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PAPER 1

ANALYSIS OF GENETIC FACTORS INFLUENCING 575 RENAL TRANSPLANTS IN A SINGLE CENTRE IN THE NORTH - WEST OF ENGLAND

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Many single and multicentre studies of renal transplantation have shown outcome to be multifactorial. The recognised problem of centre variation in multicentre studies has been avoided in this single centre study. A large number of transplants allows comprehensive analysis, but inclusion of transplants carried out during the formative years of renal transplantation introduces unintentioned bias.

Since 1968, 575 transplants have been done at this centre, and their overall one year actuarial graft survival (GS, Logrank programme) is 60.9%. Two hundred and ninety five of these transplants have been done since 1979 (GS, 65,2%).

We have searched our data for genetic factors influencing outcome. We considered donor and recipient ABO group and their combinations, donor and recipient sex and their combinations, recipient age at time of transplant, HLA-A,B and DR matching and recipient HLA-A,B and DR phenotype.

In the overall data analysis we used the Logrank statistic to compare variables. We found that female kidneys (in particular female kidneys transplanted to males) give lower survivals compared to male kidneys (GS 64.8% vs. 54.5% ;p = 0.017). We found a strongly beneficial effect of matching for HLA-A+B mismatch 67.2% and GS zero HLA-A+B match 33.3% ;p = 0.0038). The effect was strongest for matching at the HLA-B locus. As previously published, we cannot find a beneficial effect for HLA-DR matching. In addition we could find no "DR-w6" effect. The results of previous studies from this centre on matching for the Bf polymorphism are upheld in this data analysis.

Multivariate analyses (Cox regression models) on post-1978 data showed significant beneficial effects could be found only for living donor transplantation, primary grafting and the age of the recipient at the time of transplant. These three factors are by and large uncontrollable.

Reports claiming significant effects of a single factor on transplant outcome should be treated with caution. In exhaustive tests of our data, considering the most uniform clinical experience (post-1978), we can find no single genetic factor which is significantly beneficial; however we can identify clear trends towards improved graft survival through HLA-A+B matching.

PAPER 2

NON-CYTOTOXIC AUTOANTIBODIES AND ALLOANTIBODIES IN RENAL TRANSPLANTATION

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The presence of autoantibodies has been reported to be harmless or even beneficial in renal transplantation in contrast to the deleterious effect noted for certain lymphocytotoxic alloantibodies. We have already shown an association between Fc receptor blocking antibodies detected by the erythrocyte antibody rosette inhibition assay (EAI) and improved allograft survival.

In this study, the EAI and lymphocytotoxicity assays at 5°C, 23°C and 37°C were used to determine the incidence of autoantibodies in the sera of 37 patients on maintenance dialysis (Group A) and antibodies in 24 pre-transplant sera using autologous, donor, normal panel and leukaemic (CLL) lymphocytes as targets (Group B).

Neither lymphocytotoxic nor EA inhibiting autoantibodies were detected in Group A. In only one case were EA inhibiting antibodies detected against autologous cells in Group B and in this case the graft failed in less than a year and no anti-donor EAI was present. However 10/11 grafts with but only 4/13 without anti-donor EAI survived one year ($p < 0.01$). Similarly 13/15 grafts with but only 1/9 without anti CLL activity and 10/12 grafts with and 4/12 without anti-normal panel EAI survived 1 year ($p < 0.001$) and ($p < 0.05$) respectively. No autolymphocytotoxicity was detected although cytotoxicity was detected against donor, normal panel, and CLL B lymphocytes in 2, 9 and 14 cases respectively and against normal panel T lymphocytes in 10 cases.

In this study no autolymphocytotoxicity was detected. EA inhibiting antibodies directed against donor, normal panel and CLL lymphocytes correlated with good graft survival but were not directed against autologous lymphocytes.

? Fc receptor on B cells (CLL) – J. Holton

PAPER 3

α_2 MACROGLOBULIN - A POTENT NATURALLY OCCURRING IMMUNOSUPPRESSIVE AGENT

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Increasing attention is directed to the role in transplantation of substances which depress the immune response nonspecifically. Drugs have the disadvantage of side effects, particularly nephrotoxicity and metabolic disturbances. Equine antithymocyte globulin (ATG) is a potent immunosuppressive agent but limited in its usefulness by its antigenicity. We have studied the plasma protein α_2 macroglobulin (α_2M) which has been shown to have immunoregulatory activity in vitro and in vivo(1). Pure α_2M was prepared from normal human plasma and its immunosuppressive potency for normal lymphocytes in vitro (2) compared with that of ATG (Table 1).

Table 1

Agent	ID^{50} (50% inhibitory dose for lymphocyte-PPD response from 5 normal subjects) (1 ± SEM)
Equine ATG (ATGAM)	16.7 mg/100ml ±4
Pure human α_2M	3.3 mg/100ml ±1
Pure human albumin	> 100 mg/100ml ±10

α_2M was 5 times more suppressive than ATG per unit weight. Measurement of α_2M levels (Table 2) in conditions known to be associated with a degree of impaired immunity demonstrated significant increases in α_2M concentration as compared to normal controls.

Table 2

Patient Group	α_2M conc (mg/100ml) (1±SEM)	($p < 0.01$)(Wilcoxon).
Normal subjects (n=33)	195±14	
Chronic liver failure (n=12)	259±17	
Diabetes mellitus (n=20)	262±14	
Haemophiliacs (n=16)	253±18	

A method of preparing pure α_2M in large quantities by metal chelate chromatography has been developed and shown to be applicable to Cohn protein fractions which are a large potential source of human protein. Clinical evaluation of α_2M and its metabolic derivatives (2) as a potentially non-toxic non-antigenic immunosuppressive agents are now indicated.

