 8 days, n = 8) was faster than that of corneal grafts from rabbit to rat (MST: 21.5 days, median: 13 days, n = 10). The difference between these groups is statistically significant ($p < 0.01$).

Thus, these data suggest that xenografts do not obey the rules of organ xenograft rejection and indicate that the possibility exists of finding non-rejector combinations of widely divergent species.

Pretreatment with Ultraviolet-B (UV-B) irradiation to the donor corneas in rat allografts produced a significant prolongation on orthotopic corneal grafts in the rejector strain combination of DA to AO. This is thought to be due to the UV-B irradiation to antigen presenting cells in the cornea. We are currently investigating whether the UV-B pretreatment can influence corneal xenograft rejection in order to evaluate the importance of direct and indirect antigen presentation in xenograft rejection.

Protection of mammalian cells from human complement-mediated lysis by transfection of human membrane co-factor protein (MCP) and decay accelerating factor (DAF)

D J G White, T J Oglesby, I Tedja, K Liszewski, L Wright, J Wallwork, J Van den Bogaerde and J P Atkinson

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Discordant organ xenografts are hyperacutely rejected by complement mediated mechanisms. MCP and DAF, widely distributed complement regulatory membrane proteins, are postulated to protect autologous tissue by inhibiting C3 deposition. To investigate cytoprotection from complement lysis by MCP and DAF, full length cDNAs (in forward or reverse orientation) were transfected into Chinese hamster ovary (CHO) or mouse fibroblast (3T3) cell lines. Expression of human DAF and MCP on the membranes of these animal cells was quantitated by both FACS and ELISA. Human complement mediated cytotoxicity, in the presence of hyperimmune rat anti-hamster or naturally occurring human anti-mouse antibody was assessed by H^3 adenine uptake or ^{51}CR release respectively.

Possession of human DAF or MCP provided almost complete protection from lysis by human complement (but not rabbit complement) against both the hyperimmune sera (CHO cells) and naturally occurring human anti-mouse sera (3T3 cells). This protective effect was abrogated by the addition of monoclonal antibodies (10 $\mu g/ml$) to MCP or DAF at the start of the cytotoxic assay.

These data demonstrate that mammalian cells expressing human MCP or DAF are specifically protected from destruction by human complement and suggest that these proteins when incorporated into the genome of a transgenic animal could protect organ xenografts harvested from such animals from hyperacute, complement mediated rejection. These data also offer an explanation for the high expression rate of these proteins on tumour cells and reproductive tissues and the resistance of these tissues to complement attack.

THE ROLE OF $CD4^+$ T CELLS AND ALLOANTIBODY IN REJECTION OF CLASS I DISPARATE CARDIAC ALLOGRAFTS IN THE RAT

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It is widely held that rejection of class I MHC disparate grafts requires the essential participation of $CD8^+$ T cells and is effected principally by cell-mediated mechanisms. We examined the roles of $CD8^+$ and $CD4^+$ T cells in the rejection of class I disparate rat cardiac allografts (PVG R8 into PVG RT1^U) by treating recipients in vivo with monoclonal antibodies against T cell subsets.

Administration of MRC OX8 (anti- $CD8$) was highly effective at both phenotypically and functionally depleting $CD8^+$ cells from the blood and lymphoid tissue of treated rats but, unexpectedly, did not delay the rejection of class I disparate cardiac allografts when compared with animals given control antibody (MST 6 days in both groups). Conversely, administration of a mixture of MRC OX35 and MRC OX38 (both anti- $CD4$) only partially depleted $CD4^+$ T cells but markedly prolonged the survival of class I disparate heart grafts (MST > 50 days).

Anti- $CD8$ but not anti- $CD4$ treated recipients developed a strong cytotoxic antibody response to their grafts. Moreover, passive transfer of immune serum to anti- $CD4$ treated recipients promptly restored their ability to specifically reject a class I disparate heart graft (MST 6 days) suggesting a critical role for antibody in the rejection response.

These results challenge the view that $CD8^+$ T cells are essential for the rejection of class I disparate grafts and demonstrate that $CD4^+$ T cells are able and sufficient to cause rejection of such grafts by providing T cell help for the generation of alloantibody.

PAPER 15

CYCLOSPORIN TOXICITY: HAEMODYNAMICS, TUBULAR DYSFUNCTION AND ABROGATION IN THE NORMAL HUMAN KIDNEY.

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Tubular dysfunction accompanies high dose cyclosporin A (CyA) therapy and may be associated with histological change. N-methylnicotinamide (NMN) is a basic cation which reflects tubular injury in animals (Beyer K.H. et al. *Am.J.Physiol* 160: 311-320, 1950). Overnight urine clearance were measured in 9 patients receiving low dose CyA for 3 months (2.5 mg/kg/day) for psoriasis. After 3 months off therapy 8 patients received the same dose of CyA and nifedipine SR 20 mg b.d. RBF, filtration fraction (FF) and GFR were serially assessed using a Tc-99m DTPA dynamic technique together with PRA, angiotensin II (AII) aldosterone and atrial natriuretic peptide (ANP).

