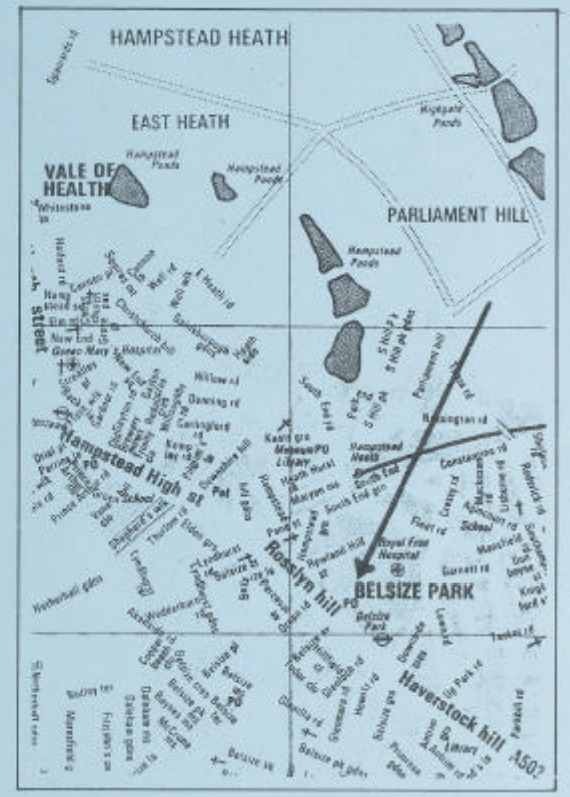
British Transplantation Society



The Royal Free Hospital, Pond Street, London NW3.

10th November, 1982

ANTIBODY THERAPY FOR CYTOMEGALOVIRUS INFECTION AFTER RENAL TRANSPLANTATION

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The current treatment of cytomegalovirus (CMV) infections after renal transplantation is unsatisfactory; the illness contributes significantly to graft loss, morbidity and mortality. Six renal transplant recipients with severe serologically proven CMV infection have been treated by passive immunisation; one patient received high-titre anti-CMV antibody plasma and five patients were given fractionated hyperimmune anti-CMV immunoglobulin. The anti-CMV immunoglobulin was prepared by screening healthy blood donors by indirect fluorescent antibody assay or enzyme-linked immunosorbent assay for ant-CMV titres greater than 1:32, and cold ethanol fractionation of the resulting pooled plasma. The resulting hyperimmune anti-CMV immunoglobulin had an anti-CMV titre of 1:900. All patients treated had been pyrexial for at least severn days before treatment, and had typical clinical and laboratory features of CMV disease including leucopenia, lymphocytosis, lung infiltrates, abnormal liver enzymes or deteriorating graft function. Two patients had been given antiviral chemotherapy without effect. Four of the six patients treated showed a complete and sustained response whithin 24 hours of antibody therapy, but the other two patients did not respond. No side effects were observed.

It is concluded that passive immunotherapy is a highly promising treatment for severe CMV infections, and merits further evaluation.

a) This work has not been previously published.

b) Some of this data has been communicated in a preliminary fashion at a workshop on viral infections in transplantation, European Dialysis and Transplant Society, Madrid, September, 1982.

Non-MHC endothelial antigens in experimental cardiac allotransplantation.

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Previous studies have shown that immunizations of MAXX rats with spleen cells from the MHC-identical BN-strain results in the formation of non-MHC endothelial antibodies. Transplantation of BN kidneys into pre-immunized MAXX recipients results in a donor-specific accelerated rejection, whereas grafting into unmodified recipients does not induce the formation of endothelial antibodies and rejection does not occur. In the present experiments the role of the endothelial antigen in cardiac allografting was studied.
Indirect immunofluorescence studies of BN hearts using MAXX anti-BN or (ACIxMAXX)F_1 anti-BN sera obtained by spleen cell immunizations did not show staining of cardiac endothelium. Grafting of BN hearts into unmodified MAXX recipients did not result in rejection, although endothelial antibodies of the IgG class were detected in 6/8 animals 3-5 weeks after grafting; additional immunizations with spleen cells 112 days after grafting also failed to induce functional rejection as did transplantation into pre-immunized recipients.
We conclude that cardiac allografts can induce endothelial antibodies but are not rejected, whereas kidney grafts do not induce endothelial antibody formation, but do undergo rejection by circulating antibodies. Quantitative differences in expression of endothelial antigens may explain the differences in rejection of both organs.