1. James K. TIBS 1980, 5; 43-47. 2. Donnelly PK. Br.J.Surg. 1983, 70; 614-622.

α_2? is at higher levels than this in Serum *

* Serum is necessary for Culture in MLR - stimulates only which at high conc. of Serum

PAPER 4

DOES A FINE NEEDLE ASPIRATION BIOPSY (FNAB) REPRESENTATIVELY SAMPLE THE INFLAMMATORY INFILTRATE IN A TRANSPLANTED KIDNEY?

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Fine needle aspiration biopsy (FNAB) is a method for monitoring human renal transplants by examination of the inflammatory cells infiltrating the graft. The advantages over needle core biopsy are that it is simple, atraumatic, and may be used daily. Needle core biopsies frequently show that the inflammatory infiltrate may form periglomerular or perivascular foci. Therefore a single aspirate may not provide a representative sample of the infiltrating leucocytes. To investigate this problem, duplicate FNABs were taken from grafts in 7 patients on 19 occasions during the first 35 days following transplation. Cytocentrifuge preparations of the paired FNABs were randomised and read blind by independent observers in 2 transplant centres (A and B) using the scoring system proposed by Hayry (1). The resulting scores for FNAB1 in each pair did not correlate with the scores for FNAB2. On cytological examination some of the FNABs were considered to be non-representative samples, but removal of these scores did not improve the correlation. FNAB scores determined by Centre A correlated well with Centre B's results for both total score (r 0.80) and analysis of individual cell types. Correlation coefficients of the scores of the FNAB pairs are summarised below.

	Centre A	Centre B
Lymphocytes	r=0.12	r=0.40
Granulocytes	r=0.26	r=0.49
Monocytes	r=0.28	r=0.52
Activated cells	r=0.67	r=0.41

	Centre A	Centre B
TOTAL SCORE	r=0.40	r=0.44

These results demonstrate that a single FNAB may not accurately represent inflammatory events occurring within the kidney.

1. Hayry P., von Willebrand E., Ahonen J. et al: Ann Clin Res 13:264, 1981.

PAPER 5

LATE ACUTE INTERSTITIAL CELLULAR REJECTION IN RENAL TRANSPLANT RECIPIENTS

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Late graft deterioration is usually attributed to chronic rejection, for which there is no treatment. Acute cell-mediated rejection in the first six months after transplantation is reversed by high dose corticosteroids. Eight patients on maintenance immunosuppression (4 male, 4 female, ages 19-48) developed sudden decline of renal function 10 months to 10 years (mean 55 months) post-transplantation. Biopsies showed acute interstitial cellular rejection. Obstruction, renal artery stenosis and non-compliance were excluded. Intravenous solumedrone improved renal function in 7 of 8 patients and arrested the decline in function in one patient, but the plasma creatinine never returned to its previous value.

Weeks post biopsy	-12	0	+2	+4	Follow up (4-92)
Plasma creatinine (umol/1)	140 (97-202)	471 (187-1069)	309 164-673)	270 (163-348)	316 (176-643)
Urinary protein (Grams/1)	0.3 (.07-.57)	1.1 (0.1-2.6)	0.88 (0-4.03)	1.14 (0.12-4.5)	0.96 (0-3)

Sequential sera were monitored for lymphocytotoxic antibodies. Five patients developed antibodies 4-60 days prior to biopsy, one had fluctuating levels of antibodies in the preceding nine months and two showed no antibody changes.

Increased proteinuria did not precede all these rejections. Premonitory events included documented urinary tract infections (3 pts), probable viral illnesses (2 pts), primary CMV infection (1 pt) and symptoms of fluid overload (1 pt). Frequent measurements of plasma creatinine are therefore indicated after such episodes.

Late acute interstitial rejection is difficult to predict. Prompt treatment might prevent permanent renal damage. In addition, screening for lymphocytotoxic antibodies may be valuable.

PAPER 6

ANTITHYMOCYTE GLOBULIN AS ADDITIONAL TREATMENT FOR REVERSAL OF ACUTE REJECTION OF RENAL ALLOGRAFTS

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During one year, 23 patients received renal allografts from 18 cadaver and 5 non-identical live donors. Immunosuppression was with azathioprine and low dose prednisone. In 13 patients, acute rejection when it occurred was reversed completely with intravenous methylprednisolone (3 Gm in 3 days; maximum 3 courses). In 5 patients acute rejection only partially responded to high dose steroid but was then completely reversed by one course of antithymocyte globulin (ATG) (3 mg/kg/day for 5-14 days) with maintenance azathioprine and steroid. In 3 patients with steroid resistant rejection, there was no response to ATG but in 1 other, steroid resistant rejection was completely reversed by two courses of ATG. One patient who received only ATG for his only rejection episode did not respond.

In summary, ATG has reversed rejection episodes in 6 patients where the kidney might otherwise have failed. In 4 other patients the kidney rejected despite ATG. There have been no deaths and 19 of 23 patients (83%) have good stable renal function three to fifteen months after transplant.