Time(months)	(0)-CyA-(3)-No CyA-	(6)-Cya+Nif-	(9)-No Cya-	(12)
NMN ml/min	157±14	89±13 ^a	89±10 ^a	128±18 ^b
GFR ml/min/1.73m ²	96±4	82±5 ^b	90±3	87±6
RBF %C.O.	17±1.3	14.4±1.3 ^b	15.4±1.4	15.2±1.2
FF %	20.5±1.6	21.1±1.9	23.7±2.8	20.7±1.5
AII(pg/ml)	8.8±1	11±1	9.3±1.1	7.8±0.8 ^b
PRA pmol/hr/ml	4.2±0.6	3.2±1.1	3.6±0.4	2.9±1.1
Aldosterone pmol/l	505±61	378±43	423±49	427±41
ANP pmol/l	3.5±0.6	4.5±0.3	3.1±0.3	4.4±0.4
Means ± SEM	* p<0.01 ^b p<0.05 ^c p<0.05 (3) vs (12) student's t			

There was a good correlation between changes in GFR, RBF ($r_s=0.58$; $p<0.05$) and changes in clearance of NMN. Overall there was a correlation between GFR ($r_s=0.65$; $p<0.01$) RBF ($r_s=0.48$; $p<0.05$) and PRA ($r_s=0.42$; $p<0.05$) and NMN clearance. Nifedipine abrogates the A-II related fall in GFR and RBF with a corresponding improvement in tubular function. NMN clearance and haemodynamic analysis permits sensitive monitoring of cyclosporin toxicity in the normal innervated kidney not revealed by standard indices of renal function.

PAPER 16

PREVALENCE OF HEPATITIS C IN RENAL TRANSPLANT PATIENTS.

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It is well recognised that haemodialysis patients are at an increased risk for developing hepatitis. With the routine screening for hepatitis B markers, the use of various precautionary and isolation methods where appropriate, and the development of an effective vaccine, the incidence of this infection among transplant population has been greatly curtailed. Non A, Non B hepatitis, however, remains a serious problem. Recently the genome of hepatitis C virus (HCV), the major causative agent of Non A, Non B hepatitis has been isolated. Antibody to this virus appears late in the course of the infection and sustained titres are detected in chronic infection. Accurate estimation of the incidence of chronic infection may be important since up to 20% of patients may develop cirrhosis.

We studied the current serum of 272 renal patients returning to transplant Outpatient clinics at our Hospital for antibodies to Hepatitis C. Patients positive for hepatitis C were retrospectively screened from stored sera in order to determine at what time in their dialysis or transplant history they acquired Hepatitis C antibodies. Mean age of these patients was 41 years (range 5 to 70). They had previously been dialysed for a mean of 2.2 years (range 1 month to 8 years) and had received a mean of 12 units of packed cells during this period (range 0 to 85). Three transplant patients (1.1%) proved positive for Hep C antibodies. From stored sera we have been able to determine at what stage these patients seroconverted for Hepatitis C antibodies.

This low incidence of HCV infection is in keeping with the low incidence of elevated serum alanine aminotransferase (ALT) (Surrogate test for Non A, Non B hepatitis) that were observed among our dialysis population. This extremely low prevalence of HCV infection in Irish Transplant patients suggests genetic or geographical variation associated with this infection.

PAPER 17

STIMULATION OF CD4⁺ T LYMPHOCYTES BY ALLOGENEIC MHC PEPTIDES PRESENTED ON AUTOLOGOUS APC.

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In transplantation, direct interactions occur between the antigen receptors of the responding T cells and the allogeneic MHC antigens on the cells of the graft. The importance of this "direct" pathway of allorecognition has obscured the potential of the "indirect" pathway, whereby the allogeneic MHC molecules are recognised precisely as conventional, exogenous antigens, i.e., as peptides in self class II MHC molecules on self APC. Because direct T-B conjugation via the helper T cell receptor and B cell class II MHC antigens with carrier peptides is essential for antibody production, we screened for potential interactions between allogeneic MHC peptides and the class II MHC antigens of various strains by measuring antibody production in animals immunised with free, unconjugated peptide. Four 12 - 21 amino acid peptides were synthesised, corresponding to the α helical regions of the α_1 and α_2 domains of an RT1.C/E class I molecule of the DA (RT1^a) rat strain, and the H-2K class I and H-2A β chain class II molecules of the C57BL/6 (H-2^b) mouse strain. In the mouse studies, BALB/c (H-2^d), DBA/1 (H-2^k), CBA/J (H-2^k) and SJL (H-2^a) mice did not respond at all to the H-2^b peptides. For the rat work, PVG (RT1^c), WAG (RT1^u), LEW (RT1^l) and BN (RT1ⁿ) rats were immunised with the RT1.C/E^a peptides. The α_1 region peptide induced a very weak antibody response only in the LEW strain. The α_2 region peptide, however, induced very strong, MHC restricted antibody responses in the PVG strain, good antibody responses in the WAG strain, but no antibody response in the LEW and BN strains. T cell proliferation studies demonstrated a strong proliferative response in PVG rats, but not BN rats, to the α_2 peptide. This was mediated entirely by the CD4⁺ T cells, and was APC dependent. These studies strengthen the possibility that the indirect pathway might contribute to allograft rejection.

PAPER 18

ACTIVATION OF HUMAN VASCULAR ENDOTHELIUM BY ALLOGENEIC PERIPHERAL BLOOD LYMPHOCYTES AND T LYMPHOCYTE SUBSETS

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The recognition of, and adhesion to, vascular endothelium by circulating T lymphocytes is a critical step in the pathogenesis of organ allograft rejection. This study examines the ability of human allogeneic peripheral blood lymphocytes (PBL) and T cell subsets to activate vascular endothelial cells (VEC) in vitro.

Human VEC were grown from umbilical veins and shown (2nd-4th passage) to be >99% pure by staining with anti-factor VIII related antibody and by the VEC specific monoclonal antibody EN-4. FACS analysis showed that resting VEC expressed both inter-cellular adhesion molecule-1 (ICAM-1) and Class-I MHC but not Class-II MHC antigens. Culture of VEC in the presence of rTNF- α or rIFN- γ increased the expression of both ICAM-1 and Class-I MHC but only rIFN- γ induced expression of Class-II MHC.

Co-culture of VEC with allogeneic PBL for 72 hrs induced ICAM-1 and both Class-I and Class-II MHC antigens. Culture of VEC with either CD4⁺ or CD8⁺ T cells alone (separated by magnetic bead depletion or panning achieving >95% purity) induced both ICAM-1 and Class-I MHC but neither subset alone induced Class-II MHC expression.

