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PAPER 1

INCIDENCE OF HLA-DPB1 MISMATCHES IN A SERIES OF HLA-A, B AND DR WELL MATCHED PATIENT-SIBLING PAIRS REFERRED FOR ALLOGENEIC BONE MARROW TRANSPLANTATION.

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The importance of patient-donor matching for HLA-A, B and DR prior to allogeneic bone marrow transplantation is well established. However the incidence and clinical significance of HLA-DP mismatches in otherwise HLA well matched BMT pairs remains uncertain.

We have previously reported a high incidence of DP mismatches in 24 HLA serologically well matched UD-BMT pairs, transplanted at the Hammersmith Hospital London (23/24 pairs mismatched), using PCR and a panel of sequence specific oligonucleotide probes (SSOP's) for DP typing. We have now utilized DNA-RFLP and PCR-SSOP typing to assess the incidence of DP mismatches between 40 patients from the north west of England and their potential, otherwise HLA well matched, sibling BM donors and to compare the efficacy of these typing methodologies.

RFLP typing was performed using 6 restriction enzymes and DPA plus DPB cDNA probes according to the Xth International Histocompatibility Workshop protocol, while PCR-SSOP was performed using a panel of 20 3' end labelled biotinylated probes combined with chemiluminescent detection. DP mismatches were detected by DNA-RFLP and PCR-SSOP in 2 patient-sibling pairs, while mismatches were detected in an additional 5 patient-sibling pairs by PCR-SSOP alone.

These results confirm the greater sensitivity of PCR-SSOP for DP typing and demonstrate a significant frequency of DP mismatches in otherwise HLA well matched patient-sibling pairs. Correlations between DP mismatches (and their origins), MLR reactivity and the clinical outcome of the grafts are under evaluation.

PAPER 2

THE EFFECT OF HLA-DP MOLECULAR POLYMORPHISM IN PRIMARY MLC AND CLINICAL GVH IN BONE MARROW TRANSPLANT OF HLA IDENTICAL INDIVIDUALS

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The proliferative response in the primary mixed lymphocyte cultures (MLC) is controlled primarily by genes within the DR region. HLA-DP molecules seem to make little or no contribution to the primary MLC. On the other hand, it has been suggested that HLA DP antigens may be partially responsible for the development of GVH in bone marrow transplantation of serologically HLA-A,B,C,DR and DQ identical individuals. We have analyzed these two aspects by oligotyping a group of 17 HLA identical bone marrow donor and and patient pairs using a panel of HLA-DP probes defining ten HLA DP alleles. The results showed that 15/17 of the HLA-DR and DQ identical pairs were also compatible for DP and in 2/17 a recombination had occurred between the DQ and DP loci. The MLCs performed between these 17 pairs were negative in both directions (GVH and HVG) in all cases except one, which was HLA-DR,DQ and DP identical. Furthermore, the MLC between one haploidentical (father-son) pair which were HLA-A,B,C,DR and DQ identical DP incompatible, was also negative.
The correlation of these results with clinical GVH was performed and the results show that after one year follow up, 10/15 HLA-DP identical patients are alive and well (four of them with no GVH), 3/15 died and 2 were not transplanted. Of the two HLA-DP recombinant patients, 1 died and in the other there was no marrow engraftment.
These results indicate that HLA-DP incompatibilities do not have a strong effect in primary MLC but they may be important in the development of GVH in bone marrow transplants between HLA identical individuals.

PAPER 3

MHC CLASS-III DNA-POLYMORPHISM IN BONE MARROW TRANSPLANTATION.

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The increased use of unrelated donors in allogeneic bone marrow transplantation has prompted the search for additional methods to better define the major histocompatibility complex (MHC) and aid in appropriate donor selection. The human MHC class-III region includes the genes for C4, C2 and factor B (Bf) all of which show extensive individual polymorphism. Although they do not encode for transplantation antigens, they are closely linked to neighbouring HLA loci and show haplotype specific polymorphism.

In this study, we have evaluated the significance of MHC class-III polymorphism in the investigation of MHC compatibility. Using two DNA probes, C4 and Bf, 65 bone marrow recipients and their HLA-identical siblings as well as 7 matched unrelated pairs were analysed. C4-RFLP showed C4-matching in all sibling pairs. However, 4/7 serologically matched unrelated pairs had C4 genotypic mismatching and 2 of these 4 also had Bf mismatching. One of the 3 pairs matched at C4 was mismatched at Bf locus. Class-II RFLP analyses were also performed in unrelated pairs. All C4 and/or Bf mismatched pairs also had DRß and/or DQß mismatching.

These results suggest that MHC class-III polymorphism may have a role as a complementary technique in determining the MHC identity. Recent identification of new genes within this region could further increase the relevance of this part of the MHC in clinical transplantation.

PAPER 4

SEROLOGICAL CONFIRMATION OF GRAFT VERSUS HOST DISEASE.

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Suspected graft versus host disease (GVHD) can be confirmed if lymphocyte chimaerism can be demonstrated in the patient. Foreign lymphocytes can be derived from lymphoid tissue transferred with a donated organ, or by blood transfusion.

Lymphocytotoxic tests were carried out using anti HLA typing sera on blood lymphocytes of potential GVHD patients. Two liver graft recipients showed a mixture of donor and recipient antigens, while in a third, at the height of GVHD, all blood lymphocytes were of donor type, but reverted to recipient type on resolution of the GVHD.

Two patients with clinical GVHD, who, after treatment for haematological malignancy, were immunoincompetent and had received blood transfusion did not show obvious chimaerism and no pretransfusion samples were available for comparison. However the results of patient HLA typing were not concordant with possible haplotypes deduced from other family members. In one case HLA tests on cultured skin fibroblasts from the patient confirmed family relationships and showed that the patient's circulating lymphocytes were not of patient origin.

These and other cases of GVHD will be discussed.

Reference. Jamieson et al. 1991. Transplant International. 4, 67-71

PAPER 5

USING VOLUNTEER UNRELATED DONORS (VUD) THE RELATIVE RESPONSE INDEX (RRI) HAS BEEN A RELATIVE RUBBISH INDEX.

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Mixed lymphocyte culture (MLC) remains our gold standard in donor selection for bone marrow transplantation (BMT). What has become the RRI was introduced by comparing the thymidine uptake of the responding cells to the VUD target with that to a single outbred control target (a huge variable). Later a pool of cells from four outbreds was used, but optimum control requires a pool of ten outbreds. At least when five or above are used 92% of the optimum plateau is reached. Our own Transformation Index (TI) limit below 1.64 was chosen from a well standardised MLC, always using a control pool of five or six outbreds and even when first published it was supported by its use in a series of actual transplants. To date the WBMT have had only three child deaths from fatal graft-versus-host disease, following BMT without T-cell depletion from over 150 donors, with a TI below 1.64.

Various limits have been set for the RRI (5 publications) from below 3.6% to below 35%, and all seemed arbitrary and unsupported by statistics from actually used donors.