This work has not been published or read at a scientific meeting previously.

USE OF MONOCLONAL ANTIBODIES IN FINE NEEDLE ASPIRATION CYTOLOGY

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Fine needle aspiration biopsy provides a simple and atraumatic method of monitoring the progress of human renal transplants and can be carried out on a daily basis. Cytolcgical examination of aspirates in 20 patients using light microscopy and conventional histological staining has confirmed the findings of Häyry and von Willebrand[1] showing increasing numbers of macrophages in severe rejection. However, the changes in early rejection are more subtle and identification of some cell types is subjective. Characterisation of lymphocyte sub-populations on the basis of morphology is clearly not possible. In a further study in 8 renal transplant recipients monoclonal antibodies have been used with an immunoperoxidase technique to positively identify and quantitate lymphocyte sub-populations within the graft.

An advantage of this method is that a single aspirate provides several cytocentrifuge slide preparations which may be treated with a range of monoclonal antibodies. This enables the relative number of B lymphocytes, T lymphocytes and sub-populations of helper and suppressor/cytotoxic T cells to be assessed. A number of locally produced monoclonals in addition to the Ortho and Coulter series of antibodies have been evaluated.

Results indicate that rejection is associated with an increase in suppressor/cytotoxic lymphocytes in the interstitial infiltrate. It is hoped that elucidation of the pattern of cellular reaction within the kidney will provide a more rational approach to immunosuppressive therapy.

1. Fall T_H/T_C not Right.

1. F. Häyry, E. von Willebrand. Monitoring of human renal allograft rejection with fine-needle aspiration cytology. Scand. J. Immunol. 13, 87-97, 1981.

2. Do not tick Blood.

Good.

This paper has not previously been published, read at a scientific meeting or submitted for consideration by another Society.

Presentation or Poster

Is the liver less immunogenic than kidney or heart?

G. Müller, U. Hopt, P.J. Morris.

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Liver allografts in the orthotopic position in several different species, are often not rejected (1,2,3,4). This apparent poor immunogenicity is strange bearing in mind the large pool of Ia bearing vascular endothelium in the liver and the extensive population of Ia bearing Kupffer cells and dendritic cells.

We have further evaluated this phenomenon by transplantation of an auxiliary liver allograft using the left renal vessels of the recipient for the vascular anastomosis and the ureter for bile drainage. The rat combination DA ($RT1^a$) to PVG ($RT1^u$) which is known to accept orthotopic liver grafts was used. 30 days after the DA auxilliary liver graft had been implanted, a DA heart allograft which was placed end/side to aorta/cava.

In contrast to findings with orthotopic liver grafts in this strain combination, all auxilliary liver allografts were rejected within 14 days.

The cardiac allografts implanted 30 days after the liver graft were rejected in a second set fashion.

These results suggest that the apparent lack of immunogenicity of orthotopic liver allografts may be related in some way as yet unexplained to the removal of the host liver.

References:

1. R. Calne et al. 1969 Nature 223, 472-476
2. FA Zimmerman et al. 1979 Trans.Proc. 11, 571-577
3. HFS Davies et al 1982. Trans.Proc. in press.
4. FA Zimmerman et al 1982 Trans. Proc. in press.

This work has not been previously published, read at a scientific meeting nor submitted for consideration of another society.

SERUM BETA$_2$ MICROGLOBULIN (B_2u) AND N-ACEYTL-B-D-GLUCOSAMINIDASE (NAG) EXCRETION? DISCRIMINANT TESTS TO DIFFERENTIATE BETWEEN REJECTION AND CYCLOSPORIN A (CyA) INDUCED NEPHROTOXICITY?