PAPER 7

INCIDENCE OF IMMEDIATE GRAFT FUNCTION FOLLOWING CONTINUOUS MACHINE OR COLD FLUSH PRESERVATION

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The advantages of immediate function following kidney transplantation are obvious. Thus any method of preservation which gives a high degree of immediate kidney function is to be preferred. Since January 1978 we have used two methods for storing kidneys. 1) Machine preservation on a Gambro Preservation Machine, 2) Cold flush preservation using either Collin's solution or Hypertonic Citrate solution. We have reviewed the outcome of all cadaveric renal transplants performed between January 1978 and December 1982 and the frequency of immediate function is shown in the Table. The mean warm ischaemic time did not differ significantly between the three groups. When machine preservation was compared with flush preservation (Citrate and Collin's combined) no significant advantage was seen at any time interval. However, when Collin's and Citrate solutions were compared at 16-24 hours, there was a significantly higher rate of immediate function in those kidneys preserved with Hypertonic Citrate. Since machine preservation is twenty times more expensive than flush preservation, we feel that for preservation periods of up to 24 hours, the flush technique is to be preferred, with Hypertonic Citrate being the solution of choice.

Total Ischaemic times Function	No.	< 480 Mins Immed.	< 480 Mins Delay	480-959 Mins Immed.	480-959 Mins Delay	960-1440 Mins Immed.	960-1440 Mins Delay	>1440 Mins Immed.	>1440 Mins Delay
Machine	26	3	0	5	1	10	3	2	2
Collins	81	8	2	27	7	21	12	2	2
Hypertonic Citrate	66	2	0	16	7	30	5	4	2

$x^2 = p < .02$

PAPER 8

THE LYMPH NODE RESPONSE TO ALLOANTIGEN: THE ENTRY OF LYMPHOCYTES AND THE SITE OF SELECTION OF ANTIGEN SPECIFIC CELLS

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During a primary immune response antigen specific lymphocytes (ASL) are delivered to organised lymphoid tissue from the recirculating lymphocyte pool — a process which is essential for an effective immune response in regional lymph nodes (LN). The conventional view is that recirculating lymphocytes in the blood enter LN randomly across the high endothelial venules (HEV). Upon encountering antigen in the tissue ASL are selectively retained and the remaining non-antigen specific lymphocytes (NSL) return to the blood via efferent lymphatics.

Recently there have been a number of papers to suggest that endothelial cells are capable of presenting antigen and that entry of lymphocytes into the LN is not random — entry of ASL being selectively enhanced by presentation of antigen at the HEV. This view is supported by experiments in sheep, where repeated antigenic stimulation of a LN during chronic drainage of the efferent lymphatic produces a specific unresponsiveness to that antigen in the whole animal.

In this study we have applied a double labelling technique to study the migratory characteristics of two populations of thoracic duct lymphocytes (TDL) that have been passaged from blood to lymph. The first population, having been passaged through a syngeneic animal, includes a normal proportion of ASL. The second population, having been passaged through a semiallogeneic animal, is negatively selected and thus totally depleted of ASL. The results indicate that entry of lymphocytes into an alloantigenically stimulated LN is a random process and confirm that ASL are selected and retained the LN parenchyma after they have left the blood stream.

PAPER 9

MIGRATION PATTERNS OF T CELL SUBSETS INVOLVED IN ALLOGRAFT REJECTION

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Synergistic functional cooperation between splenic T helper (Th) and T cytotoxic/suppressor (Tc/s) lymphocytes together with Interleukin 2 supplements has been demonstrated in B rats, by the ability of these adoptively transferred fractions to restore acute rejection in these T cell deprived hosts.These studies are further supported by ^{111}Indium (^{111}In) labelled lymphocyte migration experiments: Specifically sensitized splenic T cells were separated by monoclonal antibody techniques into W3/25+ OX8- (Th) and OX8+ W3/25- (Tc/s) subsets; these were subsequently labelled with ^{111}Indium oxine. 2×10^7 ^{111}In-labelled cells of one or other population were transferred to B rats with long functioning cardiac allografts. Animals were sacrificed at 24 hours or 5-6 days post transfer and the radioactivity of selected lymphoid and non-lymphoid organs measured as percentage of total radioactivity recovered / gram of tissue sample. After 24 hours ^{111}In-labelled Tc/s and Th accumulated in similar patterns in both lymph nodes (30%) and spleen (60-65%). After 5-6 days Tc/s migrated away from lymph nodes (13%) and further accumulated in the spleen (83%). T helpers appeared more "sessile". Minimal liver uptake was seen for either subset (3%). Cardiac allografts were penetrated by 0.4% Th compared to 0.9% Tc/s after 24 hours and 6 days. Recombining 10^7 labelled Th and 10^7 labelled Tc/s and transfer resulted in much greater lymph node penetration (55% at 24 hours; 62% at 6 days) compared with either separated T cell subset ($p<0.005$), and concomitantly lower splenic penetration (42% at 24 hours; 36% at 6 days). Furthermore graft penetration was increased to 1.3% at 6 days ($p<0.01$). These results suggest synergistic migrational behaviour as well as previously described functional cooperation between T cell subsets in allograft rejection.

PAPER 10

THE ROLE OF Fcɣ RECEPTOR BLOCKING ANTIBODIES IN RENAL ALLOGRAFT SURVIVAL

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The aim of this study was to investigate the suggestion that transfusion-induced enhancement of renal allograft survival is mediated by Fcɣ receptor (Fcɣ R)-blocking antibodies.[1] Serum from 100 patients and controls was fractionated by DE52 chromatography to yield IgG; serum from 21 transplant patients was fractionated over discontinuous sucrose gradients. All fractions and IgG preparations were tested for Fcɣ R blocking activity by a rosette inhibition assay with chick erythrocyte-antibody complexes and normal human peripheral blood lymphocytes. Following transfusion significantly more non-uraemic than uraemic subjects developed Fcɣ R-blocking IgG ($p < 0.001$). Use of a donor lymphocyte panel showed HLA-independent interdonor variation. IgG blocking lymphocyte Fcɣ R also blocked polymorph Fcɣ R and inhibited polymorph phagocytosis of IgG-coated latex beads. Monocyte Fcɣ R were unaffected. No correlation was found between pretransplant Fcɣ R-blocking IgG and graft survival at 3 months (n=52). Indeed, sucrose gradient fractionation of serum from 7 patients with subsequent graft rejection showed maximal Fcɣ R blocking by the IgG peak. Fourteen patients with successful grafts showed maximal Fcɣ R blocking by serum factors of molecular weight < 19s > 7s. Small circulating immune complexes rather than Fcɣ R-blocking antibodies may presage allograft survival.