Comparing RRI with TI to select VUD actually used for over 25 transplants, a TI below 1.64 resulted in only one death where GVHD contributed. In contrast RRI below 35% was associated with 7 deaths due to GVHD and one where it contributed, and even RRI below 1.5 was associated with 6 GVHD deaths; these RRI deaths were predicted by a TI above 1.64.

TI is better than RRI in selecting VUD.

Ref: In ed: J.R.Hobbs. Correction of genetic diseases by transplantation, 1989; COGENT/Westminster pp.147-159.

PAPER 6

PARALLEL RFLP TYPING FOR HLA-DRB AS A PERFORMANCE INDICATOR FOR THE STANDARD MICROLYMPHOCYTOTOXIC ASSAY FOR HLA-DR ANTIGENS

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To establish a definitive type for the purposes of organ matching we have undertaken routine prospective parallel studies for the definition of HLA-DR using; a standard microlymphocytotoxic assay using B lymphocytes isolated with an immuno-magnetic bead method and a linked RFLP technique using the cDNA probe pRTV1[1].

We present data from 272 consecutively typed individuals, including both patients entering the renal, corneal and cardiac transplant programmes (n=193) and a non-patient group of organ donors, patient relatives and panel volunteers (n=79). Discrepant typing between results obtained using the two techniques was observed in 19.5% of individuals. This figure includes a 16.6% discrepancy rate for the patient group and 26.6% of the non-patient group.

Of all discrepancies 27/52 involved the misassignment of HLA-DRw6 and other less frequently occurring antigens where conventional serology has been shown to be weakest. This leaves a total of 26/272 (9.6%) which could be termed "unexpected discrepancies" ie involving antigens that are not normally deemed to be problematic. This figure could therefore be used as a measure of performance for the conventional microlymphocytotoxic assay.

[1] Bidwell JL, Jarrold EA (1986). Mol. Immunol. 23: 1111-1116.

PAPER 7

IMPORTANCE OF ENDOTHELIAL LEUCOCYTE ADHESION MOLECULE (ELAM) IN RENAL TRANSPLANTATION

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White cell endothelial cell contact is mediated by various adhesion molecules which are in turn regulated by cytokine mediators. The integrity of the endothelium upon reperfusion after transplantation may determine initial graft function and possible long-term ultimate graft survival. In this preliminary study, we have investigated the expression of ELAM-1 on cultured umbilical cord endothelial (HUVEC) cells before and after stimulation with IL1 and in skin, vein and kidney from normal cadaveric donors and skin and vein from patients undergoing transplantation. ELAM-1 expression was detected by either flow cytometry or immunohistology. HUVEC cells showed staining for ELAM-1, which became more intense after incubation for 4 - 6 hours with 5 units/ml IL1, but declined upon further incubation. Addition of CyA or Methyl Prednisolone did not abolish expression of ELAM-1 in response to IL1. In contrast to previous reports, some tissues were found to be positive for the ELAM-1 antigen. This basal ELAM-1 expression may be significant in determining initial graft function. Most recipients of renal grafts are already chronically activated as indicated by increased circulating soluble IL2-R levels, reportedly due to increased IL1 production. This might cause enhanced ELAM-1 expression and consequent white cell adhesion to endothelium.

PAPER 8

TLX-B SPECIFIC ALLOANTIBODIES INDUCED BY BLOOD TRANSFUSIONS IN HUMANS

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One "member" of the family of Minor Histocompatibility Antigens, namely the complex system of trophoblast-leukocyte cross reacting (TLX) antigen with a possible functional association with the Fc receptor (FcR), Membrane Cofactor Protein and CD46 at the DNA level came into focus of interest most recently. TLX specific antibodies possess a powerful FcR and MLC blocking function, which can also be utilized as an indirect tool for TLX determination.

The blocking effect of the sera of healthy volunteers, renal transplant recipients and women with recurrent habitual abortions after blood transfusions was studied on MLC, Erythrocyte Antibody Inhibition assay. Sixty two HLA-typed random healthy individuals were tested with TLXB allosera to determine the frequency of TLX-B allotypes in the Caucasian population.

We found that both, buffy coat and platelet transfusion evoked production of IgG type alloantibodies lacking any correlation with either class I or class II specific cytotoxic antibodies. The frequency of TLX-B1, B2, B3, B4 and B5 allotypes was determined in the Caucasian population. TLX-B alloantibodies were found in the sera of pregnant women as well, but not in the cases of recurrent habitual abortions. Preincubation or isolated stimulator and effector cell population with blocking alloantibodies showed that the latter are involved in the mediation of inhibition in the MLC test. Moreover, the blocking effect on MLC is detectable only when effector cells express the alloantigens corresponding to the specificity of the sera used. These alloantibodies can be produced only in cases when donor-recipient pairs and couples mismatch in this antigen system.

PAPER 9

CYTOKINE AND NEOPTERIN PRODUCTION DURING ALLOGENEIC MIXED LYMPHOCYTE REACTIONS : EFFECT OF ENDOTOXIN CONTAMINATION.

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Cytokines and neopterin are elaborated during the course of mixed lymphocyte reactions (MLR), and there is considerable interest in the role of cytokines as mediators of allogeneic responses. Neopterin is a metabolite in the synthetic pathway of biopterin, and increased levels have been shown to correlate with immune activation, both in vivo and in vitro (1,2). In this study, the secretion of various cytokines (IL-1a & 1-b, IL-2, Il-6, IFN-g, TFN-a & -b) was monitored during MLR and control responses and compared with soluble IL-2 receptor (IL-2R) and neopterin production. It was discovered that the initial experiments had used blood contaminated with endotoxin during collection. Later experiments were performed under endotoxin-free conditions.

Under endotoxin-free conditions, allogeneic stimulation resulted in the generation of IFN-g, TNF-a & -b, soluble IL-2R and neopterin. The cytokines and soluble IL-2R were first detected between days 2 and 3 of the MLR and attained peak values by day 5. Neopterin was measurable from day 3 and gradually increased over the course of the MLR. In both MLR and control cultures, endotoxin contamination lead to enhanced cell proliferation (assessed by thymidine incorporation) and a different pattern of cytokine production. In both MLR and control cultures IL-1b, IL-2, IL-6 and TNF-a were detected from 3 hours, reaching a peak by days 1 to 2. In the MLR cultures, this early production was followed by further production of IL-2, IFN-g and TNF-a & b. Neopterin was detected in both MLR and control cultures, contaminated with endotoxin.

REFERENCES

1. Huber, C. et al (1984) J exp.Med. 160, 310-316.
2. Bron, S. et al (1988) Acta.clin.Belgica. 43, 120-126.

PAPER 10

DELAYED GRAFT FUNCTION DELETERIOUSLY AFFECTS RENAL ALLOGRAFT SURVIVAL

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There are conflicting reports regarding the effect of delayed graft function (DGF) upon renal allograft survival. The aim of this study was to analyse this possible effect in 454 consecutive transplants performed in a single centre from January 1986 - June 1990. Patients with first grafts (n=387) were given CyA and those with a subsequent graft (n=67) CyA and prednisolone or triple therapy. DGF was defined as the need for dialysis in the first week post-transplant.