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The purpose of this study was to investigate the use of serum B_2u concentration and urinary NAG excretion in combination as an early indicator of rejection and to differentiate between rejection and CyA induced nephrotoxicity in renal transplant recipients. Daily B_2u and NAG levels were measured respectively by radio immuno assay and by the automated method of Tucker et al[1]. B_2u levels were monitored in 42 patients, of whom 17 were monitored for NAG excretion. 12 of the 42 patients received standard immunosuppression. Nephrotoxicity was diagnosed histologically by exclusion and by response of renal function to reduction in CyA dose. There were 21 acute rejection episodes in this series of 42 patients. During acute rejection, elevation of B_2u occurred prior to serum creatinine elevation in 59% and on the same day in 38%. 19% of patients showed a sustained rise in B_2u concentration in the absence of rejection. 8 of the patients monitored for NAG excretion had one episode of acute rejection each. Elevation of NAG excretion occurred prior to that in serum creatinine in 3 and on the same day as serum creatinine in 2. NAG levels rose 24 hours after serum creatinine in one rejection episode. In 6 of 9 patients without rejection the NAG levels remained low. In patients with CYA nephrotoxicity, B_2u and serum creatinine either remained high or increased. NAG excretion was monitored in 3 nephrotoxic patients and rose markedly. We conclude that although the B_2u assay is useful for early detection of rejection there is a substantial proportion of false positive results. NAG excretion correlates with rise in B_2u and serum creatinine concentration during acute rejection. Since B_2u, NAG excretion and serum creatinine concentration all rise in both acute rejection and CyA nephrotoxicity they cannot be used to discriminate between these two phenomena.

Reference: 1. Tucker S.M., et al 1975 Clin. Chem. Acta. 62, 33 - 339.

This paper has not previously been published, read at a scientitific meeting or submitted for consideration by another Society.

FUNCTIONAL STUDIES OF VEILED (DENDRITIC) CELLS FROM AFFERENT LYMPH.

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Veiled or dendritic cells from afferent lymph localize in T dependent areas of lymph nodes and may be precursors of the paracortical dendritic cells. These cells may play a role in presentation of donor antigen to the host during rejection of kidney grafts[1]. We have separated dendritic cells from the afferent lymph of normal rabbits or rabbits hyperimmunized with human immunoglobulin and studied their properties in vitro. Mixed lymphocyte reactions in rabbits are generally low, and stimulation of peripheral blood lymphocytes by allogeneic veiled cells was also minimal or absent unless both cell populations were from hyperimmunized rabbits. The effect of veiled cells in modulating the responses of allogeneic lymphocytes to antigen could, therefore, be studied in the absence of allogeneic stimulation. Small numbers of autologous or allogeneic veiled cells were added to lymphocytes in 20 µl hanging droplet cultures. They enhanced the responses to stimulation with low doses of mitogen or antigen, particularly when the cells were cultured for short periods or at low cell densities. Stimulation was associated with the formation of cellular aggregates which were frequently held together by the processes of a single veiled cell.

1. R.I. Lechler, J.R. Batchelor (1982 J. Exp. Med. 155, 31.

G.F.J. Hendriks[1], A. Van Es[2], J.J. van Rood[3]

1= Eurotransplant Foundation
2= Department of Nephrology
3= Department of Immunohaematology, University Hospital, Leiden, The Netherlands

Immunosuppressive therapy, HLA-DRw6 matching and renal allograft function.

