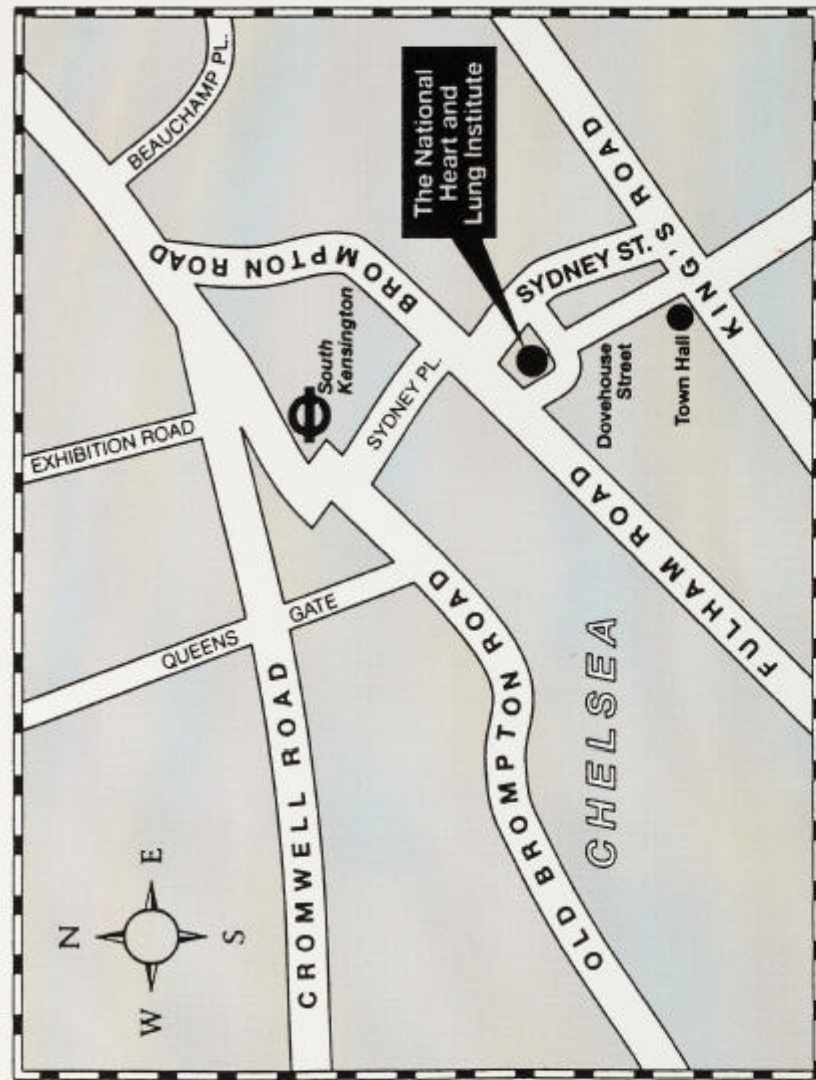


HOW TO GET THERE



THE BRITISH TRANSPLANTATION SOCIETY



NATIONAL **HEART & LUNG** INSTITUTE

POSTGRADUATE EDUCATION CENTRE
LONDON

20th & 21st October 1992

10. General Secretary's Report

The Secretary reported that there were currently 504 members of the Society (excluding the 13 applicants who had been approved at the present meeting).

Thirty one applications for bursaries to attend the next meeting of the International Transplantation Society had been received. These were being considered by a sub-committee and it was anticipated that awards would be made to most of the applicants. In addition, it had been decided to award a number of bursaries for members wishing to attend the Third Basic Sciences in Transplantation Immunology Meeting which will be held in Big Sky, Montana, during 1993. The Secretary will invite applications for these bursaries in due course.

A total of sixty six abstracts had been submitted for the present meeting of the Society. Each of these had been assessed by 4 referees and 38 (57%) had been selected for presentation.

11. Any Other Business

Mr D McWhinnie reported that the "Carrel Club" for Trainees in Transplantation had enjoyed a very successful meeting on 20th April, 1992. A further symposium entitled "Training in Transplantation" was being planned for October 1992 with contributions from the Royal Colleges, the BTS and the Surgical Advisory Committee. All those interested were invited.

There was no other business and the meeting was closed.

INVITED LECTURE

ACCELERATED CORONARY SCLEROSIS - WHERE ARE WE NOW?

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Accelerated coronary sclerosis is currently the main cause of late mortality and morbidity after cardiac transplantation and affects up to 40% of patients by 5 years. Antibody and cell mediated damage to the endothelium of the epicardial and large intra-myocardial vessels is thought to be the initial event followed by smooth muscle cell proliferation and finally lipid deposition with formation of typical atheromatous plaques in the late phases of the disease. The molecular and cellular events responsible for the initial damage is currently under intensive investigation with several exciting new developments which could have diagnostic and therapeutic implications. Increased coagulation and dyslipidaemia particularly an increase in Lp(a) appears to be associated with and predictive of coronary disease in these patients. Clinical diagnosis is still a problem because of the virtual absence of angina due to cardiac denervation and the unreliability of non-invasive tests in these patients. Quantitative angiography, intravascular echo and tests of endothelial function provide valuable information. Several avenues for prevention include matching, better immunosuppression with less use of steroids as well as several classes of drugs targeted to influence the initial stages of the disease. Further research into the basic mechanisms of this disease is required to maximise the benefit of cardiac transplantation.

INVITED LECTURE

BILATERAL LUNG TRANSPLANTATION - PROCEDURE OF CHOICE FOR SEPTIC LUNG DISEASE

J.H. Dark

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A consecutive series of bilateral lung transplants (BLT), with hilar rather than mediastinal anastomoses and preservation of the recipient's own heart, have been carried out since August 1990.

There were 11 patients (3 males), with an age range of 15 to 51 years (mean 32 years). Diagnoses included cystic fibrosis (CF) (7), other bronchiectasis (3), and one patient had emphysema with a major infective component. Ten had the operation through the transverse "clamshell" incision, and one patient had separate lateral thoractomies. Nine had varying periods (up to 120 minutes) of cardiopulmonary bypass during the implantation, and it is now our routine to use this for the left lung. All received Aprotinin perioperatively.

There was one early death, in a patient who in retrospect was septicemic at the time of transplant. One patient died of CMV pneumonitis at 44 days, having initially done well and left hospital. Two patients had biopsy proven obliterative bronchiolitis and one died of this at 11 months. The 9 survivors are however entirely well and fully rehabilitated.

Advantages over combined heart and lung transplantation (HLT) are surgical and physiological. Despite the high proportion of CF in patients, including several with previous pleurodesis, post-operative blood loss exceeded 1000 ml in only one patient and nobody required re-exploration for bleeding. The innervated recipient heart is never ischaemic and the post-operative haemodynamics have been excellent. That heart will obviously not suffer from accelerated coronary disease, a process which is now emerging as a cause of late death in HLT recipients.

When compared to HLT patients with similar pre-op pathology BLT patients have similar FEV1 and 6 minute walk times, but markedly better exercise performances measured by percent predicted maximum oxygen consumption on bicycle ergometry.

We feel that BLT should be the procedure of choice for patients with septic lung disease.

INVITED LECTURE

DONOR ORGAN PRESERVATION

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Heart/lung transplantation is now a realistic form of treatment for end-stage pulmonary vascular and parenchymal lung disease. Few donor organs are available however, and distal organ procurement is essential. Several techniques are used to preserve the lung. All have been successful but none is ideal. All are associated with early organ dysfunction and possibly also to late rejection. Ischaemia reperfusion injury and repair have been investigated *in vitro* and *in vivo*. *In vivo*, extensive pulmonary capillary remodelling occurs at low temperature together with pericapillary oedema. On rewarming, these changes reverse rapidly and there is no evidence of additional structural injury to the capillary bed during rewarming. By contrast, however the intense constriction which can develop in small muscular pulmonary arteries does not always reverse on rewarming and the small airways do show evidence of reperfusion injury. *In vitro*, both endothelial and smooth muscle cells show extensive depolymerisation on cooling of cytoskeletal and contractile proteins which repolymerise on rewarming in the order in which they depolymerised. Changes in cell composition are associated with changes in responsiveness to receptor mediated stimuli as shown by changes in cytosolic calcium concentration. The vulnerability of different receptors and their signal transduction mechanisms differ. At least *in vitro*, the type of preservation fluid in which the cells are cooled influences recovery. The clinical implications for these findings will be discussed.