274 (60%) patients had immediate function (IF) and 180 (40%) DGF. One year graft survival was significantly better in the IF group (248/274, 91%) compared to the DGF group (123/180, 68% $X^2=35.76$ $p<0.001$). When non-immunological failures and grafts that never functioned were excluded, 1 year graft survival was still better in the IF group (248/267, 93%) compared to the DGF group (123/155, 79% $X^2=16.9$ $p<0.001$). DGF lasted from 2-60 days with a mean of 13.6 days [sd 9.2], but the duration did not significantly affect one year graft survival. One year patient survival was 97% in the IF group and 90% in the DGF group ($p<0.01$).

DGF, even when non-immunological failures and non functioning grafts are discounted, has a significant detrimental effect upon both graft and patient survival at one year. Measures that are known to reduce the incidence of DGF should be aggressively undertaken.

PAPER 11

DOES EARLY REJECTION INFLUENCE RENAL ALLOGRAFT SURVIVAL?

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Acute allograft rejection remains an important cause of graft loss despite advances in immunosuppressive therapy. 454 consecutive renal transplants performed in a single centre from January 1986 to June 1990 were analyzed. Does the day of 1st rejection or the number of rejection episodes in the first month post-transplant influence graft survival. Patients with first grafts (n=387) were given CyA and those with subsequent grafts (n=67) CyA and prednisolone or triple therapy. Typically the first two rejection episodes were diagnosed on clinical grounds and treated with pulse methylprednisolone; subsequent episodes were diagnosed following biopsy and treated with ATG, OKT3 or steroids.

137(30%) of patients had no episodes of rejection in the first month and a 1 year graft survival of 77%, corrected to 88% when non-immunological failures (NIF) were excluded. For 1 or 2 episodes of rejection 1 year graft survival was 89%. Overall 1 year graft survival was significantly better in those patients with ≤2 rejection episodes (83%) compared to those with >2 (67% $X^2=7.84$ $p<0.01$, and when NIF excluded $X^2=17.7$ $p<0.001$). 1 year patient survival was 96% in both groups. The number of graft had no significant effect.

142 patients had their 1st rejection episode before day 5, 115 between days 5-10, and 60 after day 10. 1 year graft survival was not significantly different in any group even when number of graft or NIF were considered.

In conclusion >2 episodes of rejection in the first month was predictive for poor graft outcome. The occurrence of rejection before day 5 does not necessarily indicate aggressive rejection. The effect of 1 or 2 rejection episodes upon long term graft survival needs to be examined.

PAPER 12

WHAT FACTORS INFLUENCE THE SUCCESS OF SECOND TRANSPLANTS?

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In a single centre from April 1968 to December 1988, 110 patients received a second renal transplant after failure of their first graft. We have examined donor and recipient variables relating to the first and second transplants in an attempt to identify what factors affect the outcome of the second graft. Donor age, sex, blood group, length of ischaemic times, and cause of death along with recipient age, sex, blood group, cause of renal failure, duration and form of dialysis, HLA match, cytotoxic antibodies, type of immunosuppression, primary non-function, number of rejection episodes and cause of graft failure for both the first and second transplants were analysed. In addition whether or not a transplant nephrectomy had been carried out prior to the second transplant and the duration of the first transplant success were included in a Cox's multiple regression model for second graft survival.

In patients whose second graft was lost due to rejection, the number of rejection episodes associated with that transplant correlated significantly with graft loss ($p<0.0001$).

As we expected the use of cyclosporine in second transplant recipients significantly improved graft survival ($p=0.04$) when compared with second transplant recipients receiving azathioprine and prednisolone. However, we also found that the survival of the second graft was improved in those patients who had a transplant nephrectomy performed when the first graft had failed ($p=0.015$).

We conclude that the success of second transplants can be improved by not only optimising immunosuppression to reduce rejection episodes but also by removing the first graft.

PAPER 13

DUPLEX SONOGRAPHY ON DAY 2 FOLLOWING RENAL TRANSPLANTATION: A PREDICTION OF RESISTANT REJECTION?

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Thirty six renal allograft recipients were monitored by serial duplex Doppler ultrasound studies post-transplantation and during early rejection. A separate reproducibility study demonstrated no significant inter or intra-operator variability in measurements of resistive index of the interlobar artery (RI) (2.1% [1.5%] and 3.2% [2.3%] respectively, mean [standard error] of coefficients of variance).

Twenty one patients had rejection within 3 weeks of transplantation. These grafts showed greater overall rises in the RI, from day 2 to day 5 post-transplantation, than the grafts which had no rejection. Eleven of the 21 patients required more than one course of methyl prednisolone for persistent or recurring rejection. These grafts had higher RI on the day rejection was diagnosed (81 [7.3], median [interquartile range]) than the remaining 10 patients (68.6 [8.7]. The 11 grafts with persistent rejection had higher RI ($p<0.005$, Mann-Whitney U-test) on day 2 post-transplantation (76 [3.9]) than the 10 grafts successfully treated with a single course of methyl prednisolone (63.2 [10.9]).

This study demonstrates that grafts with a RI of greater than 70 on day 2 post-transplantation are likely to have rejection requiring additional treatment (sensitivity - 100%, specificity - 80%). These patients may be candidates for earlier or alternative anti-rejection therapy.

PAPER 14

PRESSURE FLOW STUDIES IN THE EVALUATION OF URETERIC OBSTRUCTION IN RENAL ALLOGRAFTS

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Dilatation of the pelvicalyceal system may occur in up to 43% of renal allografts and presently most centres investigate this dilatation by antegrade pyelography. The significance of a radiologically demonstrated ureteric narrowing is, however, unclear. During the past 16 months 13 of 117 transplants have been studied by a pressure-flow study concurrent with the antegrade pyelography to assess the functional relevance of pelvicalyceal dilatation and ureteric stenosis. After noting the resting pressure in the collecting system, contrast medium was infused through a 22G spinal needle using at a constant rate of 10 ml/min. A second needle in the pelvicalyceal system measured the pressure change induced by the infusion. A rise of more than 7 mm Hg at the equilibrium pressure was considered to show a functionally significant ureteric obstruction (Whitaker 1973)1. The results are shown in the table below:

ANTEGRADE STUDY	PRESSURE-FLOW STUDY	FINAL DIAGNOSIS
7 obstructed	obstructed	obstruction
4 obstructed	not obstructed	acute rejection
2 no obstruction	no obstruction	1 chronic rej 1 nephro-toxic

Pressure-flow studies are a useful adjunct to current methods of investigation of ureteric problems in renal allografts.