Whereas previous work^{1,2} has suggested that the CD4⁺ and CD8⁺ T cell subsets cause differential activation of VEC, the results of this investigation suggest that although either T cell subset is able to activate VEC in vitro, interaction between the subsets may be necessary for the effective induction of Class II MHC antigens on vascular endothelium.

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PAPER 19

ADVANTAGES OF REDUCING THE DOSE OF ATG DURING TREATMENT FOR STEROID RESISTANT REJECTION

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In renal transplantation treatment of Steroid Resistant Rejection (SRR) with anti-thymocyte globulin (ATG) has been widely reported. However, viral infections, particularly cytomegalovirus (CMV), remain a major complication of ATG therapy. In the first 10 patients (Group 1) we treated for biopsy proven SRR with rabbit-ATG (Institut Merieux, Lyon) three patients experienced serious viral infections with 2 deaths from CMV. ATG was administered at doses of 2.5 to 5 mg/kg per day. Doses were omitted only if thrombocytopenia (platelet count < 50x10⁹/l) or leucopenia (WCC < 3x10⁹/l) occurred. We felt this was an unsatisfactory way in which to monitor such a potent immunosuppressive agent. Therefore, in the next 15 patients (Group 2) with biopsy proven SRR, ATG was administered according to daily peripheral T cell counts. T cells were analysed using a CD3 fluorescent-conjugated monoclonal antibody (Becton and Dickinson, Oxford) and evaluating results by flow cytometry. ATG dosage was adjusted on a daily basis to keep the absolute circulating T cell count <50 cells/ul. Groups 1 and Group 2 were matched for the major variables that effect renal allograft function and survival.

Results:	GROUP 1	GROUP 2	
No. Patients	10	15	
Serious Infection	3	0	p=0.05 (Fisher's exact)
Reversal of SRR	8(80%)	14(93%)	NS
Dosage of ATG (mg/kg per day)	2.2	0.9	p<.001 (t test)

Graft survival at 1 year in group 1 was 60% and 62% in 8 patients followed to 1 year in Group 2. We conclude that flow cytometric monitoring during ATG administration enables reduction in the dosage of ATG. This avoids profound overimmunosuppression and significantly reduces serious viral infections without increasing graft loss or impairing the ability of ATG to reverse SRR. The savings accrued by reducing the dosage of ATG more than offsets the cost of flow cytometric monitoring.

PAPER 20

LONG-TERM RESULTS OF KIDNEY TRANSPLANTATION

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With one-year cadaveric renal graft survival approaching 90% in many centres, the emphasis when measuring results now lies on long-term graft function. We have examined the graft survival of 12,883 first, unrelated kidney grafts transplanted since 1971 within 52 European renal transplant centres. There has been almost a 30% improvement in five-year graft survival over the past 17 years, from 38.8% for the period 1971-1975 (with conventional immunosuppression) to 66.0% for the period 1981-1987 (with cyclosporine (CyA)). The overall gain in half-lives based on grafts functioning at one year post-transplantation was 2 years, from 9.7 years (95% confidence interval 8.4 to 11.2 years) for 1971-1975 to 11.6 years (95% confidence interval 10.0 to 13.4 years) for 1981-1987 with CyA. Within the CyA era, a difference of almost 30% in five-year graft survival has also been observed between patients transplanted with 0 and 6 HLA-A+B+DR mismatches (Table 1).

Table 1: Results by no. of mismatches, 1981-1987, with CyA

No. of HLA A+B+DR mismatches	No. of patients	1-year graft survival	5-year graft survival	Half-life in years (95% conf. int.)
0	411	90.3	79.0	13.2 (8.9-19.5)
1	1544	87.3	69.1	12.4 (10.0-15.4)
2	2034	84.8	67.8	12.8 (10.5-15.6)
3	1695	83.7	63.6	10.1 (8.3-12.4)
4	775	80.6	59.5	10.8 (8.2-14.1)
5	289	76.9	55.6	9.3 (6.4-13.5)
6	84	77.0	50.6	7.8 (4.0-15.3)

Analysis per HLA locus revealed gains in half-life for patients matched for HLA-A and HLA-B, but after the first post-transplant year the effect of HLA-DR matching was negligible. We conclude that matching for HLA continues to be of benefit, both in the short and longer term.

PAPER 21

UROLOGICAL COMPLICATIONS FOLLOWING RENAL TRANSPLANTATION

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Urological complications occur in 5-15% of patients undergoing renal transplantation and may result in significant patient morbidity.

1016 consecutive renal transplants in a single centre (1976-1990) were analyzed retrospectively to determine the incidence of urological complications and possible predisposing factors. 191 episodes of ureteric obstruction and/or urinary leak occurred in 143 patients (overall incidence 14.1%). The annual rate of urinary leak was median 5.1% [range 0 - 11.5] and of ureteric obstruction: pre-1986, 4.5% [1.4 - 6.8] and post-1986, 16.1% [14.5 - 20.5]. 48/54 (86%) primary urinary leaks and 78/104 (75%) primary ureteric obstructions occurred in the first month following transplantation. 32 (60%) urinary leaks and 89 (86%) ureteric obstructions occurred at the vesicoureteric junction/distal third of ureter. 30 (55%) urinary leaks were treated primarily by reconstructive surgery, 8 (15%) required nephrectomy for pelvi-ureteral necrosis and 2 (4%) died from associated sepsis. Prior to 1986, 10/11 (91%) patients with ureteric obstruction were treated by reconstructive surgery, but since then 74/93 (71%) have been treated by percutaneous nephrostomy and stenting with only one graft lost and no deaths.