During acute rejection episodes renal allograft recipients are exposed to increased doses of steroids. The majority of these rejection episodes occur during the first three months after transplantation. In this retrospective single centre study performed in Leiden, we examined the interactions between acute rejection episodes, the total dosis of steroids (oral and intravenous) and the renal function of the graft (creatinin clearance) in relation to the number of HLA-DR mismatches. Clinical data were collected three months postoperatively. A group of 73 successfully transplanted recipients of first renal allografts was analysed. A significantly lower incidence of acute rejection episode was observed in the DR identical group, as compared to the DR-1 and -2 mismatched group ($p<0.02$).
In addition, a highly significant correlation was observed between the number of HLA-DR mismatches and the total dose of steroids ($p<0.001$). There was also a significant correlation between the level of creatinin clearance and HLA-DR matching three months after transplantation ($p = 0.006$). HLA-DR matching is an important prognostic factor for renal allograft survival. Consequently it is not surprising that our results show that the numbers of rejection crises, the levels of corticosteroid therapy, used to reverse them, and graft function (creatinin clearance) are directly and significantly related to that factor. Our findings suggest that a higher level of DR-matching may lead to a reduction in the serious side effects of excessive levels of corticosteroid therapy. The impact of HLA-DRw6 on these findings will also be discussed.

Localisation of MHC (HLA-ABC and DR) antigens in 46 kidneys. Differences in HLA-DR staining of tubules between kidneys.

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The precise distribution of the MHC antigens was studied in biopsies from 46 kidneys which were subsequently transplanted. Monoclonal antibodies to monomorphic determinants of HLA-ABC and DR antigens were used in the peroxidase-anti-peroxidase immunohistological technique. There was no variation in the expression of HLA-ABC antigens, which were present on all cells of the renal parenchyma. HLA-DR antigens were consistently present on glomerular endothelium and mesangium, intertubular capillaries and interstitial dendritic cells. However, there was a striking variation between individual kidneys in the expression of HLA-DR on tubules. Tubular HLA-DR was present in 27 kidneys (60%) was absent in 11 (23%) and possibly weakly present in another 8 kidneys (17%). Where HLA-DR was found on tubules it appeared to be mainly on proximal tubules.

There was no correlation between tubular HLA-DR expression and donor sex, age, blood group and warm and cold ischaemia times. However, there was an increase in the frequency of HLA-DR3, 55% in the negative tubular HLA-DR kidneys compared to 15% in the positive kidneys, which, although not statistically significant, does suggest a possible genetic influence on the expression of tubular HLA-DR. Graft survival at one year was better in recipients of negative tubular HLA-DR kidneys (70%), compared to 56% in recipients of positive kidneys, but this difference was not statistically significant with the numbers studied.

This work has not been previously published, read at a scientific meeting nor submitted for consideration of another society.

POTENTIAL INVOLVEMENT OF HLA-DR POSITIVE CELLS OF DONOR ORIGIN IN RENAL ALLOGRAFT REJECTION

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The process of renal allograft rejection is the result of host immunological responses to allo-antigens expressed on the graft. Animal experiments suggest that a specialized antigen-presenting cell bearing Class II antigen plays a pivotal role in the rejection process. Using a combination of immunofluorescence and cytochemical techniques on cryostat sections of normal and rejecting human kidney we have attempted to characterize the phenotype of HLA-DR$^+$ cells. Reagents used include heterologous antisera to HLA-DR antigens and human Factor VIII, monoclonal antibodies against HLA-ABC (YE2/36), monocyte macrophage antigens (FMC-17), interdigitating cell antigens (RFD-1), adenosine triphosphatase (ATPase) and acid phosphatase (ACP) activities. We have found that normal human kidney contains a population of HLA-DR$^+$ cells in the interstitium, 80% of which have the phenotype of endothelium, i.e. Factor VIII$^+$,RFD-1$^-$,FMC-17$^-$. The remaining 20% have an interdigitating morphology and are Factor VIII$^-$,RFD-1$^+$,FMC-17$^+$. In contrast, rejecting kidney contains a vast accumulation of DR$^+$ cells. Analysis of this population reveals that it is comprised of activated T cells (T28$^+$), B cells and activated macrophages (FMC-17$^+$,ACP$^+$). A certain amount of HLA-DR antigen appears not cell bound. We hypothesize that the HLA-DR$^+$ interstitial interdigitating cells of normal human kidney are the targets of the focal inflammatory cell infiltrates which characterize the rejection process.