INVITED LECTURE

RELEVANCE OF HISTOCOMPATIBILITY MATCHING AND CROSSMATCHING IN HEART TRANSPLANTATION: TOWARDS A PROSPECTIVE TRIAL

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Selection of recipients for heart transplantation has never included HLA antigen matching in any centre worldwide. When patients have high panel reactivity due to preformed HLA specific antibodies a prospective lymphocytotoxic crossmatch is sometimes performed but transplantation may proceed even when the crossmatch is positive. The outcome of such transplants is poor, as is that of repeated heart transplantation.

Several centres in the USA and Europe have found a positive correlation of improved transplant survival and fewer rejection episodes with HLA antigen matching in patients who, by chance, received matched hearts. The data is from retrospective studies when the degree of matching achieved from random allocation with respect to HLA match is poor.

The criticism that HLA typing of heart donors is not logistically possible no longer stands since routine rapid serological techniques giving reliable results are now available. In addition HLA-DR typing at the DNA level within a 2-3 hour period is now possible.

There are strong arguments in favour of prospective HLA matching for allocation of hearts to recipients and this should always be the case when "domino donor" organs become available.

PAPER 1

POSTDISCHARGE MORBIDITY IS INCREASED IN CYTOMEGALOVIRUS MISMATCHED HEART TRANSPLANT RECIPIENTS

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The significance of complications related to cytomegalovirus infection following heart transplantation is still unclear. We examined the morbidity mortality of CMV mismatched heart transplant recipients in our programme. Between April 1986 and July 1991, 283 orthotopic heart transplants (HTx) were performed at Papworth Hospital. All patients received triple immunosuppressive therapy (Cyclosporin, Azathioprine, Prednisolone). Acute rejection episodes were treated with pulsed doses of intravenous Methylprednisolone. There were 36 hospital deaths (12.7%). Patients were divided in groups according to their donor and recipient preoperative CMV status: MISMA(R-/D+) N=43, NON-MISMA (all others) n=204, divided in two subgroups: MATCH+(R+/D+or-) n=160 and MATCH-9D-/R-) n=44. There was no statistically significant difference in donor or recipient variables. 3 year actuarial survival was 69.7% SE 7.51 in MISMA group and 79.5% SE 3.17 in NON-MISMA group (p<0.1). Infection related early deaths were more frequent in MISMA group (1 year survival 90.5% SE 4.51 MISMA group versus 96.4% SE 1/31 in NON-MISMA group, p<0.05). Rejection and non-CMV infection rates were comparable. In MISMA group 27 (62.9%) patients were ever readmitted, compared with 68 (33.3%) in NON-MISMA group (p<0.0001). Number of readmissions per patient were higher in MISMA group (2/patient vs 1.68/patient in NON-MISMA), as well as mean length of stay (16.5 days in MISMA vs 12.2 days in NON-MISMA, p<0.05). Number of additional hospital days post discharge were 24.2/1000 patient days in MISMA group and 6.55/1000 patient days in NON-MISMA group (p<0.0001). No significant difference between the two NON-MISMA subgroups could be shown. These results indicate increased morbidity in CMV mismatched heart transplant recipients in the medium and long term. Repeated readmissions are associated with poorer quality of life, as well as with increased costs. CMV matching should be considered as an important criterion in the choice of the heart transplant recipient.

PAPER 2

THE EFFECT OF THE LYMPHOCYTOTOXIC CROSSMATCH RESULT AND PANEL REACTIVE ANTIBODY STATUS ON HEART AND HEART-LUNG TRANSPLANT SURVIVAL

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Retrospective data from 699 cardiac and 290 heart-lung transplants has been analysed to determine the importance of the crossmatch result and Panel Reactive antibody (PRA) status on graft survival.

Donor reactive crossmatching was performed for 636 cardiac transplants. One year actuarial survival for a negative crossmatch (n=580) was 73.4% compared to 56.5% for the positive crossmatch recipients (n=56) $p=0.0014$. T and B lymphocyte crossmatching has been performed for 289 cardiac transplants, one year survival figures are negative (n=258) 73%, B cell positive (n=24) 62%, T cell positive (n=7) 28% ($p=0.001$). A PRA frequency above 51% was associated with a decrease in graft survival but this was not statistically significant.

Donor reactive crossmatching was performed for 283 heart-lung transplants. One year actuarial survival figures were negative (n=251) 61% and positive (n=32) 50%, $p=0.02$. T and B cell crossmatch results for 108 heart-lung transplants also showed good correlation with graft survival: negative (n=100) 68%, B cell positive (n=4) 75% and T cell positive (n=4) 0% $p=0.0047$. A significant correlation was seen between PRA status and poor graft survival in heart-lung recipients. One year actuarial survival figures were PRA negative = 64%, PRA 11-50% freq. = 37% and PRA 51-100% freq. = 40% $p=0.008$.

In conclusion, preformed lymphocytotoxic antibodies directed against donor lymphocytes are associated with a significant decrease in graft survival. Patients likely to have a positive crossmatch should be identified, and only transplanted when it is possible to perform a prospective crossmatch.

PAPER 3

ASPIRIN PROPHYLAXIS DOES NOT PREVENT CORONARY OCCLUSIVE DISEASE DEVELOPMENT AFTER CARDIAC TRANSPLANTATION.

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Coronary occlusive disease (COD), the most important long term complication after cardiac transplantation, may be a response to immunologically mediated vascular injury. Aspirin therapy has been proposed to reduce its incidence and clinical effects. We reviewed its use in our programme. Between March 1982 and June 1992, 399 patients had cardiac transplants and received cyclosporin based immunosuppression. Patients received 150mg of aspirin per day until 1988 (ASP+ Group). Prophylaxis was not prescribed to new patients after this date to assess its effect (ASP- Group). Patients were defined as COD+ if there was any evidence of coronary disease on angiography and COD- if arteriography was normal. 247 of the 399 patients have undergone coronary angiography at 2 years and 85 at 5 years. At 2 years, 39 patients (16%) were COD+ (ASP+ 28/ASP- 11) and 208 were COD- (ASP+ 146/ASP- 62) (Not significant-NS). Actuarial freedom from COD at 2 years was ASP+ 83% SEM 3%/ASP-79% SEM 5% and at 5 years was ASP+ 69% SEM 4%/ASP- 71% SEM 7% (NS). In the ASP- Group at 2 years, there were 10 graft failures: 6 from COD, 2 CVA's, 1 infection and 1 rejection. In the ASP+ Group at 2 years there were 31 graft failures: 16 from COD, 4 strokes, 4 infections, 1 rejection, 4 from malignancy, 2 from pulmonary hypertension, (COD deaths ASP+ vs ASP- NS). The actuarial survival at 2 years was ASP+ 97% SEM 1.3%/ASP- 97% and at 5 years was ASP+ 86% SEM 3%/ASP- 87% SEM 6.0% (NS). Other potential variables associated with COD development in our programme (preoperative diagnosis, donor and recipient age, lipid profile and gender) did not differ between the two aspirin groups. Aspirin prophylaxis treatment does not prevent the development of COD or reduce the risk of death after cardiac transplantation.

PAPER 4

PHARMACOKINETICS OF CYCLOSPORIN DURING THE FIRST THREE MONTHS AFTER CARDIAC TRANSPLANTATION

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Currently, cyclosporin (CYA) dosage in patients following cardiac transplantation is carefully tailored by measurement of serum trough levels. Nonetheless, progressive deterioration in renal function remains a major complication of long-term CYA administration. In 15 survivors of orthotopic cardiac transplantation, each on a standard immunosuppression regimen of CYA, azathioprine and prednisolone, serum CYA concentrations were measured, using whole blood radioimmunoassay, at 10 time intervals during the 12 hours following the patient's standard oral dose of CYA. Such CYA 'profiles' were performed 3 weeks and at 3 months after transplantation. From the profiles were computed maximum CYA concentration (C_{max}), time from dose to C_{max} and area under the time-concentration curve (AUC) which, when divided by the CYA dose, provided a measure of the overall pharmacokinetics for individual patients at a given time.