Reference

1. Macleod et al Lancet (1982) ii 468

PAPER 11

MIGRATION BEHAVIOUR AND FUNCTIONAL ACTIVITY OF ADOPTIVELY TRANSFERRED LYPHOID POPULATIONS IN B RATS

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The physiological significance of lymphocyte migration is to allow the full range of lymphocyte specificties to react with antigen deposits. We have attempted to correlate the functional ability of lymphoid cell inocula (which on adoptive transfer, restore acute rejection of cardiac allografts in T cell deprived B rats) with the migrational behaviour of these populations. Specifically sensitized thoracic duct lymphocytes (sTDL) have been shown very potent, and splenocytes (sSL) a poor source of cells for restoring allograft immunity. Therefore sSL or the T cell fraction (T sSL) and sTDL were labelled with ^{111}Indium Oxine (^{111}In) and transferred to B rats with well functioning allografts. animals were sacrificed at 24 hours and the radioactivity measured as a percentage of the total radioactivity recovered per gram of tissue sample. sTDL migrated preferentially to lymph nodes *(82%), as did T sSL (64%) when compared to sS1 *(47%), (*$p < 0.005$). Transferred sSl settled primarily in the spleen (42%) compared to sTDL (15%). The overall radioactivity index in lymph nodes divided by splenic uptake was highest following the sTDL transfer (5.4) lower for T sSl (1.92) and lowest following infusion of sSl (1.1). sTDL recirculated much more vigorously than splenic populations with 10x the peripheral blood activity ($P < 0.001$). Graft sequestration was equal and low for all inocula. Selective ^{125}iodo-2-deoxyuridine labelling of specifically sensitized T lymphoblasts and transfer to B recipients yielded different migration patterns when compared to ^{111}In-labelled populations. sTDL lymphoblasts penetrated the graft in greater numbers than T sSL ($p < 0.001$): and sTDL also settled sparsely in non-lymphoid tissues (liver, kidney, lung 4.8%) and concomitantly higher in lymph nodes and spleen (90%) compared to TsSl lymphoblasts (9.9% ($p < 0.001$) and 83% respectively). These studies suggest that high lymph node sequestration, recirculating avidity and increased specific blast activity are related to functional ability to restore allograft immunity in B recipients.

PAPER 12

GENETICS OF THE HUMORAL IMMUNE RESPONSE TOWARDS NON-MHC ENDOTHELIAL ANTIGENS IN THE RAT

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When MAXX ($RT1^n$) rats are immunized with pooled spleen and lymph node cells from the MHC-identical BN strain, antibodies are formed against non-MHC alloantigens of peritubular and venous endothelium of the kidney. In the present experiments we studied in-vivo the genetic requirements for the IgG-response towards these antigens. Since the ACI ($RT1^a$) rat is a non-responder for the endothelial antigen, MAXX and ACI rats were crossed and the antibody response upon immunization with BN cells was studied in 43 (MAXX x ACI)F_2 and 10 (MAXX x ACI)F_1 x ACI animals. The sera were tested using kidneys from BN and BN.DA ($RT1^a$) strains which carry the endothelial antigen and are MHC-identical to the responding animals. Since none of the $RT1^a$ homozygous animals responded and 30/32 animals that were $RT1^{a/n}$ or $RT1^{n/n}$ formed antibodies it appears that the immune responsiveness is encoded within the $RT1^n$ haplotype. To exclude that an MHC-incompatibility between donor and recipient influenced the response, MAXX and ACI rats were immunized with cells from BN.1L ($RT1^l$) and BN.DA respectively. Since the none of the ACI rats did respond whereas all MAXX rats remained responsive, we conclude the recipient MHC controls the IgG immune response towards non-MHC transplantation antigens. Using nude-rats it was shown that the response is T-cell dependent.

PAPER 13

THE INFLUENCE OF HLA-A, B AND -DR MATCHING AND PREGRAFT TRANSFUSIONS ON GRAFT AND PATIENT SURVIVAL AFTER RENAL TRANSPLANTATION.

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The influence of HLA-A, B and -DR matching and pregraft blood transfusions on the graft and patient survival rate of 305 recipients of first cadaver grafts was analysed.

Graft survival. HLA-A, B matching had a significant effect; 5 year survival rates for grafts mismatched for 0, 1-2, 3-4 antigens were 67%, 59%, 42%. HLA-DR matching also had a significant effect; 5 year survival rates for grafts mismatched for 0, 1, 2 antigens were 69%, 45%, 49%. The survival rate of HLA-DR compatible grafts was not improved by additional HLA-A, B matching, but that of HLA-DR mismatched grafts was. A strong transfusion effect was seen and the highest graft survival achieved was in the transfused group receiving a DR compatible kidney (80% at 5 years).