THE EFFECT OF HLA-A, -B, -DR, MB and MT MATCHING, PREGNANCIES AND PRE-TRANSPLANT TRANSFUSIONS ON THE OUTCOME OF 1,023 LONDON TRANSPLANT GROUP CADAVER RECIPIENTS

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DEPARTMENT OF IMMUNOLOGY, THE LONDON HOSPITAL MEDICAL COLLEGE, LONDON.

(a) The effect of HLA-A, -B and -DR, MB and MT matching

The results of long term follow-up of 1,023 cadaver transplants were analysed for the effect of (1) HLA-A and HLA-B antigen matching; (2) HLA-DR, -MB and -MT matching; (3) pre-transplant transfusions according to whether the recipients were well or poorly matched for HLA-A and -B; and (4) pregnancies according to the HLA-A and -B matching. The results show a marked benefit for HLA-A and -B matching indicated by a highly significant difference between observed (O) and expected (E) failures for well matched (O/E 0.73) and poorly matched grafts (O/E 1.4) (Peto analysis). Ten years' survival figures for (4+3A), (3B+2), (1+0) match grades were 44%, 28% and 16% respectively ($p<.00001$). A similar rank order of survival has been observed in DR matched cadaver grafts. The well matched group (14 patients) has done particularly well (85% at 2½ years), the 1 DR match group (104 patients - 64% at 2½ years) and the 0 DR match group (77 patients - 54% at 2½ years) - O/E 1.27, 0.87 and 0.69 respectively. But even if the DR antigens are compatible, the optimum result depends on the associated good or poor HLA-A and -B matching. MT matching showed a very poor outcome for 2 MT incompatibilities (30 patients - 40% vs 65%), for 1 (110 patients) and 0 (55 patients) MT incompatibilities at 2½ years (O/E 1.52 vs 0.86). MB also showed a difference but not as striking (68% for 2 match vs 58% for 0 match pairs).

(b) Blood Transfusions

Pre-transplant transfusions benefited only the poorly matched HLA-A and -B recipients after 5 years, the well matched recipients appeared not to benefit from transfusions after this time. This result was amplified in the recipients who had rejected kidneys or made anti-HLA-A and -B antibodies. Only patients receiving a moderate number of transfusions benefited from this regimen. Multiple transfusions, 20+ produced a result equal to that of 0 transfusions or less.

(a) The work described in this summary has not been previously published.
(b) The work contained in this summary has not been read at a scientific meeting.

Enhancement of rat kidney allografts using haptenated alloantigens and anti-hapten antibody

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Immune complexes formed with donor alloantigen and anti-donor antibody[1], or with haptenated alloantigen and anti-hapten antibody, are known to have specific immunosuppressive properties[2]. We have investigated the possibility of using the latter type of complex to enhance kidney allografts in rats.

Recipients of semi-allogeneic or fully allogeneic rat kidneys were given immune complexes formed with trinitrophenyl (TNP)-conjugated alloantigens (TNP-Ag) and a mouse monoclonal anti-hapten antibody (anti-TNP). The complexes were administered IV at the time of grafting and, in some cases, also on subsequent days. Immune complexes using TNP-modified whole donor spleen lymphocytes (T-C), cellular membrane sonicate (T-M), and papain solubilized (T-S) alloantigens were found to be effective in enhancing graft survival in specific donor-recipient combinations. Indefinite survival was obtained in some recipient groups with semi-allogeneic donors and a more modest degree of enhancement was seen with fully allogeneic kidneys. The enhancing effect of TNP-Ag + anti-TNP complexes was highly dependent on the ratio of antigen to antibody, and the optimum ratio varied among strain combinations.

The possible clinical applications of this approach to allograft enhancement will be discussed.

References:

1. Marquet RL, et al. Transplantation 24:454,1976

2. Hutchinson IV, and Brent L. Nature 292: 353, 1981.

The work described in this summary has not been previously published. Part of this work was presented at the 1982 Congress of the International Transplantation Society.

SYNERGISM BETWEEN ANTILYMPHOCYTE AND ANTIMACROPHAGE AGENTS IN SUPPRESSING ISLET ALLOGRAFT REJECTION.