Age, number or source of transplant did not significantly influence the incidence of these complications, but significantly more patients with obstruction had ≥ 2 episodes of rejection (48 [46%]) compared to those with leaks (15 [28%]) $\chi^2=5.01$, $p<0.05$.

This study has demonstrated a worrying increase in the incidence of ureteric obstruction since 1986. Technical causes may account for some cases, but the leak rate in the same period has remained constant. Cyclosporin A and aggressive diagnosis and treatment of obstruction were introduced in 1986 and may be responsible. Further study of this increase in ureteric obstruction post-transplant is warranted.

PAPER 22

IMPROVED RESULTS OF PAEDIATRIC LIVER TRANSPLANTATION

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Since the start of the combined Cambridge/King's College Hospital paediatric liver transplantation programme in 1983, 133 patients have received transplants of whom 91 are alive (overall survival 68%). More recently, despite accepting a higher proportion of high risk patients for transplantation, the results have improved. We have reviewed the results of paediatric liver transplantation over the past two years in this series.

47 children aged between 2 weeks and 15 years (mean 3.3 years) have received a total of 58 liver transplants (retransplantation rate 21%). 15 patients were aged less than 2 years (32%). Diagnoses included biliary atresia/hypoplasia (30), metabolic disease (14), fulminant hepatic failure (2), sclerosing cholangitis (1).

28 patients were listed as urgent cases; 31 patients had undergone previous upper abdominal surgery. 4 patients had significant anatomical anomalies, 3 with situs inversus and one with an absent IVC. 3 patients were cyanosed due to intra-pulmonary shunting and 1 patient had hypogammaglobulinaemia. Of the 58 livers transplanted, 43 were whole grafts and 15 reduced grafts (26%).

The actuarial one-year patient survival was 83%; 7 patients died, 3 from sepsis, 2 graft infarction, 1 cardiac arrest, 1 cerebral oedema. Of the 18 failed grafts, 5 were due to chronic rejection, 5 due to patient death with functioning grafts, 3 hepatic artery thrombosis, 2 ischaemia, 2 acute rejection and 1 portal vein thrombosis.

Despite the high proportion of complex and urgent cases, the results of liver transplantation continue to improve, as a result of the development of operative techniques and postoperative management.

*ALG induction good - now 25% rejection
CyA 150-250 whole blood
Anti thrombin 3 measures + p. given, all get aspirin
CMV proph immune job + gancyclovir UV solution*

PAPER 23

SUCCESSFUL PREGNANCY FOLLOWING TRANSPLANTATION IN WOMEN TAKING AZATHIOPRINE AND PREDNISOLONE

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Chronic renal failure is associated with a subfertile state. Following successful transplantation approximately 1 in 50 women of childbearing age become pregnant. Complications including hypertension, graft function deterioration and rejection are potential risks of each pregnancy with the fetus at risk from the effects of immunosuppressive drugs. We report the outcome of pregnancies in transplanted patients receiving prednisolone and azathioprine as their sole immunosuppressive therapy.

126 women of childbearing age (15-45 years) received renal allografts between 1965 and 1990. 27 pregnancies have occurred in 14 women. The mean age at the time of renal transplantation was 23 yr (+/- 3.7) and at first pregnancy was 27 yr (+/- 3.7) with the interval between transplantation and conception ranging between 1 and 11 years. The 27 pregnancies resulted in 23 live births including one set of twins. All the babies were born without major congenital defects and only 2 were below the 10th centile for birth weight. There were 4 documented first trimester abortions and one stillbirth at 8 months. 35% of the live births were delivered by caesarian section. Graft function remained stable during these pregnancies.

In this cohort of women maintained on azathioprine and prednisolone both the rates of conception and successful outcome were higher than other series.

PAPER 24

CYCLOSPORIN BLOOD LEVELS MAY DETERMINE GRAFT SURVIVAL OR FAILURE FIVE YEARS AFTER RENAL TRANSPLANTATION.

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Long-term deterioration in renal transplant function in patients receiving Cyclosporin prophylaxis is usually ascribed to chronic rejection or Cyclosporin toxicity. The management of such cases long-term usually consists of reduction in Cyclosporin therapy and the addition of Azathioprine and steroids. In this paper we test the hypothesis that longterm graft failure is due to inadequate dosage with Cyclosporin, and further reduction of this dose subverts the quality of immunosuppression and leads to chronic rejection, rather than reducing the incidence of Cyclosporin-induced nephrotoxicity.

Seventy-eight recipients of living and cadaveric renal transplants performed in 1983 to 1985 were studied: graft survival, and Cyclosporin trough levels were measured (HPLC). Of these, 52 continued Cyclosporin monotherapy and 26 were converted to Cyclosporin plus steroid therapy.

Results: Eight patients died with graft function. Sixteen grafts were lost after 3 years and 56 patients had functioning transplants for at least five years.

Cyclosporin blood levels (ng/ml) and graft survival were as follows:

	1 year	2 years	3 years	4 years	5 years
Functioning	300±123	245±80	272±165	233±100	226±116
	NS	p < 0.05	NS	p < 0.05	p < 0.05
Graft loss	349±241	184±68	206±86	147.5±82	126±50.5
No. lost at yearly intervals	0	0	2	6	8

Conclusion: Cyclosporin blood levels were significantly higher in the surviving transplant group compared to the group whose transplants failed. The prognosis for long-term graft function may therefore be improved by maintaining CyA trough levels at more than 220 ng/ml.