Patient survival. Both HLA-A, B and DR matching significantly increased patient survival whereas blood transfusions did not. Five year survival was 97% for the O, A, B mismatched group compared with 80% for the 3-4 mismatched group. Five year survival for the 0, 1 and 2 HLA-DR mismatched groups were 91%, 88% and 81%. In contrast the 5 year survival rate was 86% for both the transfused and non-transfused patients. The lower survival rate of patients receiving HLA poorly-matched grafts was not related to the amount of methylprednisolone received during the first 3 months after transplantation or whether the patient was considered medically high risk at the time of transplantation.

PAPER 14

EXPERIENCE WITH DR MATCHING IN 1002 FIRST CADAVER RENAL TRANSPLANTS REPORTED TO THE UK TRANSPLANT SERVICE

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The effect of HLA-DR matching in 1002 first cadaver renal transplants, reported to the UKTS between 1979 and July 1983, has been analysed. All grafts had survival data available for the first three months period. Death of recipients with functioning grafts and technical failures were included with rejection as failures. A single parameter analysis of the role of HLA-DR matching in graft survival at 3 months was carried out. The frequencies of DR antigens in the donors and recipients were comparable. The overall 3 months graft function in the 1002 recipients was 72.7%. In 541 transplants two DR antigens were detected in both the donor and recipient.
The three months graft survival in these patients was:

87	transplants with	2 DR mismatches	:	73.6%
297	"	1 DR mismatches	:	76.7%
162	"	0 DR mismatches	:	74.1%

The three months graft function in 728 recipients in whom 2 DR antigens were detected was 74.6%. The graft function in 274 recipients with only 1 DR antigen defined was 67.9%. This difference is statistically significant ($p = 0.0407$). There is no effect of the number of DR antigens detected in the donors on the graft function. The effect of matching for individual DR specificities was analysed. In the UK Transplant Service data there is no evidence that matching or mismatching for any particular DR antigen, including DRw6, is more important in the survival of first grafts.

Thus in 1002 first cadaveric renal transplants no significant role of DR matching in survival was shown.

PAPER 15

CYCLOSPORIN PHARMACO-KINETICS AFTER RENAL TRANSPLANTATION

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The use of Cyclosporin (Cy) "trough" levels has proved a relatively unsatisfactory means of regulating drug therapy because of the wide range of measured values after a standard dose. In order to elucidate the kinetics of Cy elimination we have measured the plasma concentration in the peri-operative period in 10 patients undergoing renal transplantation. Following an IV infusion of Cy (10 mg/kg) over a three hour period, multiple blood samples were collected at pre-selected time intervals up to 22 hours.

The plasma concentration versus time plot was analysed and fitted to a poly-exponential function. The terminal half life was 483 min (range 256-1078 min). Sufficient early post infusion data were collected in 6 patients to allow calculation of systemic clearance:- Mean 3.7 ml/kg/min (± 1.1 SD).

As a result of the large variation in terminal half life and clearance from individual to individual, the trough plasma Cy concentration 22 hours after this initial dose ranged from 50 to 408 ng/ml in the 10 patients. Because of these biological differences in distribution kinetics. therapeutic drug monitoring should be undertaken on a regular basis to establish the appropriate Cy therapy for the individual patient.

PAPER 16

CLINICAL TRIAL OF CYCLOSPORIN (CY) AND DONOR SPECIFIC TRANSFUSION (D.S.T.) VERSUS D.S.T. ALONE IN LIVE RELATED RENAL ALLOGRAFT TRANSPLANTATION (L.R.D.)

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D.S.T. improves graft survival in non H.L.A. identical transplants with a reactive mixed lymphocyte culture, but at the cost of sensitising the recipient in 30% of cases. We investigated the concomitant administration of Cy in suppressing D.S.T. induced sensitisation. Seventeen patients were randomised into two groups. Group I (n = 9) received D.S.T. and cyclosporin 10 mg/kg daily from one week before D.S.T. until transplantation. Group II (n = 8) received D.S.T. only. D.S.T. of 200ml fresh blood was given at fortnightly intervals on three occasions. Transplantation was performed four weeks after the third D.S.T. Cyclosporin was first line immunosuppression in both groups. Sensitisation occurred solely in 2 patients of Group II, one a persistent T warm positive crossmatch with an increase in random panel cytotoxic antibodies from 15% to 38%; the second a transient B warm positive crossmatch. Two way MLC, OKT3, 4, 8 ratios measured on paired samples from each group pre and post D.S.T. (at -8 and -2 weeks) were not significantly altered. The one way MLC in Group 1 showed suppression of donor specific responses (23% reduction $p<0.05$), in contrast to Group II (7% $p>0.1$). Good graft function has been attained in both groups at a follow-up period of two to nine months. There was one death following myocardial infarction in a 65 year old lady with good graft function. The incidence and severity of rejection is similar in the two groups. At this early stage we feel that concomitant Cy is associated with reduced sensitisation following D.S.T.

PAPER 17

A PROSPECTIVE STUDY COMPARING CYCLOSPORIN A ALONE WITH CYCLOSPORIN A PLUS STEROIDS IN CADAVER RENAL TRANSPLANTATION

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The effectiveness of CY.A as sole immunosuppressant has already been demonstrated in the European Multi-Centre Study and to our satisfaction in a continuing trial. The question remains as to whether concomitant steroid therapy would confer any further benefit and possibly reduce nephrotoxicity.

We have conducted a randomised prospective trial of CY.A alone against CY.A + steroids (prednisolone 0.5 mg/kg) in diuresing first cadaver renal allografts. Fifty transfused patients have been entered ranging in age from 20 months to 53 years (mean 31 ± 4). Sexes were equally distributed and there was no significant variation in HLA mismatches between the groups.