1) Whitaker RH. Methods of assessing obstruction in dilated ureters. BJU 1973;45:15-22

PAPER 15

HLA-DP MATCHING IN RENAL TRANSPLANTATION

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A retrospective analysis of HLA-DP matching on first cadaver renal graft survival was performed in a single centre study. HLA-DP typing was performed on DNA, amplified by polymerase chain reaction, using a battery of 19 probes recognising 19 specificities. The G.S. at 1 year for patients with 0 DP mm was 82.6% (19/23), for patients with 1 DP mm was 77.5% (69/89) and for patients with 2 DP mm 80.6% (29/36). The number of HLA-A,B,DR mismatches was similar in each group.

A further study was performed through the auspices of the CTS study. Donor and recipient pairs with 0 mm at HLA-A,B and -DR loci by serology were checked for HLA-DR by RFLP. Those pairs which had still 0 mm were examined for HLA-DP antigens. In total 25 transplants with 0 DP mm, 41 transplants with 1 DP mm and 7 transplants with 2 DP mm were found. Of these, 21 transplants with 0 DP mm, 31 transplants with 1 DP mm and 3 transplants with 2 DP mm were first cadaver transplants. No difference in graft survival was found according to the number of DP mismatches considering first or subsequent transplants.

The effect of HLA-DP matching was also examined in related (either parent to child or sibling to sibling)first transplants. Again no effect of matching was found. 52 transplants had 0 DP mm, 7 transplants had 1 DP mm and 2 transplants had 2 DP mm. These transplants had zero HLA-A,B,DR (by RFLP) mismatches.

PAPER 16

INCIDENCE OF ANTI-EPITHELIAL CELL ANTIBODY IN PAEDIATRIC RENAL PATIENTS AND NORMAL CHILDREN

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We have previously reported a correlation between an IgM antibody directed against epithelial cells and renal allograft rejection in our paediatric transplant patients. In this study we have investigated the incidence of anti-epithelial cell antibody (AECA) in all paediatric patients receiving transplants between 1987-89, in paediatric renal patients and normal children between 1989-91.

All transplanted children were tested for AECA using a serum sample taken on day of transplant or the cross-match sample. The incidence of AECA in these patients was 4/34 in 1987, 6/23 in 1988 and 9/25 in 1989. This increase in AECA positive children corresponds with a decrease in 1 year graft survival during this period.

Samples taken from 55 normal children in 1989 were obtained and 35 further normal samples were collected between Oct 90 - Feb 91. These were compared with samples from 42 paediatric renal patients awaiting transplant which were taken at comparable times. In 1989 65% of normal children and 55% of patients were AECA positive, with a larger proportion of both groups being positive in Nov/Dec as compared with Jun/Jul. In 1990/91 60% of normal children and 48.8% of patients were AECA positive.

These results confirm that the incidence of AECA increased in 1989 and that this antibody in not confined to our patient population but is also found in a comparable proportion of normal, healthy children.

PAPER 17

THE INCIDENCE OF ANTI-EPITHELIAL CELL ANTIBODIES IN PAEDIATRIC CONTROLS

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Anti-epithelial cell antibodies (AEC), reactive with the epithelial cell line A549, have been reported in association with paediatric renal transplant failure[1,2]. However, there has been no published data on the incidence of these antibodies in children who are not on renal replacement therapy.

Using an epithelial cell microcytotoxicity assay[1], we have screened 420 sera collected from children. They were collected by virology laboratories throughout England during 1989 as part of their ongoing serological surveillance for mumps, measles and rubella antibodies. They were randomly selected by the virology laboratories as residual sera from samples submitted for other investigations. There were 60 samples, 30 males and 30 females, from each of the age groups: 1<3, 3<5, 5<7, 7<9, 9<11, 11<13, 13<15. All sera were screened against A549 and those positive were further screened against peripheral blood lymphocytes (PBL) to check for the presence of HLA and autoreactive lymphocytotoxic antibodies which would also react with A549.

198 of the 420 sera (47%) were positive for the presence of AEC. The 33 sera (8%) which also reacted with lymphocytes were considered as being AEC negative. There was no difference in the number of AEC positives between males and females (49.5% and 47.6% respectively). Of the 60 samples tested for each age group, the number of positives was 1<3, 21; 3<5, 29; 5<7, 40; 7<9, 32; 9<11, 33; 11<13, 24; 13<15, 19.

This high level of AEC in a paediatric control population further emphasises the need to identify the target antigen and raises the question of its role in paediatric transplant failure.

[1] S Martin et al, Tissue Antigens 1991: 37; 152.
[2] AW Harmer et al, Transplant Int 1990: 3; 66.

PAPER 18

IS THE A549 CROSSMATCH IMPORTANT IN RENAL TRANSPLANTATION?

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An antibody reacting with the epithelial cell line A549 has been linked with a high incidence of accelerated graft failure in paediatric patients. In this report we have investigated this antibody reactivity by a conventional complement crossmatch method in transplanted patients and those awaiting transplant.

In sera from 85 chronic renal failure patients only 7% were strongly positive, 7% with weak reactivity and 86% negative for anti A549 reactivity. There was no significant correlation between anti A549 antibody and dialysis therapy nor number of graft failures. In those patients positive against A549, 5/6 were elderly males.

In transplanted patients (n=132) 27% (36/132) were positive, 19% (25/132) showed weak binding and 54% (71/132) were negative for anti A549 antibody.

Patients negative (25/73) for A549 were regrafts compared to 4/36 for patients strongly positive for A549 antibody. The incidence of graft rejection was no different in either group and there was no correlation with CMV status either of donor or recipient. There was no correlation with panel antibody reactivity.

Our experience to date, with adult transplants, is that anti A549 antibody does not contribute significantly to graft loss or dysfunction and therefore a positive A549 crossmatch is not a contraindication to transplantation.

PAPER 19

THE SIGNIFICANCE OF THE FLOW CYTOMETRIC CROSSMATCH IN SENSITISED RENAL TRANSPLANT RECIPIENTS

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Previous reports on the flow cytometric crossmatch (FACS-XM) have failed to show a significant correlation between a positive FACS crossmatch and graft failure. These studies have not made a distinction between sensitised and non-sensitised patients. We have specifically targeted those patients with high panel reactivity, previous transplants or pregnancies for FACS-XM.

Out of over 300 FACS-XM performed the data on 37 sensitised patients who proceeded to transplatation are presented. Eight of 37 patients had a positive T-Cell FACS-XM, all were negative by conventional cytotoxic crossmatch. Seven of these lost their grafts, in all but one case the graft was lost at less than 30 days. The one graft which is currently surviving at 2.5 years was an HLA identical graft and the positive crossmatch cannot, therefore, be attributed to HLA specific antibodies. Five grafts were lost in the 29 FACS-XM negative patients.

These results demonstrate a significant correlation ($p=0.0005$) between a positive FACS-XM and graft failure in sensitised patients and we would regard a positive crossmatch in such patients as a contraindication to transplantation.

PAPER 20

RENAL TRANSPLANTATION AFTER IMMUNOADSORPTION IN HIGHLY SENSITISED RECIPIENTS

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Twenty per cent of potential renal allograft recipients possess high titres of pre-formed cytotoxic antibodies (CAB) which preclude transplantation. Immunoadsorption (IA) with protein-A is a novel strategy to de-sensitise these patients and we report three cases who have been transplanted following treatment.