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We have previously demonstrated the ability of the antimacrophage agent silica to prolong islet allograft survival in an F1 hybrid to parent strain model (ASxAUG)→AS). In a stronger strain combination (Wag→AS) silica was found to be ineffective. The aim of this project was to study the efficacy of silica in combination with antilymphocyte agents in suppressing the rejection of islets in this stronger strain combination. The islets were harvested using a collagenase digestion and ficoll gradient technique and injected intraportally into the streptozotocin-induced diabetic recipients. Group 1 acted as controls; group 2 received 50mg/100g of silica by intraperitoneal injection on day -6; group 3 received 1ml of antilymphocyte serum (ALS) on days -1,1,3 and 5; group 4 received Cyclosporin A(CyA), 20mg/kg dissolved in olive oil, by gavage for 7 days; group 5 received silica and CyA; group 6 received silica and ALS; group 7 received CyA and ALS and group 8 received silica, ALS and CyA. The blood sugar persistently raised above 10mmol/l was regarded as evidence of rejection. The results are shown in the table:

Group	No. of rats	No. of days before rejection
1	10	0(9),2
2	9	0(6),4,4,9*
3	7	0(7)
4	5	0,9,10,11,12
5	4	0,6*,11,12
6	6	0,0,5,6,6,6
7	5	14,22,23,30,33
8	6	28*,29,49, >100(3)

* died normoglycaemic

In conclusion in this model there is synergism between ALS and Cyclosporin A and between these two agents and silica. This study provides more evidence of the importance of macophages in islet rejection.

a) The work described in this summary has not been previously published.
b) The work contained in this summary has not been read at a Scientific meeting.

Synergistic immunosuppressive action of procarbazine hydrochloride (PCH) and antilymphocyte serum (ALS) in a rat renal allograft model

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The median survival time (MST) of BN ($RT1^n$) to Lewis ($RT1^1$) kidney transplants is greatly improved from 11 days to 82 days by treatment of recipients with both PCH (50 mg/kg on days 1,3 and 5) and ALS (5 ml/kg on days 2,4 and 6 after grafting). PCH or ALS alone are weak agents in this combination, giving MSTs of 11 and 14 days respectively. Recipients treated with PCH + ALS fail to make specific antibody and blood or spleen cells from these rats are non-specifically deficicnt in GVH reactivity. Graft survival is not due to graft adaptation or to opsonization of antigen-reactive cells. Suppressor T cells are present which can specifically prolong the survival of allografts in 400 R irradiated syngeneic recipients and, in mixing experiments, can modulate the GVH reactivity of normal syngeneic lymphocytes. Thus it appears that, as in the mouse, PCH + ALS treatment has a powerful immunosuppressive effect leading to a state of graft acceptance mediated, at least in part, by specific suppressor T cells.

This work has not been previously published, read at a scientific meeting nor submitted for consideration of another society.

Prostacyclin, aspirin and salicylate in rat cardiac allograft rejection.

Platelet accumulation in acute rejection may contribute to graft failure. This study compared prostacyclin (PGI_2) infusion in rat cardiac allograft rejection with aspirin. DA ($RT1^a$) hearts in untreated PVG ($RT1^c$) recipients rejected in 7.33 ± 0.86 days. PGI_2 i.v. infusion in glycine, 250 ng/kg/min, from day 1 prolonged graft survival to 8.71 ± 0.75 days (Wilcoxon $P < 0.01$ v glycine controls). PGI_2 from day 5 prolonged graft survival to 9.45 ± 1.37 days ($P < 0.01$ v controls). Aspirin, 200 mg/kg/day s.c. injection from day 1 prolonged graft survival to 17.27 ± 16.33 days and from day 5 to 12.17 ± 4.82 days ($P < 0.01$ v controls). Histology showed that beneficial effects were not due to reduced vascular occlusion.