C_{max} varied from 387ug/l to >1600ug/l at 3 weeks and from 603ug/l to >1600ug/l at 3 months following transplantation. T_{max} varied from 120min to 720min at 3 weeks and from 30min to 720 min at 3 months. There was weak correlation between C_{max} at 3 weeks and at 3 months ($r=0.5$, $p=0.06$) and none for T_{max}. There was no correlation between CYA trough levels and AUC. However, there was strong correlation when 3 week AUC/CYA dose was compared with 3 month AUC/CYA dose ($r=1.17$, $p<0.0001$).

CYA trough levels appear to be a poor measure of overall exposure to CYA during twice daily dosage compared to AUC. The finding of a marked degree of consistency in handling of CYA in patients at the two time points after transplantation, suggests that CYA profiles might provide a better means of controlling dosage. It remains to be seen whether such improved control will have any influence upon the development of renal dysfunction.

INVITED LECTURE

LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS IN INFLAMMATION

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The adhesion of leukocytes to endothelium is the first step in their migration into the tissues in inflammation and may be a rate-limiting step in the evolution and progression of inflammatory responses. Leukocyte-endothelial cell (EC) adhesion is dependent upon interactions between leukocyte adhesion molecules and ligands expressed by EC. Thus the number and type of leukocytes entering the tissues in different forms and stages of inflammation is related to (a) the profile of integrins and other adhesion molecules expressed by leukocytes of different lineage, (b) the responsiveness of leukocytes to lineage-selective chemoattractants (eg interleukin-8, macrophage chemotactic and activating factor) and (c) the expression of adhesion molecules on endothelium. The talk will focus on the mechanisms by which adhesion molecule expression by EC is regulated by rapidly-acting mediators (histamine, thrombin) and by the delayed effects of cytokines such as interleukin-1, tumour necrosis factor, interleukin-4 and interferon gamma. Evidence will be presented that the molecules identified using tissue culture technology are relevant to inflammation in the clinical setting.

INVITED LECTURE

REPROGRAMMING THE IMMUNE SYSTEM WITH MONOCLONAL ANTIBODIES

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It would be highly desirable to find a means of giving short-term therapy to establish transplantation tolerance to bone-marrow and organ grafts. Current drug immunosuppression tends to be relatively non-specific and consequently the whole immune system is penalised for the unwanted activities of just a minority of lymphocytes. Studies in rodents have shown that short courses of CD4 and CD8 antibodies can guide the immune system towards long-term tolerance by amplifying natural regulatory processes. This can be achieved with minimal T-cell destruction and operates through peripheral T-cell (ie post-thymic) tolerance mechanisms. These mechanisms have been analysed and presently include tolerance by "helplessness", anergy and dominant suppression. A fuller understanding of mechanisms may enable more rationale intervention to achieve transplantation tolerance and for the reversal of autoimmunity.

INVITED LECTURE

IMMUNOSUPPRESSIVE MACROLIDES

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The two immunosuppressive macrolides, FK 506 and rapamycin, are powerful inhibitors of T-cell function and more potent suppressants of cell-mediated immunity than cyclosporin A (CsA). Although structurally similar, FK 506 and rapamycin inhibit T-cell proliferation at different stages in the cell cycle and act by distinct molecular mechanisms. In a fashion analogous to the binding of CsA to cyclophilin, FK 506 is bound intracellularly to a specific, cytosolic binding protein or "immunophilin" (FK binding protein or FKBP). The FK 506-FKBP complex (but not FK 506 alone) then binds to and inhibits the phosphatase activity of calcineurin. This activity of the FK 506-FKBP complex has been linked to inhibition of dephosphorylation of the cytoplasmic component of a nuclear gene regulatory protein, nuclear factor of activated T cells (NF-AT). Dephosphorylation of NF-AT is essential for its translocation from the cytoplasm to the nucleus, where it regulates the transcription of the interleukin-2 (IL-2) gene. Recent evidence indicates that, although FK 506 inhibits production of IL-2 and other T_{H1} cell cytokines (such as IFN-gamma), it does not inhibit production of IL-10 (a T_{H2} cell product). Since IL-10 suppresses T_{H1} cytokine production, this may in part explain the immunosuppressive action of FK 506. Although rapamycin also binds to FKBP, the FKBP-rapamycin complex fails to inhibit calcineurin activity and does not suppress cytokine production. Rapamycin however (unlike FK 506), inhibits strongly the responses of T-cells to IL-2 and other cytokines.

FK 506 is effective in prevention and reversal of organ allograft rejection in all species tested. In human cardiac transplantation, its steroid-sparing activity, low incidence of hypertension and lack of hirsutism are notable benefits, especially in children. Significantly, low doses of rapamycin act synergistically with subtherapeutic doses of CsA to prolong cardiac allograft survival in the rat. Phase I clinical trials with rapamycin have recently begun.

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PAPER 5

ANTI-ENDOTHELIAL ANTIBODIES: THE ACCELERATING FACTOR FOR TRANSPLANT-ASSOCIATED CORONARY ARTERY DISEASE

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The most serious long-term complication of heart transplantation is the development of accelerated coronary artery disease (CAD). Although the precise aetiology of the disease is unknown, immune mechanisms have been implicated. We have previously shown a strong positive correlation between the presence of anti-endothelial cell antibodies and the development of CAD. Using the techniques of SDS-PAGE and Western immunoblotting, we found that 15/21 patients with angiographic evidence of CAD had antibodies reactive with a doublet of polypeptides of approximately 60 and 62 kD. Only 1/20 non-CAD patients had antibodies of this specificity.

Additional studies have shown that these polypeptides are associated with the endothelial cell membrane. The temporal pattern of the appearance of the antibodies reactive with these polypeptides has been investigated in 9 CAD patients and 10 non-CAD patients. Serum samples were analysed at 1, 3, 6, 9, 12 and 24 months after transplantation. Six out of 9 CAD patients produced antibodies against the 60 and 62 kD doublet within the first 6 months following transplantation and once produced, they could be detected in all subsequent sera studied. Only 2/10 non-CAD patients produced these antibodies and in both cases these could only be detected in a single serum sample.

The production of anti-endothelial cell antibodies by a group of non-transplant patients with angiographic evidence of CAD has also been investigated. Although 26/51 of the patients studied were found to have circulating anti-endothelial cell antibodies, only 3/51 of the patients had antibodies reactive with the 60 and 62 kD proteins. It appears therefore that the production of antibodies against these proteins is specific for transplant-associated CAD and may be an accelerating factor causing rapid disease progression.

PAPER 6

PRIMARY PROLIFERATIVE ALLOGENEIC RESPONSES OF CD4⁺ AND CD8⁺ T CELLS TO HUMAN VASCULAR ENDOTHELIAL CELLS

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Endothelial cells (EC) express MHC class II molecules in response to IFN- γ and provide costimulatory signals to CD4⁺ T cells. To examine EC immune accessory function further, we examined the proliferative response of allogeneic CD4⁺ and CD8⁺ T cells to EC obtained from human umbilical veins. CD4⁺ T cells proliferated in response to IFN- γ pretreated EC (1000 IU/ml x 72 hours) which expressed class II molecules, but not to untreated EC. CD8⁺ T cells proliferated to MHC class I molecules expressed on both untreated and IFN- γ treated EC. Combined populations of CD4⁺ and CD8⁺ T cells showed synergistic, rather than additive, responses to both untreated and IFN- γ treated EC. Induction of MHC class II molecules by CD8⁺ T cells as an explanation for this synergy seems unlikely since only CD16⁺ T cells induced class II expression on EC.

In summary, CD4⁺ and CD8⁺ T cells recognise and proliferate to allogeneic MHC class II molecules expressed by EC. CD4⁺ and CD8⁺ responses are synergistic under the conditions tested but the synergism did not appear to be due to induction of MHC class II antigens on EC by CD8⁺ T cells.

PAPER 7

ABILITY OF ENDOTHELIAL CELLS TO CAUSE DIRECT ALLO-STIMULATION OF PURIFIED T CELL POPULATIONS.