PAPER 25

SMALL VESSEL CORONARY OCCLUSIVE DISEASE
AFTER CARDIAC TRANSPLANTATION

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The functional effects of coronary occlusive disease (COD) on small resistance coronary vessels are unclear. We investigated the changes in coronary flow reserve (CFR) to the non specific smooth muscle vasodilator papaverine. A 3F Doppler probe was inserted into the left anterior descending (LAD) coronary artery in 46 patients following orthotopic heart transplantation. Studies were performed in 42 males and 4 females with a mean age of 46.8 years (range 20-61 years). The median time from operation was 4 years (range 3 months to 10 years). Coronary blood velocity was measured at rest and at maximum hyperaemia produced by intracoronary papaverine. Coronary flow reserve (CFR) was defined as the ratio of peak to resting velocities. Minor COD was defined angiographically as LAD stenosis < 25% and/or small vessel disease

	CFR	Patient Numbers
"Minor" COD	Mean 2.6 ± SD 1.1	21
Normal	Mean 4.1 ± SD 1.1	25
		p < 0.00003

Therefore in the presence of minor epicardial or small vessel disease, coronary flow reserve in resistance vessels is markedly impaired. This demonstrates that non endothelial dependent coronary vasodilation is abnormal prior to the development of severe epicardial lesions in COD.

PAPER 26

ASSESSMENT OF MYOCARDIAL PRESERVATION DURING HEART
AND HEART-LUNG TRANSPLANTATION: A STUDY OF 317
HUMAN DONOR HEARTS

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The International Heart Transplant Registry has shown that 48% of deaths occurring < 30 days after HTx are due to cardiac failure. To ascertain the cause of this mortality accurate evaluation of the functional status of the heart prior to excision and its preservation during Tx is vital. Quantitative birefringence measurements (QBM) assess the response of muscle fibres to ATP and they correlate with measurements of cardiac function (p < 0.001). On the basis of QBM 300 hearts monitored during HTx could be separated into 3 groups: Group 1: (poor donors). 110 (51%) classed as poor before excision (QBM 1.04 ± 0.01 compared with 1.41 ± 0.01, p < 0.001 for remaining 190). Group 2: (deteriorated during storage and implant). 108 initially satisfactory hearts deteriorated from 1.43 ± 0.01, to 1.14 ± 0.01, p < 0.001. Group 3: (good throughout). The remaining 82 (27%) did not alter significantly. Mortality: Deaths due to cardiac dysfunction (organ failure, dysrhythmia, acute rejection, coronary occlusive disease) were more frequent in recipients of hearts from Groups 1 and 2 (24% and 20% compared with 3% from Group 3, p < 0.001). In contrast, of 17 hearts monitored during HLTx 14 (82%) remained good throughout; 1 deteriorated, 2 were classed as poor at excision - one of these recipients died from acute rejection. Ischaemic times were similar during HTx and HLTx (178 ± 6 and 177 ± 11 minutes respectively). The study suggests that inadequate myocardial preservation before or during transplantation affects both early and long-term survival of the recipient. Since preservation was superior in HLTx further studies are needed to ascertain which aspects of donor organ management were beneficial in this group.

PAPER 27

THE EFFECT OF SYSTEMIC DRAINAGE OF THE PANCREAS ON INSULIN SENSITIVITY

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To assess the metabolic impact of the site of venous drainage of the pancreas, the pancreaticoduodenal and splenic veins were surgically transposed from the portal vein to the inferior vena cava in five dogs. The remaining portal-systemic connections were severed by carefully dissecting the pancreas from the duodenal wall except for the entrance of the common bile duct. Insulin sensitivity was assessed by concurrently infusing somatostatin (0.5ug/kg-min) and insulin (800uU/kg-min) both before and two weeks after the venous transposition. Normoglycemia was maintained using a variable glucose infusion. The metabolic clearance rate of glucose (MCR) was determined using an infusion of tritiated glucose. Before surgery, fasting insulin concentrations were 6.3 ± 2.2 uU/ml and rose to 12.8 ± 1.5 uU/ml ($p < 0.05$) after transposition. During insulin infusion, these rose to 30.6 ± 2.9 and 32.2 ± 3.7 uU/ml respectively. MCR rose from 3.4 ± 0.6 to 15.4 ± 2.1 ml/kg-min in the normal animal and from 3.8 ± 0.6 to only 9.6 ± 1.7 ml/min after surgery. The index of insulin sensitivity: (increment in MCR)/(increment in insulin level) fell from 0.51 ± 0.07 to 0.28 ± 0.05 , a decrease of 45% ($p < 0.02$). These results indicate that with other factors such as innervation intact, drainage into the systemic circulation alone decreases insulin sensitivity to about half the value when the pancreas drains portally.

PAPER 28

CONTRIBUTION OF OPTIMAL HLA ANTIGEN MATCHING TO HIGH SURVIVAL OF 275 CADAVER KIDNEYS TRANSPLANTED IN 1989 & 1990.

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During 1989 & 1990, 275 cadaver kidney transplants were performed at this single centre with an actuarial transplant survival (Log Rank) at 12 months of 91%. Recipient selection throughout was based on HLA-A,B,DR antigen matching avoiding mismatches whenever possible. As participants in the UK 'Beneficial matching' scheme we transplanted 51 (18.5%) kidneys with no more than 1 HLA-A or B mismatch (25 UKTS, 26 local). Survivals at 12 months were 94.4% for 'Beneficially' matched first transplants and 89.7% for 195 other first transplants. Survivals for DR mismatches were 94.5% (152 - 0 DR MM), 85.9% (120 - 1 DR MM) and 66.7% (3 - 2 DR MM); $p = 0.02$.

Arguments against prospective matching have included unacceptable cold ischaemia and longer recipient waiting times. An analysis of donor kidney origin did show slightly higher survivals for local kidneys (+12%); there was no difference in survivals for recipients waiting more or less than 6 months for a well matched kidney.

We conclude that a kidney transplant survival rate of over 90% is attainable but this is strongly influenced by HLA antigen matching. Furthermore, the UK 'Beneficial matching' scheme has made a positive contribution to the high degree of matches and survivals achieved at this centre in the last 2 years.