All three patients were highly sensitised with no evidence of spontaneous reduction in CAB titres. IA was performed on a Citem 10 system and there were no adverse affects. Treatment of 18-40 (mean 27) litres of plasma over 4-7 (mean 6) treatment sessions reduced the titre of CAB to <1:8 in all patients and abolished reactivity to non-prohibited antigens. One patient required re-treatment with IA following CAB resynthesis. Immunosuppression, from day 1 of IA, was prednisolone and cyclophosphamide. Transplantation was performed at 125, 6 and 270 days after starting therapy, when immunosuppression was changed to prednisolone, cyclosporin-A, and anti-lymphocyte globulin - which was replaced by azathioprine after 10 days. Satisfactory allograft function has been maintained for 26 and 20 months in two patients, and the third patient still has primary graft dysfunction, due to acute tubular necrosis, at one week post transplantation.

IA permits transplantation of selected highly sensitised patients.

PAPER 21

DISCREPANCIES IN PERIPHERAL BLOOD LYMPHOCYTE CROSSMATCH RESULTS AS COMPARED WITH SPLENIC LYMPHOCYTE CROSSMATCHES

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The use of cadaver donor peripheral blood lymphocytes (PBL) for prospective crossmatching may be beneficial for cardiac and pancreas transplantation when ischaemia times must be minimised. Following a report of inaccuracies in crossmatching using PBL[1], this study was undertaken to assess the suitability of PBL as compared with splenic lymphocytes (SPL) for crossmatching.

10 donors (D) were retrospectively crossmatched against 23 renal patients (R) (7D each vs. 2R and 3D each vs. 3R) who had been selected at the time of organ retrieval as having ≤ 3 HLA antigens mismatched. D/R pairs were selected so that for each donor a positive and a negative initial SPL crossmatch were represented. 241 sera from 23 patients were crossmatched against Dynabead separated PBL T and B cells and SPL.

For 18 of the 241 (7%) sera from 6 of the 23 (26%) patients the PBL crossmatch was negative whilst the SPL was positive at >50% kill. For 3 of these 6 patients all of the sera tested were negative with PBL, so a PBL crossmatch would have been falsely reported as negative.

These results strongly support the previous report[1] that PBL are not suitable as the cellular material for D/R prospective crossmatching.

We will continue to use splenic lymphocytes for all prospective crossmatching prior to cadaveric transplantation.

[1] S Hibbett, D Phelan, T Mohanakumar
Clinical Histocompatibility Workshop, Palm Springs, February 1991

PAPER 22

ALLORECOGNITION OF ISOLATED DENATURED CHAINS OF CLASS I AND CLASS II MHC MOLECULES

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The heavy chain of RTI-A classical class I and the α and ß chains of RTI-B class II molecules of the DA (RT^{av1}) strain were prepared from DA spleens by a combination of monoclonal antibody affinity, lentil lectin affinity and gel permeation chromatography followed by boiling in SDS and preparative electrophoresis in SDS polyacrylamide gels. LEW RTI^{1} rats immunised with the isolated chains in Freunds adjuvant produced strong antibody responses in the denatured chains, but these antibodies did not cross-react with whole RTI-A class I or RTI-B class II molecules of the DA strain. Immunisation with each of the 3 chains resulted in second set rejection of DA skin allografts. Immunisation with the isolated chains, class I as well as the α and ß chains of class II, resulted in accelerated antibody responses to whole, undenatured DA class I molecule after DA skin grafting, suggesting "carrier" priming of the T helper cells. These results demonstrate that indirect allorecognition can play an important role in allograft rejection.

PAPER 23

ALLOGRAFT REJECTION BY INDIRECT ALLORECOGNITION OF DONOR MHC PEPTIDES.

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LEW (RTI^{1}) rats were immunised with free, unconjugated, 22-24 amino acid peptides corresponding to the α helical region of the first domain (peptide 1) and the ß sheet (peptide 2) and α helical region (peptide 3) of the second domain of the RTI-A classical class I molecule of the DA (RTI^{av1}) strain. Peptides 1 and 3 induced strong primary and secondary antibody responses, while peptide 2 induced only weak secondary antibody responses. None of the anti peptide antibodies cross-reacted with whole RTI-A^{av1} class I molecules. All 3 peptides induced a LEW APC-dependent $CD4^{+}$ T cell proliferation response, this being very strong with peptide 1 and weak with peptide 2. LEW rats immunised with peptides 1 and 3, and especially with a combination of peptides 1 plus 3, showed accelerated rejection of DA skin grafts. Interestingly, peptide immunised rats had accelerated kinetics of antibody production to the RTI-A^{av1} class I molecule, in response to the DA skin graft, suggestive of "carrier" type priming for antibody production. These results demonstrate that indirect allorecognition can play an important role in allograft rejection and point to some possible mechanisms whereby it can influence effector mechanisms.

PAPER 24

ORGAN SPECIFICITY OF SUPPRESSOR T CELLS

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Suppressor T cells (Ts) are present in the spleens of rats which have accepted an allogeneic kidney or heart graft under cyclosporine immunosuppression. These Ts are demonstrated in adoptive transfer experiments.

Origin of Ts cells	Fate of grafts in DA rats given Ts cells			
	LEW kidney	3° party	LEW heart	3° party
DA rat tolerant of LEW kidney	Accept	Reject	Reject	Reject
DA rat tolerant of LEW heart	Reject	Reject	Accept	Reject

Suppression is donor specific, as expected but, remarkably, is also organ specific. The simple interpretation is that organ-derived peptides are seen in association with allogeneic MHC molecules so that DA Ts have receptors for LEW plus kidney peptide or LEW plus heart peptide rather than just the LEW alloantigen molecule alone. This exquisite fine specificity of T cells could be generalised. Hence, extrapolating, it might be that different components (vascular endothelium, parenchymal cells) of an organ graft might be recognised by different subsets of allospecific T cells.

PAPER 25

T CELL OLIGOCLONALITY IN HUMAN KIDNEY TRANSPLANT BIOPSIES

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There have been several reports that T cells from human kidney biopsies stimulated with donor alloantigens in vitro show oligoclonal patterns of T cell receptor (TCR) gene rearrangements. However, it is not certain whether T cells expressing particular TCR V genes are specific for particular HLA mismatches. The aim of this study was to investigate whether T cell populations infiltrating kidney transplants during a rejection episode express a limited range of TCR Vβ genes. T cells were extracted from needle biopsies taken from kidneys of transplant patients, in most cases during a rejection episode. They were stimulated polyclonally and grown in the presence of interleukin-2 in vitro for 2-3 weeks. DNA was extracted and subjected to Southern blot analysis using TCR Cβ and Jγ gene probes. T cells were also cloned directly from biopsies by limiting dilution andCD4 and CD8 expression determined prior to DNA analysis. Around 50% (n=60) of biopsies generated T cell lines and in all cases an oligoclonal pattern of TCRβ gene rearrangements was found as compared to peripheral blood. However, $CD4^+$ or $CD8^+$ T cell clones from the same biopsy mostly had different rearrangements. This would suggest that although biopsy T cell populations are oligoclonal these are not composed of cells expressing the product of a single TCRβ gene.