All patients received 500 mg Solumedrol interoperatively; immunosuppressive therapy was commenced 6 hours post, when diuresis was established. CY.A was given I.V. (6 mg/kg) for the first 12 hours then orally (17 mg/kg) reducing to 6 mg/kg at 4 months.

Results There were no deaths in either group. Four grafts have been lost in each group giving an actuarial graft survival of 84% at one year for both groups. Nephrotoxicity and gastrointestinal intolerance of CY.A were the two most troublesome problems resulting in a forced change to conventional therapy for 6 patients in the CY.A alone group and 8 patients in the CY.A + steroids group. A conversion rate of ≡ 27%.

Graft survival in both treatment groups is highly significantly better than historical controls on azathioprine and steroids. We have found no advantage for combining CY.A with oral steroids.

PAPER 18

LOW DOSE STEROIDS DO NOT AUGMENT CYCLOSPORIN IMMUNOSUPPRESSION, BUT DO DIMINISH CYCLOSPORIN NEPHROTOXICITY

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Steroids are frequently administered with Cyclosporin although there is no evidence that immunosuppression is improved. In a controlled clinical trial, all 61 patients undergoing cadaveric renal transplantation were randomised to receive Cyclosporin (tapering from 15-17 mg/kg) alone or with low dose Prednisolone (0.3 mg/kg). Rejection was treated with 3-5 grams i.v. Methylprednisolone. The two groups were well randomised and have been followed up for 3-15 months. To date there has been no difference in the frequency of rejection episodes and although more kidneys were lost in the steroid group, this was not significant.

	Cyclosporin alone	Cyclosporin + Steroids	
First/second grafts	23/6	26/6	
Kidneys rejected	2	8	N.S.
Graft survival (6 months)	24(83%)	22(69%)	N.S.

A detailed analysis of the first six transplant months revealed a greater incidence of Cyclosporin nephrotoxicity in the patients receiving Cyclosporin alone.

	Cyclosporin alone	Cyclosporin + Steroids	
Patients having toxicity	81%	48%	$p < .01$
Toxic episodes/patients	1.73	0.77	$p < .001$
% whole blood levels >1000 ng/ml	30%	38%	N.S.
% levels >1000 ng/ml that were toxic	38%	16%	$p < .01$

High blood Cyclosporin levels were equally common, but in the steroid group these were better tolerated and caused toxicity less often. We conclude that steroids reduce nephrotoxicity possibly by exerting a protective effect upon the kidney.

PAPER 19

DISPLACEMENT BONE MARROW TRANSPLANTATION

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Human bone marrow donors have since 1968 been selected by tissue-typing and MLC, and used following recipient induction schedules evolving from nil, through TBI (introduced to try to eliminate residual leukemic cells) and Cyclophosphamide (CY) 40-50 mg/kg x 4 to Busulphan 80 mg/m² x 4 followed by CY x 4 to intentionally displace the recipient's marrow and achieve 100% of donor type marrow, post-graft.

Personal observations since 1971 of enzyme-donation from donor cells to deficient recipient cells, especially for lysosomal enzymes, have been revealed (1) the inborn error has to be expressed in leucocytes (about 7% of those 1,300 currently understood); (2) the need to displace the recipient's abnormal marrow; (3) replacement with normal donor marrow can provide (4) a lifelong source of cells; (5) donor-type tolerance for the previously missing activity (e.g. no antibodies to the normal enzyme); (6) large numbers of circulating cells (e.g. 50-300 g. leucocytes daily); (7) release of active component; (8) transfer of active component into deficient host tissues.

This concept of displacement BMT (Hobbs, 1981, Lancet ii, pp. 735-739) has been applied to 36 patients with inborn deficiencies, has provided circulating normal cells for 33, and 23 (69%) are alive and well. In the world, 36 of 44 different, otherwise fatal, diseases are now curable, with a further 6 awaiting the long-term mental assessments.

SCID - Can get defective host cells to respond to mitogen post-BMT - transfer for - graft cells - after a while host cells are ~~of~~ crowed out by graft cells ∴ donation of a factor can occur.
Need to eliminate host BM cells to allow graft cells to "take" - displace aberrant cells first
Recent habit WBI - causes cancer.
∴ uses Busulphan + Cy - Donor lymphocytes given pre-Cy to ablate host graft ! Response to new enzyme - then graft marrow - host tolerant to new enzyme

PAPER 20

SOY BEAN LECTIN SEPARATION OF ALLOGENEIC BONE MARROW IN ADULTS: T CELL DEPLETION AND STEM CELL RECOVERY

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Fractionation of donor marrow to remove immune competent T lymphocytes has been used to prevent graft versus host disease (GVHD) following allogeneic bone marrow transplantation (BMT). Loss of stem cells during fractionation has resulted in graft failure in children with immune deficiency. We have evaluated the technique in 7 consecutive patients (6 adults, 1 child) undergoing BMT following large volume fractionation in leukaemia.

Harvested marrow was fractionated using soy bean agglutinin to a volume of 50 mls; containing a mean of 0.2×10^8 nucleated cells/kg with 2.6×10^4 CFU^{GM}/kg recipient weight. These fractions contained a mean of 3.7% and 12.2% of the initial starting values representing a threefold relative enrichment.

Assessment of the final fraction with monoclonal antibodies, E rosetting and PHA transformation showed detectable T cells in only one case. Engraftment occurred without delay (>1.0×10^9/1 leucocytes by mean 24 days) and without acute GVHD in any patient. We have shown that fractionation of large volumes of marrow with substantial T cell depletion can result in successful engraftment despite the low cell numbers infused. The method is time consuming and labour intensive but analysis of T cell removal and CFU^{GM} loss at each stage is helping us devise further modifications of the basic technique.