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The role of endothelial cells in stimulating the anti-allograft response is of considerable interest. Previous studies have reported that purified human T cells give a proliferative response when cultured with passaged Human Umbilical Vein Endothelial Cells (HUVEC), but studies to date have not rigorously excluded the possibility that contaminating monocytes or dendritic cells in the responder or stimulator populations respectively were causing the response.

Here we have used positive selection on antibody coated Dynabeads to produce CD4 and CD8 T cells which were 99% pure by FACS analysis. These cells were functionally depleted of monocytes as demonstrated by their inability to proliferate in response to OKT3, PHA or act as stimulator cells in a Mixed Lymphocyte Response. HUVEC were collected at the fifth passage and immunocytochemical analysis revealed contamination by leukocytes to be less than 1/10,000. When cultured with cytokine (γ IFN, IL-1 β and TNF α) treated irradiated HUVEC or non cytokine treated unirradiated cells for six days CD4 and CD8 T cells gave unequivocal proliferative responses. Untreated irradiated HUVEC were significantly less efficient at allopresentation.

In conclusion purified HUVEC can cause direct allostimulation of T cells but this response requires active participation by live endothelial cells.

PAPER 8

THE INFILTRATION OF SERINE PROTEASE+ CELLS IN HEART TRANSPLANTS

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Leukocytes are commonly observed in heart transplants, but the degree of their cellular infiltration often correlates poorly with either the immunological status or the eventual survival of the graft. Consequently, the differentiation between donor-reactive infiltration and non-specific cellular sequestration would enhance the early diagnosis of rejection. In the present study we examined the expression of granzyme A, a functional marker of cytotoxicity, in a rat heart transplant model. Granzyme A is a serine protease found only in activated cytotoxic T cells and NK cells. Its expression correlates well with the episodes of cell killing, and its protein product has been shown to induce target DNA fragmentation in an apoptotic reaction. Using *in situ* hybridization, we measured the expression of granzyme A in rat heart transplants either untreated or immunosuppressed with cyclosporine (CsA) or anti-CD4 monoclonal antibody (OX-38). The frequency of granzyme A-expressing cells was compared to 1) the CD4:CD8 ratio of infiltration; 2) the histopathology grading; 3) the palpation grading of the graft contraction. Results showed that granzyme A was expressed 10-folds higher in rejecting allografts as compared to syngeneic grafts. The frequency of granzyme A+ cells in rejecting allografts was also three-folds higher than that found in indefinitely tolerized allografts immunosuppressed with CsA or OX-38. Furthermore, in donor-recipient combinations where the immunosuppressive regimens fail to induce graft tolerance, the level of granzyme A was as high or higher than that observed in untreated allografts. The specificity and the early expression of granzyme A shows strong potential as a clinical diagnostic marker for acute cellular rejection. We are currently conducting studies to examine that possibility.

PAPER 9

A PROSPECTIVE ANALYSIS OF CYTOKINE PROFILES IN CARDIAC ALLOGRAFTS

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Cytokines are thought to play a central role in the inflammatory and allospecific components of allograft rejection, particularly the acute rejection episodes which occur early after transplantation. Consecutive endomyocardial biopsies collected from cardiac allografts were analysed for expression of genes for inflammatory and immunoregulatory cytokines by the polymerase chain reaction (PCR). RNA extracted from each biopsy was converted to cDNA and subjected to PCR amplification, using primers specific for IL1 β , IL2, IL4, TNF α and TNF β . Cytokine profiles have been performed on all biopsies collected during the first four months (0-4m) post-transplantation from a total of 13 cardiac allograft recipients. A total of 85 biopsies were analysed, 37 of which were designated histologically as mild or moderate rejection by the Billingham criteria.

	Number of Positive Biopsies	
	Rejecting (n=37)	Non-rejecting (n=48)
TNF β	16 (43%)	9 (19%)
TNF α	8 (22%)	4 (8%)
IL2	13 (33%)	2 (4%)
IL4	10 (27%)	2 (4%)
IL1 β	3 (8%)	7 (15%)

Thus IL2, IL4, TNF β and TNF α are most commonly associated with rejection episodes. The presence of cytokines in non-rejecting biopsies, particularly IL2, was often correlated with infection, eg CMV. All biopsies are currently being analysed for IL10, a cytokine which has the ability to suppress the production of IL2 and TNF β (ie T_H subset) and also monokine production. Detection of intragraft cytokines may lead to an improved understanding of immunosuppressive therapies.

PAPER 10

IN VITRO CHARACTERISTICS AND CLINICAL RELEVANCE OF PRIMED ALLOREACTIVE CTLs IN HEART AND RENAL TRANSPLANTATION.

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Previous studies in highly sensitized patients showed that cytotoxic T cell precursor (CTLp) against HLA class I antigens, towards which patients had formed antibodies (not-acceptable mismatches, NAM), were similar to those against HLA antigens, towards which no antibodies were present (acceptable mismatches, AM). Limiting dilution assays performed in the absence or presence of antibodies against CD8 showed that CTL directed against NAM were significantly less inhibited by anti-CD8 compared to those directed against AM. Those "primed" CTL could also be distinguished from the more "naive" CTL on the basis of their resistance to cyclosporine A (CsA). In contrast to CsA, therapeutic concentrations of FK506 were able to inhibit both "naive" and "primed" CTLs. A clinical relevance of these "primed" cells is suggested by the observation that in heart transplantation a good anti-CD8 inhibition of donor-reactive graft infiltrating CTLs coincides with good graft function, while in the presence of CTL resistant to anti-CD8 rejection occurs.

Furthermore in renal patients, transplanted across a positive historical cross-match, an association was found between the presence of CsA resistant donor specific CTLs with rejection and graft loss, while the presence of CsA sensitive CTLs was correlated with good graft function.

PAPER 11

OUTCOMES OF RENAL TRANSPLANTATION ACROSS POSITIVE HISTORICAL CROSSMATCHES

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We investigated the influence of positive historical crossmatches on the outcomes of 138 patients receiving cadaveric transplants.

16 received second and 4 third grafts. All patients received prednisolone, azathioprine and cyclosporin. ATG was given prophylactically to patients with PRA >60% and previous graft failures. Acute rejections were treated with methylprednisolone and ATG for steroid resistant rejections.

T- and B-cell crossmatching was performed by standard NIH technique and B-cell crossmatching by two colour fluorescence technique. DTT (30 minutes pre-incubation) crossmatching (defining IgG or IgM antibodies) was also carried out. Historical sera are sera collected >6 months before transplantation.

81(59%) had negative current and historical crossmatches and 57(41%, 10% were IgG positive) had negative current but positive historical crossmatches. Positive historical crossmatches (IgG or IgM) had no effect on 1 year graft function, once graft failures were excluded. However, increases in the number of rejection ($p < 0.001$) and patients with >1 rejections ($P < 0.05$) were seen in the IgG positive group, as was the number of immunological graft failures ($p < 0.04$).

In conclusion, IgM positive historical crossmatches appeared to have no effect on 1 year graft function, the number of rejection episodes or early immunological graft failures. In contrast, IgG positive historical crossmatches appeared to have a significant effect on the number of rejection episodes and early immunological graft failures.

PAPER 12

SURVEY OF PATIENT SELECTION FOR CADAVERIC RENAL TRANSPLANTATION

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Not all dialysis patients are selected as suitable for cadaveric renal transplantation. To assess the impact of various adverse features on the likelihood of selection we circulated a questionnaire with 20 case histories of potential transplant recipients. 190 UK nephrologists and transplant surgeons were asked if they would offer transplantation to each patient (a) at present and (b) if an adequate supply of donor kidneys were available. The response rate was 75%. The median (range) number of patients selected was (a) 12 (4-20) and (b) 15 (8-20). For 16/20 patients the selection rate was significantly higher in (b) than (a), with the largest differences for non-compliant ((a) 47% (b) 87%) and elderly patients ((a) 33% (b) 72%). There were significant differences in selection rates amongst the groups of doctors surveyed. Patients were more likely to be selected by nephrologists working in Renal Units with transplantation than by either transplant surgeons or nephrologists working in the Units without transplantation.

This survey highlights the constraining effect of the shortage of kidneys on the selection of patients for transplantation. The wide variation in responses suggests that some suitable patients may still be denied the opportunity of transplantation.