PAPER 26

THE INFLUENCE OF THE ANGIOTENSIN CONVERTING ENZYME INHIBITOR ENALAPRIL AND A THROMBOXANE RECEPTOR ANTAGONIST (L607596 MERCK, SHARPE AND DOHME) ON CYCLOSPORIN NEPHROTOXICITY

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Using a new model employing repeatable single injection isotopic techniques for measuring glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) we have previously shown that low doses of cyclosporin reduce GFR, ERPF and filtration fraction (FF), and that this was incompletely reversed by Nifedipine. Here we gave enalapril or L607596.

Six groups of 8 Sprague-Dawley rats received cyclosporin 7.5 mg/kg/day or cremaphor vehicle ip, with either enalapril 5 mg/kg/day, L607596 20 mg/kg/day or their vehicles. GFR and ERPF were measured before and after 14 days of treatment.

The double vehicle group and the L607596 group showed no change in GFR, ERPF or FF, whereas ERPF increased in the enalapril group ($p< 0.05$ paired T-test). The cyclosporin group suffered falls in GFR (mean 59%), ERPF (mean 41% both $p < 0.0001$) and FF (mean 30%, $p < 0.001$). In the cyclosporin and enalapril group, ERPF was restored but there were still large falls in GFR (mean 72%, $p < 0.005$) and FF (mean 42%, $p < 0.005$). The L607596 and cyclosporin group had normal GFR and FF, and although there was a fall in ERPF (mean 10%, $p < 0.05$), this was smaller than in the cyclosporin alone group ($p < 0.0001$, unpaired T-test).

Whilst enalapril did not protect GFR or FF from the effects of cyclosporin, L607596 was highly effective.

THE HUMAN FOETAL PANCREAS: BENEFITS AND DANGERS OF PRETREATMENT BY TISSUE CULTURE

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Clinical transplantation of the human foetal pancreas (HFP) has been unsuccessful so far. Rejection was probably a major cause of failure as there is evidence that the HFP is immunogenic soon after its formation at 7 weeks gestation. Conventional tissue culture using 5% CO_2 in air at 37°C is generally regarded as a safe form of immunomodulation. We investigated its effects on the expression of the major histocompatibility complex antigens in the HFP as these have not been documented previously. In addition, we compared the responses of fresh and cultured HFPs to stimulation with Interferon-gamma (IFN-g). Our results showed that (1) conventional tissue culture depleted the HFP of Class II* interstitial cells, (2) loss of these cells was more complete in first trimester HFPs, (3) IFN-g induced the expression of cryptic Class I and Class II antigens in the fresh HFP and that, surprisingly, (4) preliminary culture of the HFP markedly accelerated and enhanced this response.

Our findings might explain the lack of benefit from immunomodulation of the HFP by conventional tissue culture despite its ability to reduce the number of immunostimulatory cells in the explants.

PAPER 28

FIVE YEAR EXPERIENCE OF WHOLE ORGAN PANCREATIC TRANSPLANTATION IN DIABETIC RECIPIENTS

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Eleven patients have received whole organ pancreas grafts in our institution in the last 5 years. These patients were carefully selected as being those most likely to benefit from the procedure. The Sollinger technique was used, with exocrine drainage being directed into the bladder via a duodenal button (7 cases) or a duodenal segment (4 cases). There were 6 males and 5 females with a mean age of 38 years. Seven had had previous kidney grafts and were transplanted with a pancreas only. The last 4 received a kidney and a pancreas from the same donor.

The first 3 pancreatic grafts were lost after 2 days, 4 months and 2 months respectively, from portal vein thrombosis (1) and rejection (2). Seven of the 8 subsequent grafts are currently functioning 3 months to 4½ years post-transplantation. Five have survived for over 3 yeasrs. All are insulin independent with a mean serum glucose of 5.2 mmol/L, and a mean serum creatinine of 157 umol/L. Two of the early patients who rejected their transplants died over two years later of unrelated causes. Kidney graft survival was 100% at 2 years. In 22 diabetic patients who received just a kidney transplant during the same period, it was 76%.

It is our experience that pancreatic transplantation can be safely performed in selected diabetic recipients, and can achieve long term reversal of the diabetic state.

PAPER 29

PANCREATIC TRANSPLANTATION IS THE BEST TREATMENT FOR END STAGE RENAL FAILURE DUE TO TYPE I DIABETES

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Between 1983 and 1991, 20 simultaneous pancreaticorenal transplants were performed in patients with end stage renal failure due to type I diabetes mellitus using Cyclosporin monotherapy as first line immunosuppression. Multiple pre-operative factors were assessed with respect to outcome in pancreaticorenal recipients (Gehan-Wilcoxon Test). Seven received thorough cardiac assessment pre-operatively. The outcome was compared to 23 diabetic recipients on CAPD who received renal transplants only. Three year patient (100%) and renal allograft (75%) survivals were similar in cardiac screened pancreaticorenal transplant recipients and renal only recipients. Both groups were significantly better than non-cardiac screened pancreaticorenal allograft recipients and patients maintained on dialysis. Pre-operative history of cardiac disease ($p = 0.013$) and CVA ($p = 0.007$) adversely affected prognosis in pancreaticorenal recipients, and younger recipient age was associated with favourable outcome ($p = 0.008$).

Improved outcome of combined pancreaticorenal transplantation can be achieved by careful pre-operative cardiac assessment. With the improved quality of life and good renal allograft survival in these patients, a controlled trial of renal allograft versus pancreaticorenal allograft in type I diabetic renal failure patients would be justified.

PAPER 30

SEQUENTIAL SINGLE LUNG TRANSPLANTATION: PROCEDURE OF CHOICE FOR END-STAGE SEPTIC LUNG DISEASE

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Since the summer of 1990 we have applied a new procedure, the sequential single lung transplant (SSLT) to patients with end-stage suppurative respiratory failure.

There have been three male and three female recipients, mean age 32, with pre-transplant diagnoses of cystic fibrosis (CF) in four and bronchiectasis of unknown aetiology in two. There was one early death from multi-organ failure in the first 24 hours, in a recipient who was septic at the time of transplantation. Follow-up is from one to eleven months: the longest survivor is currently in hospital with respiratory failure from obliterative bronchiolitis (OB) but the other four are fully rehabilitated. Pulmonary function in these four patients is normal.

The transplant was performed through a "clam-shell" transverse sternum splitting thoracotomy in five patients, and separate postero-lateral thoracotomies in one: cardio-pulmonary bypass was only required in four patients. The separate bronchial anastomoses have healed without difficulty although one distal endobronchial stent was required.