PAPER 21

STANDARDISATION OF SPECIFIC ANTI-T CELL MONOCLONAL ANTIBODIES FOR IMMUNOTHERAPY; THE FIRST CLINICAL EXPERIENCE

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In human allogeneic bone marrow transplantation (BMT), acute graft versus host disease (aGvHD), a potentially lethal complication, may develop in spite of methotrexate or cyclosporin A prophylaxis. Preclinical studies have for more than 15 years suggested that aGvHD might be prevented by the removal of immunocompetent (IC) T lymphocytes from the donor marrow inoculum. In our study a series of T cell specific monoclonal antibodies have been made and standardised for the elimination of T cells from the bone marrow. Two combinations have been found to deplete virtually all identifiable T cells from the bone marrow: OKT3 + OKT11a and MBG6 (a T12-like antibody) + RFT8. We have therefore utilized the latter cocktail together with rabbit complement. Acute GvHD has been successfully abrogated in 15 (evaluable) successive patients despite the fact that patients were given no additional immunosuppressive prophylaxis. Other specific anti-T cell reagent (RFT2), prefentially active against activated T blast cells has been found to be especially efficient in eliminating all identifiable T-blasts in the thymic form of acute lymphoblastic leukaemia (T-ALL). This antibody has therefore been tried in autologous bone marrow transplantation for cleaning of the marrow. After incubation with RFT2 + complement excellent bone marrow regeneration was observed. These reagents may serve equally useful purpose as specific immunosuppressive agents during acute rejection episodes after kidney, liver and heart transplantation.

99% depletion of OKT8+. Grafts
Regeneration delayed a few days.
MBG6 clone if difficult to grow (IgM Ab)

PAPER 22

DEOXYADENOSINE AND 2 ' DEOXYCOFORMYCIN SELECTIVELY KILL CLONOGENIC MALIGNANT T.LYMPHOBLASTS FROM HUMAN BONE MARROW

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It is established that human T.ALL cell lines are extremely sensitive to growth inhibition by adenosine deaminase inhibitor such 2'deoxycoformycin (dCF), these studies have shown that prolonged (48-72 hours) incubations were required for complete cell kill. This in vitro sensitivity is paralleled by the excellent in vivo responses of some patients with T.ALL to dCF therapy. These observations have led us to evaluate the possibility of using these drugs for the chemo-manipulation of remission bone marrow prior to autologous marrow transplantation for T.ALL. Using a clonal assay for the T.ALL line — Molt 4 we have demonstrated that these cells are highly sensitive to short incubations with dCF and AdR. Incubation of Molt 4 cells with dCF (10^{-5}M) and AdR (5 x 10^{-5}M) for 2 - 4 hours prior to washing and culture in a drug free semi-solid medium killed 100% of clonogenic cells (n = 6), lower concentrations of AdR proved less toxic. Similarly, when studying growth of Molt 4 cells in suspension culture a 4 hour pre-incubation with dCF (10^{-5}M) and AdR (5 x 10^{-5}M) proved as toxic to cell growth as continuous exposure for 72 hours. In contrast at these concentrations of dCF and AdR normal marrow CFUc was inhibited by a mean of 15%. Also when mixtures of Molt 4 with normal marrow cells were incubated with these concentrations of dCF and AdR the drugs proved equally toxic to Molt 4 cells as in unmixed experiments. These results demonstrate that short incubations of T.ALL blasts with these reagents are highly toxic and suggest that they could be exploited for the elimination of neoplastic T.cells from bone marrow prior to autologous bone marrow transplantation.

PAPER 23

ARE ISLET ALLOGRAFTS ABLE TO MAINTAIN A STATE OF UNRESPONSIVENESS?

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In the rat islet allografts are rapidly rejected, and immunosuppression with cyclosporin A does not prevent rejection. In contrast, cyclosporin A treatment induces indefinite survival of renal allografts with the production of an unresponsive state that then allows transplantation of islets from the same donor without rejection. It is possible that islets do not contain some factor that is responsible for maintaining this unresponsive state. We performed an experiment where DA rats were given auxillary LEW renal allografts and cyclosporin A 10 mg/kg for 14 days. Long-survivors were then given LEW islets under the recipients own left kidney capsule without further immunosuppression. 100 days after islet transplantation the LEW renal allograft was removed, leaving only the islet allograft in situ. Function of the islet allograft was monitored for a further 60 days, proof of function then being confirmed by removing the recipient kidney bearing the LEW islets. Strain specificity was investigated using PVG and then BN islets. The results show that LEW islets given to rats bearing long-surviving auxillary LEW kidneys are accepted and subsequent removal of the renal allograft does not lead to rejection of the islet transplant. We conclude that islet allografts are capable of maintaining an unresponsive state in rats.

PAPER 24

TRANSPLANTATION OF CRYOPRESERVED ISLET GRAFTS IN DOGS

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Successful cryopreservation of a dispersed islet graft would provide time for tissue typing, recipient preparation and graft transfer and might reduce immunogenicity through differential sensitivity of passenger leucocytes.

This work examines the feasibility of cryopreservation of such a graft in the large animal. Mongrel dogs underwent total pancreatectomy and dispersed islet grafts were prepared by the technique of Kretschmer. Group 1 received fresh intrasplenic autografts. Three study groups received identical but cryopreserved grafts (10% dimethylsulphoxide as cryoprotectant) with differing cooling and thawing rates; Group 2 cooled at 5°C/min and thawed at 80°C/min, Group 3 cooled at 0.5°C/min and thawed at 80°C/min and Group 4 cooled at 0.5°C/min and thawed at 8°C/min.