PAPER 29

LEUCOCYTE INFILTRATION IN RENAL ALLOGRAFT BIOPSIES: SIGNIFICANCE OF ACTIVATED CELLS.

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Renal allograft rejection is usually accompanied by high levels of infiltrating leucocytes. Indeed the magnitude of the infiltration within a biopsy can be used prospectively in the diagnosis of rejection, but high levels of infiltration occasionally occur in the absence of rejection. We have quantitated the expression of markers of activation, CD25 and CD69 to look for variation in the composition of the leucocyte infiltration in different clinical circumstances. Cryostat sections of needle core biopsies (n=60) from 34 recipients of cadaver renal allografts were stained for the leucocyte-common antigen, CD25 and CD69.

Significantly higher levels of infiltration were detected in biopsies taken during (29.9± 2.0%) and in the 6 days preceding rejection (23.3± 3.4%) than in biopsies obtained after rejection, >6 days before rejection or from patients with no rejection episodes (11.7± 2.1%, 10.6± 2.6%, 9.1± 1.3% respectively).

There was invariably a higher level of expression of CD25 than CD69. While there was no consistent increase in the proportion of the infiltration expressing activation markers in the 6 days preceding rejection, the expression of CD25 during rejection (30.8± 8.9%) was significantly greater than in biopsies with no rejection (10.7± 1.7%) and in biopsies obtained after a rejection episode (8.5± 2.2%; p<0.008).

These data demonstrate that the proportion of activated cells within the transplant infiltrate varies, rising significantly during clinical rejection.

PAPER 30

TNF ALPHA PRODUCTION BY IL-1 ALPHA STIMULATED PROXIMAL TUBULAR EPITHELIAL CELLS (PTEC) CAN BE INHIBITED BY PRE-TREATMENT OF PTEC WITH CYCLOSPORINE A

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In previous reports, we have shown that human proximal tubular epithelial cells (PTEC) may function as a target for graft infiltrating cells (GIC) during allograft rejection. We recently found that IL-1 α stimulated PTEC can produce TNF- α a cytokine known to have immune modulatory capacity. TNF- α production was measured using a standard TNF- α Bio assay with the TNF- α sensitive L929 mouse fibroblast cell-line. A TNF- α standard with well known concentrations of this cytokine was included in each experiment to quantify the amounts of TNF- α produced. Time response experiments revealed that PTEC already show significant production of TNF- α within two hours, after IL-1 α (1 ng/ml) stimulation, reaching a plateau at 48 hours. Blocking experiments showed that the TNF- α production could be completely inhibited by anti-IL-1 α MoAb. Furthermore we showed that pretreatment of PTEC with the immunosuppressive drug Cyclosporine A for 72 hours completely blocked the TNF- α production by IL-1 α stimulated PTEC. Currently experiments are undertaken to see if TNF- α production is the result of 'de Novo' synthesis or release from pre-existing pools. In addition, we are investigating if PTEC also express the membrane bound form of TNF- α and if this can be upregulated or induced by IL- α . From the data we conclude that PTEC produce TNF- α upon stimulation by IL-1 α . This may have implications for the immune response during allograft rejection, since it is known that TNF- α promotes renal injury and can activate T-cells. Cyclosporine A inhibits TNF- α production by PTEC in vivo.

PAPER 31

DONOR-SPECIFIC SYSTEMIC TOLERANCE IN MICE INDUCED BY HEART ALLOGRAFTING UNDER COVER OF ANTI CD4 PLUS ANTI CD8 MONOCLONAL ANTIBODIES

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AIM: To block allorecognition in such a manner that allospecific systemic tolerance is induced.

METHODS: Cocktails of anti-CD4 and anti-CD8 monoclonal antibodies (mabs) were used to block (IgG2a isotype, non-depleting) or remove (IgG2b isotype, depleting) CD4 and CD8 bearing cells in mice receiving a heart allograft into the peritoneum (Balb/c (H2^d) to CBA (H2^k)). Mab therapy was given three times weekly for 21 days only. After 100 days systemic tolerance was tested by skin grafting from donor type, or from third party type (C57Bl. (H2^b))mice. Some mice bearing a functional heart allograft also received a second Balb/c heart allograft to the neck after 130 days.

RESULTS : Control CBA mice without mab therapy rejected Balb/c hearts at around 11 days. Recipients treated with either non-depleting or depleting anti-CD4 plus anti-CD8 mabs retained functional allografts for over 100 days. Third party skin was rejected acutely, whilst donor-type skin was chronically rejected in 5 of the 8 mice which had received non-depleting mabs; the other two skin grafts were accepted and grew hair. 6/9 mice in the depleting mab group accepted donor-type skin. When those mice which had rejected donor type skin were tested with a second heart allograft, this heart was accepted.

TABLE:	N ^o mice	graft survival time (days)			
		1st heart	Balb/c skin *	C57B skin	2nd heart
non-depleting mabs	8	>100 (x8)	<i>20,21,21,25, 24,>40(x3)</i>	8,9,9,10,10, 10,11,13.	<i>>12,>13 >28,>5</i>
depleting mabs	9	>100 (x9)	<i>20,20,21, >40(x2)>60(x4)</i>	9,10,11(x3), 12(x3),13.	<i>>21,>20 >26</i>

*italics indicate those mice which received a second Balb/c heart allograft

CONCLUSIONS: Limited therapy with mabs which block or deplete CD4 and CD8 bearing cells at the time of allografting is associated with the generation of systemic tolerance to donor whilst retaining immune competence. Depleting mabs were the more effective. In those mice which had rejected donor type skin a second heart allograft was accepted, showing that the first heart was not retained simply because passenger cells (including antigen-presenting cells) had left the graft.