This procedure has several advantages over the conventional combined heart/lung transplant. The transverse incision gives excellent access and bleeding from dense pleural adhesions, a major problem in this group of patients, was minimal except for the septic patient who died. The recipient retains their own innervated heart: all donor hearts were used for other recipients. With anastomoses at hilar level the mediastinum is hardly entered and vagal damage avoided. CF patients in particular provide a major challenge for lung transplantation and the SSLT is a significant advance in their management.

PAPER 31

BILIARY ANASTOMOSIS FOLLOWING LIVER TRANPLANTATION DOES NOT BENEFIT FROM T TUBE SPLINTAGE

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The results of different techniques of biliary anastomosis were assessed in seventy-four consecutive liver transplants. In sixty-five patients a direct end-to-end anastomosis was fashioned between donor and recipient common bile ducts. The first 16 anastomoses were splinted with a T tube; no splint was employed in the remaining 49 patients. In two patients early in the series a biliary conduit was fashioned from the gall bladder, and in six patients biliary drainage was via a Roux-en-Y jejunal loop. One patient succumbed to pulmonary hypertension during surgery and no anastomosis was performed. There were no biliary leaks from the two conduits or from the six jejunal loops. Three of 16 patients with splinted duct-to-duct anastomoses sustained leakage, requiring Roux loop conversion in two and removal of the T tube in one. Seven of 49 unsplinted anastomoses leaked, necessitating Roux loop conversion in 6 and percutaneous drainage in the remaining patient. Biliary leakage was invariably due to donor duct necrosis. No strictures occurred following duct-to-duct anastomosis.

There was no difference in leak rate between splinted and unsplinted anastomoses ($p = 0.98$, corrected Chi-square test). The groups were well matched for age, sex and indications for transplantation. It is concluded that T tube splintage confers no advantage in direct duct-to-duct anastomosis following liver transplantation.

PAPER 32

DYNAMICS OF SERUM IL-I AND IL-2R-CELLS IN PATIENTS BEFORE AND AFTER RENAL ALLOTRANSPLANTATION

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Quantitative parameters of various subpopulations of immunocompetent cells were investigated by means of cytochemical ANAE (alpha-naphtylacetate esterase) test and indirect immunofluorescence method using OKTII, OKT4, OKT8 and OKT26a monoclonal antibodies to determine the percentage of cells expressing IL-2 receptors (IL-2R). IL-I blood concentration was determined by ELISA method using specific test-systems. 2I healthy subjects, I8 pre-transplant chronic renal failure (CFR) patients and 20 posttransplant patients have been examined.

It was found that the percentage of IL-2R-carrying cells in primary recipients (5.I3+I.7I%) did not differ from the norm actually (8.33+I.2I%) while in secondary recipients it was decreased significantly (2.2I+0.8%).

In patients with a renal transplant the number of Tac-positive cells increased, as a rule, during the development of rejection reaction (6-I0%). In the case of Cyclosporin A (Cs-A) overdosage detected by Cs-A serum concentration no IL-2R-positive cells were found actually. Increased IL-2R expression was also noted when posttransplant infectious complications developed. The said cases of increased percentage of IL-2R carriers were often accompanied by an elevation of OKT8 cells values and/or those of lymphocytes with diffuse ANAE enzyme distribution which is typical of suppressor cells population.

Blood IL-I was lowest in early posttransplant period with prevetive immunosuppression and normal transplant function (0.025-0.I ng/ml). A high IL-I level was noted during the development of rejection reaction in patients with infectious complications (up to I60 ng/ml).

The findings showed that the determination of IL-2R-positive cells and serum IL-I level could be used in the diagnosis of posttransplant complications (acute rejection reaction, Cs-A overdosage, infectious complications).

POSTER

FREQUENCY OF TYPE 1 DIABETES IN AN AMERICAN HISPANIC GROUP IS DETERMINED BY ETHNIC ADMIXTURE AND THE PRESENCE OF THE CAUCASIAN A1,B8,DR3 HAPLOTYPE.

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Type 1 (insulin-dependent) diabetes is caused by both environmental and genetic factors. We have measured the frequencies of HLA antigens in diabetics and non-diabetics from two different ethnic groups, non-Hispanic whites (NHW) and Hispanics living in the same area of Colorado, USA. There is a significant difference in incidence of the disease in the two groups: NHW, 15.4/100,000/year and Hispanic, 9.7/100,000/year. The frequencies of certain HLA antigens, significantly, HLA-A3, -A24, -B8, -B35, -B38, -DR3 and DQw6 differ between the groups (non-diabetics; $P<0.05$) confirming the native Indian origin of the Hispanic group. There are no significant differences between antigen frequencies in NHW and Hispanic diabetics, except for those antigens that differ in frequency between the control groups, implying that the disease is immunogenetically similar in both groups. The DR3 antigen and the HLA-DQA1 allele, A3 were associated with type 1 diabetes ($P<0.05$) in NHW and Hispanics. DQw8 was increased in Hispanic diabetics only ($P<0.001$). DR5 and DQw7 were decreased in frequency in Hispanic patients only ($P<0.05$). DQw6 was decreased in frequency in NHW diabetics only ($P<0.001$). Most strikingly, despite the absence of the diabetogenic haplotype A1,B8,DR3 in the Hispanic controls (0/47 compared to 9/65 NHW controls), 13/65 (20%) of the Hispanic diabetics are positive for this haplotype (OR=27.0; CI 13.2-54.8; $P=0.02$). The absence of B8,DR3 haplotypes in the Hispanic population could be a major cause of the lower incidence of type 1 diabetes. Oriental and native Indian populations, which do not have admixture have even lower frequencies of type 1 diabetes and are devoid of the A1,B8,DR3 haplotype. These data indicate that a significant portion of HLA-associated susceptibility to type 1 diabetes in this Hispanic group is of European origin and support a role of genetic determinants in ethnic and geographical variation in the incidence of type 1 diabetes.

POSTER

HLA AND HEPATITIS B VIRUS INFECTION

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Approximately 90% of adult Hepatitis B Virus (HBV) infections are self-limiting, resulting in protective immunity and the development of antibodies against the HBs and HBc antigens. The remaining 10% of adult infections show incomplete immunity and the development of a chronic, infective carrier state, characterised by the persistence of detectable HBsAg. These may be asymptomatic or be associated with chronic active hepatitis. Chronic carriers of HBV have normal immune function against a standard range of stimuli; suggesting a specific anergy or immune deficiency.

This presumably represents a specific inability in certain individuals to generate an effective immune response to HBV, classically a T cell mediated reaction subject to MHC restriction via class I and class II HLA determinants; this suggests a potential role for particular HLA antigens in susceptibility to chronic HBV infection. In the gulf state of Qatar, HBV is endemic with an estimated 16% of the population exposed to infective doses at some time in their adult life.