PAPER 13

THE CLINICAL SIGNIFICANCE OF ALLO SPECIFIC ANTIBODIES AGAINST ENDOTHELIAL CELLS DETECTED WITH AN ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC) ASSAY FOR VASCULAR REJECTION.

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THE CLINICAL SIGNIFICANCE OF ALLO SPECIFIC ANTIBODIES AGAINST ENDOTHELIAL CELLS DETECTED WITH AN ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC) ASSAY FOR VASCULAR REJECTION.

F.J.van der Woude, B.A.Yard*, F.Claas*, J. Thorogood*, J.A.Bruyn*, M.E. Paape*, L.A. van Es, J.H. van Bockel*, M.Kooymans-Coutinho* and M.R.Daha*, Department of Nephrology, University of Leiden, Leiden, The Netherlands.

Vascular or chronic rejection is thought to be mediated by antibodies bound to endothelial cells (EC). However, relatively little is known about the target antigens recognized on EC. In this study serum samples of 64 consecutive renal transplant patients were analysed for ADCC activity against a panel of HLA typed human umbilical vein endothelial cells (HUVEC).

Serum samples were obtained immediately before transplantation and 1 week, 1 month and 1 year after transplantation. The results were evaluated in the context of classical tests to measure donor-specific immunity and related to parameters of presentation, graft survival and histology. There was a highly significant correlation between ADCC positivity and vascular rejection ($p=0.015$). ADCC positivity did not correlate with primary non function, the number of rejection episodes or with interstitial rejection. Graft survival was better in the ADCC negative than in the positive group ($p=0.0004$). Although there was a difference in graft survival between the high sensitized (PRA > 85%) and low sensitized (PRA < 85%) group, this did not reached statistical significance. Anti EC-ADCC positivity was not related to anti lymphocyte ADCC or positive monocyte crossmatches. From these data we conclude that the anti-EC ADCC has a higher sensitivity than PRA or lymphocyte/monocyte crossmatches for graft survival and vascular rejection. Under the current "Eurotransplant" organ exchange policy, antibodies against this newly defined type of alloantigens seem to have greater clinical relevance for graft survival than anti-HLA antibodies.

PAPER 14

CYTOMEGALOVIRUS INFECTION AS AN EXPLANATION OF ANTI-EPITHELIAL ANTIBODIES IN RENAL TRANSPLANTATION

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Several studies have addressed the possible importance of antiepithelial antibodies in kidney transplantations using the A549 cell line. Our study throws new light on this problem using a newly developed ELISA method.

Sera from 128 kidney transplant patients were tested for IgM antiepithelial antibodies prior to transplantation; only 4 sera were positive (3%). 102 patients were followed up post transplantation; sera were collected at 2, 6 and 12 weeks and at the suspected rejection episodes. All samples were tested for CMV IgM antibodies. 15 patients developed anti A549 IgM antibodies, but there was no correlation with acute rejection. Antiepithelial antibodies showed no binding to sections of normal kidney or biopsies of rejected kidneys.

11/102 patients were positive for anti CMV IgM antibodies while 15/102 were positive for IgM antiepithelial antibodies. In 9 cases both antibodies were found, which indicated a highly significant association ($p<0.001$). Although it was difficult to identify the cytomegaloviral protein by Western blot analysis of the cell lysate, PCR analysis showed the presence of the CMV DNA in A549 cells of several batches from different sources.

These data suggest that the A549 cell line may be infected with CMV virus and anti A549 antibodies may be directed in part against the viral antigens.

PAPER 15

THE DIFFERING ABILITIES OF CD45RC⁺ CD4 T CELL SUBSETS FROM BLOOD TRANSFUSED DONORS TO REJECT CARDIAC ALLOGRAFTS IN ATHYMIC NUDE RATS

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We have investigated CD4 T cell subsets in blood transfused (BT) rats for their ability to induce cardiac allograft rejection after adoptive transfer. Intact PVG rats (RT1^b) rejected heterotopic DA strain (RT1^a) heart grafts in 7-8 days. Graft destruction was significantly delayed (MST >30 day; 20% no rejection) in PVG recipients injected with 1.5 ml DA blood 7, 14, or 30 days before. To investigate T cell activity in BT rats, CD4 T cells were purified from thoracic duct lymphocytes and separated into CD45RC⁺ and CD45RC⁻ subsets by staining with mAb OX22 (anti-CD45RC). Since the CD45RC⁻ subpopulation contains both Thyl⁺ recent thymic emigrants (virgin) and Thyl⁻ antigen-experienced T cells, Thyl⁺ cells were removed. Athymic PVG nude rats were pre-grafted with DA hearts prior to syngeneic CD4 T cell transfer. Transfer of the CD45RC⁺ subset (20x10⁶) from non-transfused or third-party (BN strain blood) transfused donors induced DA heart graft rejection approximately 12 or 25 days later, respectively. CD45RC⁺ T cells from 7-day BT donors showed reduced allograft activity (MST 45 days). In contrast, the CD45RC⁻ subset from non-injected donors was unable to induce allograft rejection. However, as few as 2x10⁶ CD45RC⁻ CD4 T cells from 7-day BT donors rejected DA cardiac allografts in the nude recipients (MST 20 days). These results are discussed in relation to the differing lymphokine profiles associated with the different CD45RC subsets.

PAPER 16

INDIRECT PRESENTATION OF ALLOGENIC MHC CLASS I PEPTIDES IN A MODEL OF CARDIAC ALLOGRAFT PROLONGATION

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Cardiac allograft prolongation can be achieved by the preoperative injection of recipient type L cells transfected with donor type class I MHC molecules. We have shown that these L cells traffic to the spleen and that preoperative splenectomy abrogates this effect. We wished to determine whether class I MHC alloantigen is presented directly by the transfected L cells or indirectly as peptide by recipient splenic cells. Two weeks after IV injection with Kb-transfected L cells, spleens from C3H mice (H-2k) were harvested and low density adherent cells (Lowdacs) isolated according to the dendritic cell enrichment protocol of Steinman. These were used as stimulators in a primary mixed lymphocyte reaction (MLR) against naive C3H splenic T cells. Strong proliferation was seen above control levels from mice injected with L cells transfected with Kb but not from those transfected with an irrelevant non-MHC gene. The following results suggest indirect presentation of Kb derived peptide: 1) Transfected L cells alone did not stimulate naive T cells. 2) When L cells were subjected to the Lowdacs isolation protocol, no cells were recovered. 3) Contamination of the harvested splenocytes with transfected L cells did not significantly alter the counts. 4) Adding monoclonal antibody directed at the responding population during the MLR showed that anti-CD4 but not anti-CD8 antibody significantly reduced the counts. 5) Negative selection by antibody mediated complement lysis of the stimulators showed the stimulating cell to be class II positive and not a T cell, B cell or macrophage. The use of dendritic cell antibodies will allow a positive characterisation in the near future. In summary, we have demonstrated indirect presentation of a class I MHC alloantigen by host class II positive splenocytes in a model of cardiac allograft prolongation, the first reported instance of indirect MHC presentation in immunologic tolerance.

PAPER 17

TREATMENT OF SEVERE DIABETES MELLITUS FOR MORE THAN ONE YEAR USING A VASCULARIZED HYBRID ARTIFICIAL PANCREAS

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We report the successful application of a hybrid artificial pancreas for the treatment of severe diabetes mellitus induced by total pancreatectomy in two dogs (the first one-year successes in a 65 dog allo/xeno-graft series). The device consists of a coiled ultrafiltration membrane (nominal molecular weight cut-off 80 kDa), surrounded by an annular islet chamber and contained within an acrylic housing. Each end of the membrane is connected to a vascular graft with a matched internal diameter, and anastomosed to the iliac vessels. Pancreatic islets were isolated using collagenase digestion and a ficoll density gradient and suspended in a nutrient matrix for device seeding.

Control of the blood sugar was achieved for more than one year in these two animals without any immunosuppressive therapy. Although exogenous insulin (<12 units/day) was required during the latter part of the study period, removal of the devices resulted in a rapid increase in the fasting blood sugar levels and the exogenous insulin requirements (>30 units/day; $p < 0.001$ versus weeks 1-52 in both dogs). Metabolic studies, post-explant in vitro studies, and histological analyses confirmed islet cell survival and insulin production by the devices. This hybrid artificial pancreas has a clear clinical potential for islet cell transplantation without immunosuppression.