Results at one month:

	Success (Euglycaemic. No insulin)	Partial Success (>50% reduction in insulin demands)	Failure (50% reduction in insulin demands)
Group 1 (n=12)	12	0	0
Group 2 (n=5)	0	0	5
Group 3 (n=7)	3	3	1
Group 4 (n=8)	1	3	4

All fresh graft recipients were euglycaemic at one month. All grafts failed in Group 2. Of grafts cooled at 0.5°C/min, 3 of 7 of those thawed rapidly were successful while only 1 of 8 grafts thawed slowly were a success. An IVGTT performed at one month on successful animals demonstrated identical glucose handling by the recipients of fresh and cryopreserved grafts.

A large animal collagenase-dispersed islet graft can, therefore, be successfully cryopreserved and the function of such a graft is identical to that of a fresh graft.

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PAPER 25

THE USE OF CYCLOSPORIN IN PARATHYROID TRANSPLANTATION IN RATS

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Despite claims that allografted parathyroid tissue has a reduced ability to elicit a rejection response (1) its prolonged function normally requires host immunosuppression (2). The dose of cyclosporin required to maintain parathyroid allograft function has been investigated in a vigorously rejecting rat strain combination. Lewis (Ag-B^l) rats underwent total parathyroidectomy and were fed a diet deficient in calcium, hypocalcaemia being confirmed. Each animal subsequently received 2 parathyroid glands from DA (AG-B^4) donors, implanted into a groin muscle. Forty seven grafts were technically successful. In Group I, 16 rats received 17 mg/kg cyclosporin daily for 28 days. All retained graft function, as shown by weekly serum calcium estimations, for 4 weeks. In Group II, 18 rats received 8mg/kg cyclosporin daily. Eight grafts were rejected and 10 still functioned after 4 weeks. Group III consisted of 13 animals acting as controls, treated daily with the solvent used for drug administration. All rejected their grafts, returning to a state of hypocalcaemia within 3 weeks. This study shows that 17mg/kg cyclosporin daily, but not 8mg/kg cyclosporin daily, reliably permits parathyroid allograft function across a major histocompatibility barrier. Allografted parathyroid tissue would seem to require immunosuppressive therapy in doses similar to those required in other tissue transplants.

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"Very few people would benefit from this approach"
Start with be OK with Vit D

PAPER 26

CHYLO-OESOPHAGEAL FISTULA: A NEW APPROACH TO THORACIC DUCT DRAINAGE

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Thoracic duct drainage, although an effective immuno-suppressive procedure, has not been widely adopted because of the considerable technical difficulties of fluid and protein loss, cannula blockage, local infection and poor patient acceptance. We report an experimental chylo-oesophageal fistula in the sheep which overcomes many of these problems. The isolated subclavian and jugular veins were used as a conduit between the thoracic duct and the oesophagus and patency was demonstrated radiographically for 1-6 weeks. Sham operated animals acted as controls. Lymphocyte counts and biochemical analyses were performed on peripheral blood samples. The animals remained healthy and no parenteral fluid or protein replacement was necessary at any stage. In 16 animals, lymphocyte counts fell by 50% from 8.4 ± 3.1 x 10^9/1 pre-operatively, to 3.7 ± 1.4 at 1 week, 4.1 ± 1.4 at 2 weeks, and 4.3 ± 2.4 at 3 weeks ($p<0.01$). By comparison, there was no significant change in peripheral blood lymphocyte counts in control animals (n=5). There were no other significant changes, apart from transient falls in plasma protein levels and weight. These preliminary results suggest that chylo-oesophageal fistula may be an effective method of internal thoracic duct drainage without the need for vulnerable catheters or specialised equipment for the re-infusion of lymphocyte-poor lymph or expensive protein or fluid replacement.

PAPER 27

RED CELL SURVIVAL AND THE TIMING OF THE BLOOD TRANSFUSION EFFECT

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The blood transfusion effect (BTE) may be attributable to the development of high plasma suppressive activity (PSA)(1) associated with the reticulo endothelial system (RES) blockade following rapid red cell destruction (2). Renal transplants performed within 3 months of recipient blood transfusion are most successful (3). We studied the effect of blood transfusion on i) the development of PSA, ii) red cell clearance and iii) cardiac allograft survival in WAG rats. (i) Rats (n=6) given 2ml Cr-labelled allogeneic but blood group compatible DA rat blood developed high PSA (maximum 7-14 days post transfusion). ii) High PSA coincided with maximum red cell destruction ($t\frac{1}{2}$ 7 days). Transfusing 2ml syngeneic WAG blood caused no significant change in PSA and red cells were cleared at normal rate ($t\frac{1}{2}$ 14 days). iii) Cardiac allograft survival transplanted across a major histocompatibility barrier PVG/C x WAG were studied. WAG rats were transfused with 4ml DA blood (in 2ml aliquots 3 days apart) either 2 weeks or 4 weeks before transplantation. After transplantation half the rats received immunosuppressive (IS) therapy (azothiprine, methylprednisolone). DA blood transfusions prolonged allograft survival (median 22 days) significantly longer ($p<0.01$, Wilcoxon) than WAG transfused control groups (median 9 days) but only when transfusion was given 2 weeks prior to transplantation and in the presence of IS.

Observations: The BTE is a time dependent phenomenon. It is associated with development of high PSA which may follow accelerated clearance of blood group compatible but not syngeneic red cells. Could this be a model for the human BTE in which RES blockade is aggravated by renal failure and IS (4)?

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