We have studied the HLA phenotype frequencies of three groups of Qataries, consisting of 31 patients with acquired immunity to HBV following infection, 21 HBsAg +ve chronic carriers and 100 normal, healthy controls. All were from the indiginous population, with ages ranging from 18 to 72. The frequency of DR2 was significantly reduced in chronic carriers compared with controls (46% vs 13%; $C^2=7.8$; $p<0.01$). RFLP analysis of Taq 1 digests demonstrated no particular prevalence of DRw15 or DRw16 in either group. There was a slight reduction in both subtypes of DR2 in the immune group also, although this was not significant. We have also observed a statistically significant increase in the frequency of DR7 in the chronic group compared with the immune (54% vs 16%; $C^2=8.7$; $p<0.01$) group. In the chronic group 25% of DR7 positive individuals are homozygous for DR7, compared with 0% in the immune group and 3% in the controls.

From these preliminary data, we suggest that DR7, or genes in close linkage disequilibrium with the HLA DR7 genes, confer susceptibility to chronic HBV infection, possibly by an inability of the DR7 molecule to present HBV antigen to effector T cells. The possession of DR2 may similarly bestow a degree of resistance to HBV infection.

POSTER

MULTIPLE SCLEROSIS IN JORDANIAN ARABS

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Considerable evidence exists to indicate genetic predispositions in the aetiology of MS. A gene or genes within the HLA region of the human MHC on chromosome 6p have been consistently implicated as factors governing susceptibility in Northern Europeans. In a recent study of 71 patients with MS from an area around London in the South East of England, using conventional serological methods and analysis of RFLP patterns of Taq 1 digested fragments hybridising with DRB and DQB cDNA probes, we demonstrated a statistically significant increase in the frequencies of HLA DRw15 and the associated HLA DQw6 compared with 100 healthy control subjects. These data agree with a number of previous reports implicating DR2 and DQw1 in MS.

A high incidence of MS is seen in the Jordanian Arab population. Our very early study in 1977 (Lancet i:1123) suggested an increase in the frequency of DR4 in this population compared with a control group.

In a recent study, we have analysed the HLA phenotype frequencies of 36 Jordanians with MS by serological and RFLP typing, and the complement C4 and factor B allotypes by high voltage gel electrophoresis.There were no significant differences in HLA or complement allotype frequencies between these patients and a control group consisting of 69 healthy volunteers. These results argue against the previously reported association with DR4. This may reflect the relatively crude serological definition of HLA class II alleles which existed for the early study.

However, the evidence from these and the London study is consistent with the hypothesis that the true disease susceptibility gene for MS is not a class II HLA allele, but lies elsewhere within the HLA region of the human MHC and in Northern European populations is found in significant association with DRw15 and DQw6.

POSTER

HLA ASSOCIATIONS WITH MENIERE'S DISEASE

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Meniere's disease is a disorder of the inner ear leading to sensorineural hearing loss and tinnitus and may manifest as unilateral or bilateral disease. Although the precise aetiology of the disorder remains unclear, the significant differences in the levels of circulating immune complexes and complement between Meniere's patients and controls, and the reports of significant HLA associations suggest that autoimmune reactions are involved. There is also a noted hereditary predisposition.

In a previous study (Xenellis J. *et al.* J. Laryngol Otol. 100 (1) 21-24: 1986) a strong positive correlation was observed between Meniere's disease and HLA Cw7. We have studied a consecutive series of 60 caucasian patients with Meniere's disease, 20 cases of bilateral disease and 40 unilateral cases. Diagnosis was based on the results of a full neuro-otological protocol and conformed to AAOO criteria. There were 31 males and 29 females with a mean age of 53.2 years. HLA A, B, Cw, DR and DQw typing was performed by conventional microcytotoxicity testing, complement C4 and factor B allotyping was by high voltage gel electrophoresis and immunofixation techniques.

Distinct differences were observed between the patients and a control group of 82 normal caucasians, and also between the bilateral and unilateral patient groups. We noted a highly significant ($p<0.01$) positive correlation with Cw7 and bilateral Meniere's patients, which agrees with previous reports, but only an association trend ($p<0.1$) in unilateral cases. There was also a highly significant ($p<0.01$) negative association in bilateral cases with HLA B12. In contrast, there was a highly significant positive correlation of B12 with unilateral cases. The incidence of B12 in the total Meniere's group was not statistically different from the control group. This possibly explains the absence of similar observations in previous studies. There were no differences in C4 or factor B allotype frequencies between disease and control subjects, or between bilateral and unilateral cases.

These results suggest that autoimmune factors are important in the pathogenesis of bilateral Meniere's disease. Our study confirms previous reports that Cw7 confers susceptibility to Meniere's disease , but suggests a possible protective influence for B12. These data also indicate that different HLA alleles may be involved in the pathogenesis of unilateral and bilateral Meniere's disease.

POSTER

GRAFT REJECTION ASSOCIATED WITH HLA-DP MIS-MATCHING IN ALLOGENEIC BONE MARROW TRANSPLANTATION.

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Using conventional criteria for establishing MHC identity in allogeneic BMT HLA mis-matching at the DP locus, secondary to genetic recombination may be overlooked. Although DP genes are expressed at lower levels than other class II genes they are known to be involved in GVHD and possibly also in graft rejection. We have recently observed a case of graft failure following allogeneic BMT from an apparently HLA matched sibling donor where HLA-DP disparity was retrospectively revealed by DNA analysis.
A 30 year old woman with AML(CR1) received an allogeneic BMT from her HLA matched sister. Conditioning consisted of cyclophosphamide and fractionated TBI(14.4Gy), donor marrow was T-cell depleted as prophylaxis against GVHD. On day 35 post BMT, following initial engraftment, her WBC fell dramatically and graft rejection mediated by recipient cells was documented by PCR analysis.
Genotypic identity was confirmed by Southern blotting at DQα, DQß and DRß, however genotypic disparity was noted at both DPα and DPß. This case demonstrates the occurrence DP mis-matching in the context of intrafamilial BMT secondary to genetic recombination and its potential role in causing graft rejection. The findings in a number of other matched related and matched unrelated transplants will be discussed.

POSTER

AN ANALYSIS OF THE SEROLOGICAL FINDINGS IN 21 CONSECUTIVE CASES OF NEONATAL THROMBOCYTOPENIA

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Between April 1990 and June 1991, 21 cases of Neonatal Thrombocytopenia were referred for serological investigation. Maternal platelet counts were universally normal (over 150 x 10^9/l) with the platelet count of the babies variably reduced. Tests on maternal samples included PLA1 phenotyping; anti-PLA1 screening and HLA screening by lymphocytotoxicity. When available, paternal blood samples were also investigated. Nineteen of the 21 cases are fully evaluable. A diagnosis of Neonatal Alloimmune Thrombocytopenia (NAIT) was established in 11/19 (57.9%) of cases. In the remaining 8, where maternal platelets typed as PLA1 positive, a non immunological cause for neonatal thrombocytopenia was established. Anti-PLA1 antibody was present in the sera of 9 of the 11 (81.8%) NAIT cases. Our findings in these cases are in keeping with other reported series. The number of cases of NAIT diagnosed in the period under review reflects the population of 3 million served by this Regional Transfusion Service.