PAPER 18

THE EFFECT OF HLA MISMATCHING ON REJECTION EPISODES AFTER CARDIAC TRANSPLANTATION

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Data from 501 adult cardiac transplants has been analysed to determine the effect of HLA incompatibilities on the frequency and severity of rejection episodes within the first 3 months after operation. Patients were included if survival exceeded the 3 months after operation. Rejection was diagnosed by histological evaluation of endomyocardial biopsies. The influence of both HLA Class I (HLA-A, HLA-B) and Class II (HLA-DR, HLA-DQ) loci have been investigated.

No correlation was seen between HLA Class I mismatching and rejection. A significant effect, however, was seen with HLA-DR mismatching on the number of rejections experienced (OMM = 1.67 ± 0.27 , 1MM = 3.26 ± 0.15 , 2MM = 3.56 ± 0.16 , $p < 0.001$). The time period between operation and the first rejection was found to be decreased with increasing HLA-DR mismatch (OMM = 27.95 days ± 4.57 , 1MM 18.70 ± 1.42 and 2MM 16.35 ± 1.26 $p < 0.05$). Furthermore, the proportion of patients with no or mild rejection within 3 months of operation was seen to correlate with the degree of HLA-DR incompatibility (OMM = 54%, 1MM = 22%, 2MM = 19% $p < 0.001$).

In conclusion, we provide further evidence for a role of HLA-DR matching in cardiac transplantation. Prospective matching is now performed at our institution for adult cardiac transplants whenever logistically possible.

PAPER 19

IMMUNOGENICITY OF CORONARY ARTERIES AND THE DEVELOPMENT OF TRANSPLANT ASSOCIATED CORONARY SCLEROSIS

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Transplant associated accelerated coronary sclerosis (TX-ACS) is the most serious complication following heart transplantation, affecting 6% of our patients at one year and progressing to 17% after three years. We have characterised the antigens present on endothelium from coronary arteries at time of surgery from patients who required re-transplantation (n=4) or at post mortem (n=10) from transplant patients who died. Coronary arteries from unused donor hearts (n=4) or heart transplant recipients whose original disease did not involve the coronaries (n=8) were used as controls.

All control coronary arteries strongly expressed PECAM, ICAM-1, ELAM-1, MHC class I and DR antigen. Expression of VCAM-1, DP and particularly DQ antigen was more patchy. There was no significant difference in the expression of MHC or adhesion molecules between control arteries and those from transplant patients. Only 5/14 transplanted coronaries showed intimal cellular proliferation typical of TX-ACS. Two of these also showed evidence of intimitis as did one other specimen.

Normal coronary arteries appear to be highly immunogenic prior to transplantation. This suggests the endothelium would need little, if any, stimuli to initiate as well as be a target for an immune response ultimately leading to TX-ACS. Factors such as MHC mismatch, a high frequency of precursor cytotoxic T cells and anti-endothelial antibodies may pre-dispose the transplant recipient to the disease.

PAPER 20

PROGNOSTIC VALUE OF LUNG FUNCTION MEASUREMENTS FOLLOWING HEART-LUNG TRANSPLANTATION (HLT).

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We have studied lung function in 82 patients who have survived at least 6 months following HLT in an attempt to relate early lung function to long-term survival. Actuarial curves calculated using the life table method were used to study the relationship between lung function and long term survival. Forced expiratory volume in one second (FEV_1) was used to assess lung function. Average lung function (ALF) at any time was defined as the average of the previous 4 measurements, or the average of the previous 30 days measurements, whichever involved the greater number of readings. Patients were divided into 3 groups: those who obtained normal lung function ($FEV_1 > 80\%$ of predicted normal), those who demonstrated abnormal lung function ($FEV_1 40-80\%$ of predicted normal), and those with irreversible obstruction of airflow ($FEV_1 < 40\%$ of predicted normal). The latter group were patients with obliterative bronchiolitis (OB). Of the 82 patients who have survived longer than 6 months following surgery, 55% obtained normal FEV_1 by 3 months, 80% by 6 months and 88% by 12 months. The median time to achieving normal FEV_1 was 83 days. 71 patients obtained normal FEV_1 by 12 months and in 70% of these patients, lung function remained normal 12 months after they first obtained normal FEV_1 . 33 of the patients who had obtained normal predicted FEV_1 by 12 months subsequently reverted to the abnormal lung function group. The median time for this to occur was 33 months from the time they had first achieved normal FEV_1 . 12 of these 33 patients with abnormal lung function ultimately developed irreversible obstruction. The median time for this to occur was 18 months from the time their lung function first became abnormal. One year actuarial survival for patients from the time they first enter the normal, abnormal and irreversibly obstructed groups are 92%, 60% and 40% respectively. We conclude that patients who achieve normal lung function following HLT have a survival advantage and that deterioration in ALF into the abnormal category is associated with a much poorer prognosis. The first 6 post operative months appears to be the critical period when lung function must be maximised by close monitoring and aggressive investigation of suboptimal graft function.

PAPER 21

EVALUATION OF CYTOMEGALOVIRUS BY DEAFF TEST, POLYMERASE CHAIN REACTION AND SEROLOGY

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CMV infection causes significant morbidity and mortality after heart or lung transplantation. Three methods for the identification of CMV in the laboratory were evaluated. Blood, urine and bronchoalveolar lavage (where indicated clinically) specimens were monitored during the first 6 months following transplantation for:

- CMV early antigen by DEAFF test using commercially available antibodies;
- CMV DNA by polymerase chain reaction (PCR) using a "nested" technique;
- an increase in titre of IgM and IgG antibodies by serology.

Patients in this study included 19 heart, 3 single-lung and 8 heart-lung transplant recipients. Eleven patients (37%) were positive during the first 3 months after transplantation for CMV by one or more of these tests.

	PCR +ve	PCR -ve
DEAFF +ve	7	1
DEAFF -ve	3	19

Two of the 7 patients who were positive by both DEAFF and PCR did not have elevated IgM at the time of testing. The DEAFF test detected one sample which was not positive by PCR, while 2 samples positive by PCR were negative in the DEAFF test. However, samples remained positive by PCR for 2 weeks after initiating of gancyclovir therapy, whereas patients immediately reverted to being negative by DEAFF test.

In conclusion there was a good correlation between the PCR and DEAFF tests. Serology was less sensitive than either DEAFF or PCR as 6 patients who were positive by one or both of these tests were negative by serology.

PAPER 22

INFLUENCE OF CYCLOSPORIN ABSORPTION PROFILE ON THE OUTCOME OF RENAL TRANSPLANTATION: NEED OR LUXURY?

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Pre-dose whole blood Cyclosporin A (CyA) measurement is the established method for monitoring CyA therapy. Use of CyA absorption profiles has been advocated as the optimal method. We therefore evaluated CyA absorption profiles of renal allograft recipients in relation to graft outcome over a 5 year period.

100 patients on CyA monotherapy had a 4 hour CyA absorption profile analysis using whole blood HPLC at 3-12 months after transplant. Patients were classified as a "Fast" absorber (group I) who had a rapid rise in CyA blood concentrations peaking at 2-4 hours and "Slow" absorbers (group II) with no rise up to 4 hours. Subsequent therapeutic monitoring was done by using pre-dose whole blood CyA levels only. The groups were of similar age, sex, body weight, kidney origin and post-transplant mortality.

Gp.	n	CyA Dose		CyA Level ng/ml		Rej. No.	5 year creat.
		1 yr	5 yr	1 yr	5 yr		
I	59	7.99	5.15	250	204	1.2	246
II	41	8.91	7.40	379	220	1.4	239
p		NS	NS	0.002	NS	NS	NS

There was a significantly greater mean trough CyA level in Group II compared to group I at one year (despite similar CyA dosage) but no difference at 5 years. The outcome of renal allografts was similar in the two groups in terms of graft failure, rejection episodes and 5 year creatinine.

Pre-dose CyA measurement is therefore satisfactory for routine monitoring requirements and there would be no additional benefit from the use of CyA absorption profiles in renal allograft recipients.

PAPER 23

IS E-SELECTIN A MARKER FOR ACUTE CELLULAR REJECTION IN RENAL TRANSPLANTATION?