Sodium salicylate, 200 mg/kg/day by s.c. injection from day 1 gave graft survival from 11 days to over 6 months (median 90 days; $P < 0.01$ v aspirin and v controls). ADP-induced platelet aggregation was inhibited by 22% ± 2% in the aspirin treated group but was normal in the salicylate treated group. When added to PVG platelets, 50% inhibition of aggregation was caused by 1.1 mmol/l aspirin or 14.5 mmol/l sodium salicylate. Prolongation of graft survival by salicylate was unlikely to be mediated by reduced platelet activity.

SHAW, J.F.L. Department of Surgery, Addenbrooke's Hospital, Cambridge.

a) The work described in this summary has not been previously published.

b) A small part of the work contained in this summary (concerning PGI_2 and aspirin) has been read at the Surgical Research Society, Sheffield, 9th July 1982.

IN VITRO INTERFERON STIMULATION OF NK AND ADCC EFFECTOR CELLS FROM IMMUNOSUPPRESSED PATIENTS.

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Natural killer (NK) cell function is impaired in transplant recipients receiving conventional immunosuppression and this may partially explain the susceptibility of such patients to viral infections. Interferon (IF) is a potent stimulator of NK and ADCC effector cell function and may have a therapeutic role to play in the treatment of certain viral infections. In these studies we have examined the effects of in vitro IF stimulation on NK and ADCC mediated by the PBL of the following groups: (1) a healthy control group (n = 14); (2) a group of renal allograft recipients receiving conventional Immunosuppression (n = 17) and (3) a group of allograft recipients receiving Cyclosporin A as their sole immunosuppression (n = 11). Cytotoxicity was measured in a short-term chromium release assay using K562 as the NK target and a rabbit-antibody-coated lymphoblastoid cell line (LHN13) as the ADCC target. NK and ADCC were measured before and 1 hour after incubation with 1000 u/ml of pure human lymphoblastoid interferon. The results were as follows:

Mean % Specific ^{51}Cr release at E:T ratio of 50:1

	K562		Ab-LHN13	
	Pre-IF	Post-IF	Pre-IF	Post-IF
Controls	34.9 ± 7.5%	50.6 ± 8.3%	53.3 ± 6.4%	60.4 ± 7.8%
Az + P	10.7 ± 9.9%	24.9 ± 19.8%	31.7 ± 16.1%	43.3 ± 15.9%
Cy.A	23.1 ± 10.0%	33.6 ± 13.2%	57 ± 6.5%	59.9 ± 12.9%

Although NK function is impaired in both conventionally immunosuppressed patients and those receiving Cyclosporin-A, this may be partially restored by IF-stimulation. Impaired ADCC after conventional immunosuppression may also be restored but Cyclosporin A does not appear to alter ADCC function.

These data provide some rational basis for the use of IF in the treatment of certain viral infections in immunosuppressed patients.

a. The work described in this summary has not been previously published.

b. The work contained in this summary has not been read at a scientific meeting.

Presentation

CYCLOSPORIN A SERUM LEVELS AS A MONITORING ASSAY - FACT OR FICTION?

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Serum estimations of Cyclosporin A (CyA) by radicimmunoassay [1] may be a useful guide to effective immuno-suppression (I-S) on one hand and the avoidance of nephrotoxicity on the other. A comparison was made between samples obtained with known values from the Hammersmith Hospital. This showed a mean difference in the estimations of 31.2 ± 30.3ng/ml. 26 patients had serum CyA assay performed at frequent intervals 1-5 weeks after cadaveric renal transplantation (CRT). Oral, intramuscular and intravenous CyA was given according to our recently published protocol [2].

RESULTS: CyA levels of 6 patients with rejection episodes before day 25 and 6 patients with rejection episodes later than 25 days after CRT showed no statistical difference at any time compared to 12 patients without rejection although there was a trend towards an increased mean CyA level in the first week in the patients without rejection (Table 1).