PAPER 32

REGULATION OF MURINE SPLENOCYTE FUNCTIONS BY HEPARAN SULFATE

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Lymphocyte and macrophage functions are known to be regulated by cytokines released as a consequence of lymphocyte-APC interactions. Heparan sulfate (HS), a biologically active acidic polysaccharide, is released from endothelial cells (EC) as a consequence of interactions with lymphocytes or antigen presenting cells (APC). We therefore tested the potential influence of HS on the function of murine lymphocytes. HS (10 µg/ml-100 µg/ml), when present for the first 6-24h of a mixed lymphocyte culture (MLC), augmented the proliferative response of splenocytes to allogeneic stimuli, as measured by [³H]thymidine incorporation. HS also increased up to 10-fold proliferative responses to the mitogens Con A, PHA, anti-CD3, and ionomycin. These effects appeared to be mediated by IL-1, since increased IL-1 synthesis by APC and B cells was detected and since the enhanced response was impaired by anti-IL-1 antibody. Increased allospecific cytotoxicity (3-fold to 10-fold) was also observed, however the mechanism for this effect is uncertain. Addition of HS to a MLC 48 to 72 h after establishment of the culture stimulated the production of PGE₂, which in turn inhibited proliferative and cytolytic responses. These findings indicate that HS may significantly influence lymphocyte and APC functions and suggest a model in which HS, released from EC as a consequence of interaction with T cells, polymorphonuclear cells, or antibody and complement, contributes to the activation of APC or EC thereby promoting cell mediated immune responses.

PAPER 33

ALLOANTIGEN-PRESENTATION BY HUMAN ENDOTHELIAL CELLS

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We have investigated the ability of human endothelial cells (EC) to present alloantigens to CD4+ T cells. IFN- γ -treated EC, but not control EC, express class II MHC molecules and stimulate CD4+ T cell proliferation; proliferation is blocked by MAb to HLA-DR and CD4. MAb to LFA-1, CD2, LFA-3 and CD44, but not HLA-A,B, CD8, VLA-4 or VCAM-1, also inhibit. IFN- γ -treated fibroblasts or smooth muscle cells express comparable levels of class II but do not stimulate CD4+ T cells to proliferate.

The capacity of EC to present alloantigen may relate to the greater ability of EC to provide costimulation, which we measure as augmented IL-2 production by PHA-stimulated CD4+ T cells. EC costimulation in this system depends on cell contact and is partly blocked by MAb to CD2 and LFA-3, but not LFA-1 or CD44. Micelles of LFA-3 partly replace EC, but EC also interact through an, as yet, unidentified ligand. EC, but not LFA-3, can enhance T cell c-fos mRNA levels, which may contribute to augmented IL-2 synthesis through an AP-1 site in the IL-2 promoter.

Alloantigen presenting functions of EC may contribute to accelerated graft arteriosclerosis in cardiac transplantation. Graft coronary arteries consistently show infiltrates of CD4+ T cells juxtaposed to luminal arterial EC expressing class II MHC molecules. Histologically, this lesion suggests a chronic delayed hypersensitivity response to graft endothelium; intimal smooth muscle hyperplasia results as a consequence.

We conclude that EC can express MHC alloantigens and display costimulatory molecules which enable these cells to stimulate an allogeneic T cell response *in vitro* and *in vivo*.

PAPER 34

ALLOANTIGEN PRESENTATION BY CULTURED RENAL TUBULAR EPITHELIAL CELLS.

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It has been observed that epithelial cells within a rejecting renal allograft express high levels of Class II major histocompatibility (MHC) antigens. In this study the ability of Class II MHC antigen expressing epithelial cells to activate proliferation of allogeneic peripheral blood mononuclear cells (PBMC) was examined.

Lines of human renal epithelial cells were established and their expression of Class II MHC antigens was upregulated by culture with gamma-interferon. Allogeneic PBMC were cultured with either these epithelial cells, with endothelial cells or with splenic cells. Lymphoproliferation was stimulated by both endothelial and splenic cells but cultured renal epithelial cells were non-stimulatory. Flow cytometry demonstrated similar levels of expression of Class II MHC antigens by both epithelial and splenic cells. The failure of proliferation by lymphocytes cultured with allogeneic epithelial cells was not overcome by treatment with IL-1 or indomethacin; however, addition of IL-2 stimulated proliferation and allowed generation of a cytolytic cell line.

These findings indicate that epithelial cells expressing Class II MHC antigens may be unable to directly stimulate the proliferation of naive allospecific recipient lymphocytes present within a renal allograft. However within a rejecting graft it is likely that the local concentration of IL-2 is high. Therefore, expression of Class II MHC antigens by epithelial cells within a renal allograft may render such cells immunogenic and able to play a role in the rejection process.

PAPER 35

IN VITRO IMMUNOMODULATION OF DOG ISLETS BY SYNERGISTIC MONOCLONAL ANTIBODIES

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Reducing the antigenicity of donor tissue by removing donor passenger leukocytes acting as antigen presenting cells (APC) could help diminish the immunosuppressive therapy needed to achieve graft acceptance.

Immunomodulation of islet tissue by pretreatment with specific anti-dog monoclonal antibodies (Mabs) directed against passenger leukocytes has been tested in *in vitro* experiments using islets isolated by collagenase digestion and purified by Ficoll gradients from dog pancreata. The outbred dog model resembles the clinical situation and has been used widely in the development of islet isolation and transplantation and was therefore chosen as a preclinical test model.

Islets were pretreated with single, pairs or a cocktail of 3 different, complement-binding anti-dog Mabs (identified by complement mediated cytotoxicity) together with autologous dog serum as a source of complement. After extensive washings to remove free, unbound Mabs these treated islets were tested for their stimulatory capacity in mixed allogeneic lymphocyte-islet-co-cultures. Treatment with a cocktail of Mabs could diminish the stimulatory capacity which could be restored by the addition of small numbers of donor type leukocytes. Functional tests after Mab treatment are in progress. Currently we know that Mab cocktails in the presence of autologous complement lyse leukocytes but have a negligible effect on islet targets.