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E-Selectin (ELAM) is an Endothelial cell membrane glycoprotein; part of a diverse family of cellular adhesion molecules which regulate the interaction of leucocytes and vascular endothelium. They are central in the host response to foreign antigens and tissue injury. ELAM may be present in small amounts in normal tissues, but is highly inducible in areas of inflammation by cytokines such as Interleukin 1. This aids the binding and passage of leucocytes into areas of inflammation thus amplifying the host response.

We have used a highly sensitive alkaline phosphatase anti-alkaline phosphatase (APAAP) immunohistological method to detect ELAM on renal transplant biopsies, employing a mouse monoclonal antibody (British Biotechnology). Biopsies were also stained to detect lymphocyte infiltration. Sections were scored blind by one observer and then correlated with independently reported histological findings. Presence of ELAM was scored as + (present) or - (absent). Lymphocyte infiltration was scored as a percentage of the total area of the biopsy. Biopsies with less than one glomerulus were excluded.

	ELAM +	ELAM -
Acute cellular rejection	7	0
No rejection	4	14

In the group who were ELAM positive with acute cellular rejection the average lymphocyte infiltration was 25% (range 1-50%). In the ELAM negative group with no rejection the lymphocyte infiltration was 1.8% (range 0-10%).

These results show that the presence of ELAM may be a significant marker for acute cellular rejection ($p < 0.001$, Chi Squared Test) and may aid diagnosis in histologically uncertain cases.

PAPER 24

OXYGEN FREE RADICALS, AND PLATELET AND GRANULOCYTE AGGREGABILITY IN RENAL TRANSPLANT PATIENTS

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Patients with renal transplants are still at increased risk of morbidity and mortality from vascular causes. In recent years, interest has focussed on the role of microvascular factors and free radical activity in the genesis of such events.

Oxygen free radical reaction products (plasma malondialdehyde-like material), the free radical scavengers plasma thiol and red cell superoxide dismutase, and whole blood platelet and granulocyte aggregation were measured in 23 stable renal transplant patients (duration of transplantation, six months to 8 years, median 2 years), and 23 age-matched normal controls.

Malondialdehyde-like material, measured using a high performance liquid chromatography method, was significantly increased in transplant patients compared to controls (transplants, median, range: 2.54(2.09-3.66)nmol/ml; controls: 2.36(1.77-2.85)nmol/ml; $p < 0.001$). The patients also had increased red cell superoxide dismutase (transplants: 128.1(89.4-193.8)U/l/2ml red cells; controls: 95.9(62.0-132.6)U/l/2ml red cells; $p < 0.001$), and reduced plasma thiol (transplants: 428(266-496) μ mol/l; controls: 445(358-501) μ mol/l; $p < 0.05$) irrespective of plasma albumin concentration (thiol/albumin, transplants: 9.24(7.12-10.72) μ mol/g; controls: 10.34(8.52-12.43) μ mol/g; $p < 0.001$). These factors were not influenced by immunosuppressive therapy, duration of transplantation or plasma creatinine concentration.

Transplant patients had significantly higher levels of collagen-induced and spontaneous whole blood platelet aggregation compared to controls (collagen, transplants: 72(4-93)%; controls: 43(6-94)%; $p < 0.001$; spontaneous, transplants: 46(11-93)%; controls: 37(10-75)%; $p < 0.05$). There was no significant difference in ADP-induced platelet aggregation. Granulocyte aggregation was increased in patients receiving cyclosporin A (transplants, cyclosporin A (n=15): 57(36-66)%; no cyclosporin A (n=8): 45(37-62)%; controls: 39(31-61)%; $p < 0.01$).

Renal transplant patients are subject to oxidative cell damage and may be at increased risk of vascular thrombosis. Possible contributory factors include an immunological reaction to the graft, and/or the effects of immunosuppressive therapy.

PAPER 25

S-ADENOSYLMETHIONINE IMPROVES HEPATIC FUNCTION DURING LIVER PERFUSION AFTER SEQUENTIAL COLD AND WARM ISCHAEMIC INJURY.

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S-Adenosylmethionine (SAME) has proven benefit in the treatment of cholestatic liver dysfunction and in preventing experimental hepatotoxicity. We have studied its effects in reducing preservation injury during an ischaemic regimen simulating that used in rat liver transplantation: 24h cold storage in University of Wisconsin solution (UW) at 4°C followed by 20 min warm ischaemia at 37°C (CI+WI). SAME was administered to male Lewis rats (250-350g; n=7) 16-18h before hepatectomy and included in the UW storage solution and added as a bolus to the perfusate just before reperfusion. CI+WI injury (n=6) profoundly impaired haemodynamic variables throughout the 3h perfusion with median liver blood flow, oxygen delivery, oxygen consumption and oxygen extraction ratio 62%, 53%, 142% and 260% of control values (n=11) at 30 min, respectively. SAME restored corresponding values towards normal in all cases: median 90%, 88%, 120% and 130% of control. Bile production to 15 min was increased 5-fold by SAME treatment (median 12.3 vs 2.3 mg/g/h in CI+WI) and rose progressively towards control values at 3h (23.5 vs 27.4 mg/g/h, respectively). SAME substantially decreased both glucose release and acid production (titrated with bicarbonate) over 3h: glucose 900 vs 1440 vs 880 and bicarbonate 0.122, 0.189 and 0.094 mmol/g.h for SAME, CI+WI and control, respectively. SAME had no benefit to parenchymal or endothelial cell damage as judged by perfusate levels of purine nucleotide phosphorylase and aspartate aminotransferase. **Conclusions:** SAME reduced the metabolic injury resulting from CI+WI as judged by bile flow, acid production and glucose release. SAME was also shown to be a novel potent agent for the improvement of haemodynamic function following ischaemic damage.

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PAPER 26

PORTAL VEIN THROMBOSIS AND LIVER TRANSPLANTATION

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Portal vein (PV) thrombosis found at the time of orthotopic liver transplantation (OLT) or developing in the post operative period may be a difficult problem to manage and few reports on outcome are available.

100 consecutive patients undergoing OLT were reviewed to assess the incidence of PV thrombosis and the outcome of surgical management.

Eleven patients (6M, 5F, age 18-59, median 45 years) had partial (n=5) or complete (n=6) occlusion of the portal vein at the time of surgery. Surgical thrombectomy by flushing or Fogarty catheter was possible in 10/11 patients, the remaining patient undergoing PV to right gastroepiploic vein anastomosis. On follow up 5 patients have died of unrelated causes (PV patency confirmed in 4/5). Of the remaining 6 patients (median follow up 29 months), one has developed PV thrombosis 12 days following retransplantation which was successfully treated by surgical thrombectomy (re-thrombosis rate 1/11 = 9%). Two patients with normal PV at time of OLT developed PV thrombosis at 24hrs and 14 days post op and both were successfully treated by thrombectomy. One is well 25 months following OLT with patent PV and the other died 1 month post OLT of systemic aspergillosis (PV confirmed patent). Of the 100 patients investigated the three who developed post OLT PV thrombosis all received intra-operative aprotinin (Trasylol) (3/43). None of the 57 patients who did not receive this treatment developed PV thrombosis (X^2 with Yates correction = 2.05, $p=0.1$, N.S.)

We would conclude that PV occlusion is relatively common (11%), surgical thrombectomy effective (10/11) and re-occlusion rare (1/11).