	MEAN SERUM CyA LEVEL ± SD (ng/ml)		
	Early Rejectors	Late Rejectors	Non Rejectors
WEEK 1:	266.3 ± 266.0	436.1 ± 355.1	702 ± 710.4

CyA levels of 13 patients in whom CyA nephrotoxicity was proven by either biopsy or the therapeutic effect of dose rejection were compared with 13 patients who showed no evidence of nephrotoxicity. There was no significant difference between the mean CyA levels at any time after CRT. There was marked variation in the serum level of those patients receiving identical oral doses of CyA. Of 13 courses of methyl prednisone pulse therapy 11 showed a mean increase in CyA level of 249%. Our experience with CyA serum levels at present does not allow interpretation of any given level as being definitely nephrotoxic or as correlating with an I-S efficacy. Levels may be helpful as one factor of many in reaching a decision about these two crucial parameters in renal transplantation.

Ref. 1. Donatsch, P. et al. J. Immunoassay 2 (1) 19-32 (1981)
2. Lancet (II (8289), 57-60 (July 1982).

This paper has not been previously published or read at a scientific meeting or submitted for consideration by another society.

EARLY EXPERIENCE OF CYCLOSPORIN A IN CLINICAL HEART TRANSPLANTATION

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Since March 1982 Cyclosporin A has been incorporated into the immunosuppressive regime of 11 (10 male and 1 female) consecutive cardiac recipients. Follow up is from 1 month to 7 months with 9 current survivors.

Previous experience with Cyclosporin A and other additional immunosuppressive agents in cardiac transplantation suggested that patients were over-immunosuppressed. As a result our initial protocol was Cyclosporin A (18 mg per kg per day initial dose), in conjunction with low dose Prednisolone (0.3 mg per kg per day). Two severe and two moderate early (within 2 weeks) rejection episodes occurred in 6 patients on this regime, with one death at 8 days post-transplantation. As a result additional antithymocyte globulin (ATG) to maintain T cells at 10% for 10 days were incorporated into the immunosuppressive regime for subsequent patients. There have been 2 mild rejection episodes prior to discharge in 5 patients on this regime. Rejection episodes have been treated with methylprednisolone ± equine ATG, or augmentation of Prednisolone.

Five infective episodes have occurred in 4 patients (2 bacterial, 1 CMV + fungal, 1 toxoplasma) in the early post-operative period (0-3 months) with one death from disseminated toxoplasmosis. An additional late infection at 6 months (pneumocystis) has occurred.

Cyclosporin A nephrotoxicity of mild or moderate nature has occurred in all patients, responding to reduction in Cyclosporin A dose and serum levels. 80% of patients have left hospital with a mean stay of 33% less than 29 previous transplant patients. Refinements of Cyclosporin A immunosuppressive therapy will be made as a result of continued evaluation.

Synergism between Salicylate and Cyclosporin A in rat cardiac allografts

Although Cyclosporin A (CyA) is a powerful immunosuppressant the dose-related nephrotoxicity in humans can present clinical problems. Perhaps a combination of CyA with other non-steroidal drugs would allow a non-nephrotoxic dose of CyA to be used, yet still avoiding steroidal side-effects. DA ($RT1^a$) hearts were transplanted heterotopically to PVG ($RT1^c$) rats and cessation of graft beat taken as the end-point of rejection. Treatment was given by daily subcutaneous injection for 30 days unless grafts rejected earlier than this.

Results:

DRUG THERAPY	NUMBER OF RATS	MORTALITY	SERUM CyA LEVEL DAY 7 (ng/ml)	GRAFT SURVIVAL (DAYS)	MEDIAN	WILCOXON COMPARISON WITH CONTROLS
Saline 0.6 ml	10	0	< 20	7(x6),8(x4)	7.4	-
CyA 2mg/kg in olive oil	7	0	133 ± 128	7(x3),8(x3),10	7.9	N.S.
Sodium Salicylate 100 mg/kg in saline	8	0	< 20	7,8(x2),9(x4), 10	8.6	N.S.
CyA 2mg/kg + Sodium salicylate 100 mg/kg	11	0	131 ± 89	9,>60(x10)	> 60	P<0.01

There was a definite synergistic effect between sodium salicylate and CyA in the prolongation of heart allograft survival. Similar results are being obtained in other rat strain combinations.

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a) The work described in this summary has not been previously published.

b) The work contained in this summary has not been read at a Scientific Meeting.