These *in vitro* results show promise for transplantation of dog islets using immunomodulation by simple Mab plus complement pretreatment. Thus this model may serve as a useful indicator for clinical application. *In vitro* immunomodulation experiments using human islets and anti-human Mabs with specificity for CD45 are in progress.

PAPER 36

IN VITRO ASSESSMENT OF ISLET IMMUNOGENICITY AFTER IMMUNOMODULATION BY POLYCLONAL OR MONOCLONAL ANTIBODY

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Isolated pancreatic islet transplantation has been proposed as a future way of treating diabetes. The removal of antigen presenting cells (APC, passenger leukocytes) residing within donor tissue may help to reduce the recipients immune response and thence reduce immunosuppressive therapy to achieve graft acceptance.

Immunomodulation of purified rat islet tissue by monoclonal antibody (Mab) pretreatment was investigated in the strong histoincompatible rat strain combination DA - WAG (RT1^a - RT1^u). Commercially available anti-rat Mabs with specificity for Ia antigens, macrophages, dendritic cells were compared with non-commercially available polyclonal antibodies with congenic anti class I,II or I+II specificity for their effect on intra-islet APCs. A range of concentrations of Mabs alone or cocktails of Mabs or polyclonal antibodies were tested previously by ⁵¹Cr release studies using donor mononuclear cells suspensions. Treatment of purified islets with chosen antibodies were carried out at +4°C with and without preabsorbed rabbit complement added subsequently at 37°C at a non toxic concentration. Mixed islet lymphocyte cultures were used as the functional *in vitro* indicator of depletion or inhibition of APC cells. Mab treated islets showed no effect on the proliferation rate of co-cultured allogeneic lymphocytes whereas polyclonal antibodies reduced the proliferation rate to 50 % of their untreated islet control value. Reconstituting treated islets with a small number of spleen cells containing APC cells restored the stimulatory capacity to its normal control level. Insulin secretion studies of islets after treatment showed a two fold stimulation of insulin release when challenged with high glucose solutions.

These *in vitro* studies suggest that whilst commercially available anti rat Mabs did not inhibit or deplete intra-islet APC cells, polyclonal antibodies have shown a substantial immunomodulatory effect in reducing islet antigenicity.

PAPER 37

EXPRESSION OF VASCULAR ADHESION MOLECULES IN NORMAL AND TRANSPLANTED HUMAN HEART AND LUNG.

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Adhesion of leucocytes to vascular endothelium is a necessary step leading to the migration of cells into underlying tissues. Immunocytochemical studies have been used to investigate the expression of vascular adhesion molecules (ICAM-1, PECAM, VCAM and ELAM-1) in normal heart (n=6), cardiac biopsies from heart transplant patients with acute rejection (n=6), normal human lung (n=6) and lung from recipients who required retransplantation because they had obliterative bronchiolitis (n=6). These antigens have been identified on untreated (ICAM-1, PECAM) or cytokine activated (VCAM, ELAM-1) cultured human umbilical vein endothelial cells. In normal heart ICAM-1, PECAM and to a lesser extent VCAM are expressed on venules and arterioles whereas ELAM-1 is restricted to venules. No differences occur during rejection. Capillary and endocardial endothelium normally express ICAM-1 and PECAM but little, if any, VCAM or ELAM-1. During rejection, however, there is an increase in the expression of all adhesion molecules especially VCAM. This is paralleled by an increase in von Willebrand Factor expression on endothelial cells and an induction of ICAM-1 on intercalating discs and myocardial membrane.

In normal lung, endothelium lining large and small vessels and alveolar capillaries expressed ICAM-1, PECAM and to a lesser extent VCAM and ELAM-1, whereas, epithelial cells only expressed ICAM-1. There was an increased expression of ELAM-1 on vascular endothelium and ICAM-1 on bronchiolar epithelium in the transplanted lung.

In conclusion, the induction of vascular adhesion molecules in rejecting heart and transplanted lung is evidence of endothelial activation during rejection.

PAPER 38

CYTOMEGALOVIRUS DIRECTLY INDUCES MHC CLASS I AND ICAM-1 EXPRESSION ON CULTURED HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS

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Human proximal tubular epithelial cells (PTEC) can be infected with Cytomegalovirus (CMV) *in vivo* and *in vitro*. Clinically, there is an association between CMV infections and the occurrence of rejection after renal allotransplantation in man. Adhesion molecules such as intercellular adhesion molecule (ICAM) and MHC antigens are thought to be important in the induction and amplification of rejection. Therefore, we studied the ICAM-1 and MHC expression on cultured PTEC after CMV infection or stimulation with cytokines. Class I and β 2-microglobulin (β 2M) antigen expression was increased by all tested cytokines (α -interferon [α -Inf], γ -interferon [γ -Inf], interleukin-1 α [IL-1 α], tumor necrosis factor- α [TNF- α]). ICAM-1 expression was induced by γ -Inf and TNF- α , class II expression was induced only by γ -Inf. Small concentrations of α -Inf inhibited the γ -Inf induced class II expression. PTEC infected with CMV displayed increased class I, β 2M and ICAM-1 expression while class II expression was not induced. Immunoprecipitation studies revealed no class I antigens encoded by the CMV genome. CMV infection did not inhibit the γ -Inf induced expression of class II molecules. There was no evidence that the increased antigen expression after CMV infection was due to soluble factors such as α -Inf or β -Inf. Our findings suggest that CMV infection may trigger rejection by directly increasing the cell surface expression of adhesion molecules and MHC antigens within the transplanted organ.