CILAG LECTURE

PEPTIDES NATURALLY PRESENTED BY MHC CLASS I MOLECULES

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The peptides naturally presented by MHC class I molecules can be isolated from whole cells or from purified MHC molecules by acid extraction followed by HPLC separation. Analysis of such peptides representing normal self peptides, viral peptides, minor histocompatibility peptides, and peptides recognized by alloreactive T cells indicated the following: (i) Each normal class I-expressing cell simultaneously presents hundreds or thousands of peptides derived from cellular proteins. (ii) The peptide content of cells is dependent on the expression of MHC class I genes. For example, the H-2K^b-restricted minor H peptide H-4^b is not detected in H-4^b cells not expressing K^b. (iii) Cells may contain peptides that are not dependent on coexpression of MHC molecules. For example, H-4^b cells (irrespective of MHC expression) contain an additional peptide recognized by H-4^b-specific, H-2K^b restricted CTL with low efficiency. Such MHC-independent peptides may be precursors for the peptides finally presented by MHC molecules. (iv) Several peptides naturally presented by MHC class I molecules have been identified, for example, ASNENMETM (D^b-restricted) and TYQRTRALV (K^d-restricted) are naturally presented by influenza-infected cells. (v) The peptides presented by class I molecules adhere to allele-specific rules or motifs, which require allele-specific lengths (8 residues for K^b, 9 for K^d, D^b, and HLA-A2.1) and correct occupancy of two anchor residues, one of which is always C-terminal and is aliphatic for the alleles mentioned. Based on these data, we propose the following model for peptide processing in the MHC class I pathway. Proteases in various cellular compartments (eg cytosol) are degraded by an endopeptidase cutting C-terminal of aliphatic residues. The resulting peptides that share the C-terminus but not the N-terminus with the final product are then translocated to the compartment of MHC class I assembly and bind there to MHC molecules. Binding requires accommodation of side chains of the allele-specific anchor residues into the allele-specific pockets of MHC molecules. Finally, the N-terminal residues of the precursors are trimmed by an hypothetical protease activity. Thus, class I molecules have an instructive as well as a selective role in processing.

PAPER 27

NEONATAL TOLERANCE OF CARDIAC ALLOGRAFTS

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Cardiac transplantation in the newborn period is one of the few surgical options for certain otherwise lethal congenital cardiac malformations. Induction of permanent and specific tolerance to a transplanted heart, obviating the need for immunosuppressive drugs, may be easier to accomplish in foetuses and newborns than in adults, due to the immaturity of their immune system. Neonatally induced tolerance to skin grafts has previously been achieved in various murine strain combinations by intravenous inoculation of semiallogeneic bone marrow and spleen cells within 24 hours of birth. We have used this model to establish tolerance to fully vascularised cardiac allografts in 2 murine donor-recipient strain combinations not previously used: BALB/c (H-2^d) to C3H/He (H-2^k) and C57BL/10 (H-2^b) to C3H. An inoculum of equal numbers of bone marrow and spleen cells from (C3H X BALB) F1 or (C3H X C57) F1 adult female mice was injected intravenously into C3H neonates within 24 hours of birth. At 8-12 weeks of age, a fully vascularised heterotopic cardiac allograft was transplanted, from BALB or C57 respectively. Prolongation of graft survival was achieved in each strain combination, in a dose-related fashion, with 5/5 C3H recipients of C57 hearts and 5/6 C3H recipients of BALB hearts accepting the allografts indefinitely, at a dose of 15×10^6 total cells. We are now attempting to induce permanent tolerance of a transplanted cardiac graft by neonatal injection of donor antigen in forms that may be more directly applicable to the clinical situation. Fibroblasts (L cells) derived from the C3H recipient strain were transfected with genes for donor (C57 or BALB) MHC class I antigens, singly and in various combinations with class II antigens, and injected intravenously into C3H newborns. Foetally-derived fibroblasts are readily available by amniocentesis and could be an accessible source of recipient tissue from infants diagnosed antenatally with congenital heart lesions. In preliminary experiments, this has resulted in varying degrees of prolongation of the survival of BALB or C57 heart grafts. The reasons for the variable degree of success with this mode of antigen administration may include insufficient density of the selected MHC antigen(s) expressed by the transfected cells, inadequate persistence of the antigen or poor localisation of the antigen to a necessary anatomic site. The fate of the injected cells *in vivo* was studied by labelling with the fluorescent dye DiI. In addition, the importance of chimerism to the induction of tolerance was established.

PAPER 28

ANTI-CD4 ANTIBODY AND RANDOM BLOOD TRANSFUSION PROLONGS MURINE ALLOGRAFTS INDEFINITELY

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We have previously shown in a murine model that a 250µl donor specific transfusion (DST) under the cover of anti-CD4 antibody (anti-CD4 Mab) prolongs indefinitely the survival of cardiac allografts transplanted 28 days later. Pre-operative blood transfusion from random blood donors has been shown to improve graft survival in clinical kidney transplantation. We therefore sought to determine whether random blood transfusion would be as effective as DST when combined with anti-CD4 therapy in this allograft model.

Methods C3H/He (H2^k) mice were given 50µg of YTA 3.1.2 anti-CD4 Mab i.v. on two consecutive days. On the second day they received 250µl of whole blood combined equally from H2^b, H2^s, H2^d and H2^q donors. 28 days later they received a C57BL/10 H2^b heart graft.

Results	MST (days)				
no treatment	12	anti-CD4 alone	26	DST alone	21
RT alone	9	anti-CD4 + 1/4 volume DST	24.5		
anti-CD4 + donor specific transfusion	≥100				
anti-CD4 + random transfusion	≥100				

Conclusions

DST combined with anti-CD4 Mab clearly prolongs the survival of murine cardiac allografts but any potential extrapolation to clinical cadaveric transplantation is difficult given the practical problem of matching blood and subsequent organ donors. However, our data in this murine model show that random blood plus anti-CD4 Mab is equally effective probably because of antigen sharing between the blood donors. Since fortuitous sharing of HLA antigens between blood and kidney donors is thought to explain the success of random blood transfusion in clinical transplantation we suggest that the benefits of DST and anti-CD4 therapy (neither of which is effective alone) might be provided by a combination of random blood transfusion and anti-CD4 antibody.

PAPER 29

Activation of T suppressor cells: the requirement for lymphokines

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The differential activation of CD8⁺ T cells to function as T cytotoxic (Tc) or T suppressor (Ts) cells probably depends on the lymphokines present during their activation. This is suggested by the observation that addition of 0.5 µg/ml cyclosporine to a mixed lymphocyte culture (MLR) inhibits the generation of Tc but not of Ts cells. To test this further we stimulated CD8⁺ T cells, purified from lymph nodes using immunomagnetic beads, in lymphokine-containing media and assayed for Tc and Ts activation. By FACScan analysis the T cells were 98% CD8⁺, 2% CD4⁺. The CD8⁺ cells were activated *in vitro* with dendritic cells (DC) isolated from the thoracic duct lymph of mesenteric lymphadenectomised, irradiated rats, in the presence or absence of 0.5 µg/ml cyclosporine. As a source of lymphokines, the culture supernates of mixed lymphocyte cultures (MLR) performed in the absence (MLR-supt) or presence of cyclosporine (MLR-CsA supt) were added to the DC+CD8⁺ T cell cultures. The generation of Tc was dependent on the addition of MLR-supt and did not occur when MLR-CsA supt was added. The activation of Ts was lymphokine-dependent, too, but occurred when MLR-CsA supt was added. Other studies in the rat and mouse have revealed that cyclosporine preferentially affects the production of Th1 (IL-2 and Interferon) rather than Th2 (IL-5) lymphokines. Hence, we can suggest that Tc and Ts activation is differentially controlled by lymphokines produced by different subsets of T helper cells. Attempts to identify the Th2 lymphokine(s) involved are in progress.

**THE HUNTERIAN PROFESSORSHIP LECTURE OF THE ROYAL
COLLEGE OF SURGEONS OF ENGLAND**

MALIGNANCY FOLLOWING RENAL TRANSPLANTATION

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While an increased risk of cancer in immunosuppressed organ graft recipients is widely accepted, the risks are generally believed to be small. Frequently cancer incidences of 2-5% are quoted. However, such estimates do not take into consideration duration of survival following transplantation. In Australia and New Zealand information concerning all renal transplant recipients (ISTR) has been documented in a unique Dialysis and Transplantation Registry for the past 28 years. The information includes details of all malignancy which develops. There now appear to be firm indications that, with time, the risks of aggressive neoplasia in these patients are substantial. The enhanced risk exists for virtually all types of malignancy and persists indefinitely. There is a multi-factorial aetiology of neoplasia following organ transplantation but the greatest increased risks are for those malignancies where viruses are implicated. Virtually all malignancies can occur with a short latent period but there is a timing differential for different cancers with lymphomas and Kaposi sarcoma appearing earlier than visceral malignancies or those of the blood. Most cancers are aggressive including those of the skin. There is no discernible change in the rate of malignancy in those patients treated with cyclosporin. Malignancy is established as a major cause of late failure in organ transplantation.

NOTES