British Transplantation Society

The Medical School
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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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(2) Department of Statistics, University of Manchester
(3) Renal Transplant Unit, Manchester Royal Infirmary, Manchester.

Many single and multicentre studies of renal transplantation have shown outcome to be multifactorial. The recognized 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 unintentional 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 64.6% 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 53.3%: 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.
NON-CYTOTOXIC AUTOANTIBODIES AND ALLOANTIBODIES IN RENAL TRANSPLANTATION

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(1) Blood Transfusion Department, Aberdeen Royal Infirmary
(2) Department of Surgery, University of Aberdeen

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 EAI 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 EA was present. However, 30/11 (26%) without anti-donor EA survived one year (p<0.001). Similarly, 13/15 (87%) with anti-donor EA and 10/12 (83%) with and without CLL activity and 10/12 (83%) with and without normal panel EA survived 1 year (p<0.001) and (p<0.05) respectively. No autoelymphocytotoxicity was detected although cytotoxicity was detected against donor, normal panel, and CLL lymphocytes in 29 and 14 cases respectively and against normal panel T lymphocytes in 10 cases.

In this study no autoelymphocytotoxicity 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.

α2 MACROGLOBULIN - A POTENT NATURALLY OCCURRING IMMUNOSUPPRESSIVE AGENT

Department of Surgery, University of Newcastle upon Tyne, Newcastle upon Tyne.

Increasing attention is directed to the role in transplantation of substances which depress the immune response non-specifically. Drugs have the disadvantage of side effects, particularly nephrotoxicity and metabolic disturbances. Equine antihymocyte globulin (ATG) is a potent immunosuppressive agent but limited in its usefulness by its antigenicity. We have studied the plasma protein α2 macroglobulin (α2M) which has been shown to have immunoregulatory activity in vitro and in vivo(1). Pure α2M was prepared from normal human plasma and its immunosuppressive potency for normal lymphocytes in vitro (2) compared with that of ATG (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>Equine ATG (ATGAM)</td>
</tr>
<tr>
<td>Pure human α2M</td>
</tr>
<tr>
<td>Pure human albumin</td>
</tr>
</tbody>
</table>

α2M was 5 times more suppressive than ATG per unit weight. Measurement of α2M levels (Table 2) in conditions known to be associated with a degree of impaired immunity demonstrated significant increases in α2M concentration as compared to normal controls.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient Group</strong></td>
</tr>
<tr>
<td>Normal subjects (n=33)</td>
</tr>
<tr>
<td>Chronic liver failure (n=12)</td>
</tr>
<tr>
<td>Diabetes mellitus (n=20)</td>
</tr>
<tr>
<td>Haemophilia (n=16)</td>
</tr>
</tbody>
</table>

A method of preparing pure α2M 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 α2M and its metabolic derivatives (2) as a potentially non-toxic non-antigenic immunosuppressive agents are now indicated.

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

Nuffield Department of Surgery, John Radcliffe Hospital, Oxford.

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 30 days following transplantation. 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 cytochemical 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.

<table>
<thead>
<tr>
<th>Centre A Centre B</th>
<th>Centre A Centre B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>r=0.12 r=0.40</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>r=0.26 r=0.49</td>
</tr>
<tr>
<td>Monocytes</td>
<td>r=0.28 r=0.52</td>
</tr>
<tr>
<td>Activated cells</td>
<td>r=0.67 r=0.41</td>
</tr>
</tbody>
</table>

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


PAPER 5

LATE ACUTE INTERSTITIAL CELLULAR REJECTION IN RENAL TRANSPLANT RECIPIENTS

Renal Unit, Manchester Royal Infirmary, Manchester.

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 solamine 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.

<table>
<thead>
<tr>
<th>Weeks post biopsy</th>
<th>-12</th>
<th>0</th>
<th>+2</th>
<th>+4</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine (umol/l)</td>
<td>140</td>
<td>471</td>
<td>309</td>
<td>270</td>
<td>316</td>
</tr>
<tr>
<td>(187-160)</td>
<td>(164-473)</td>
<td>(163-348)</td>
<td>(176-443)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary protein (Grams/l)</td>
<td>0.3</td>
<td>1.1</td>
<td>0.88</td>
<td>1.14</td>
<td>0.96</td>
</tr>
<tr>
<td>(0.1-2.6)</td>
<td>(0.1-2.6)</td>
<td>(0.1-2.6)</td>
<td>(0.1-2.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Renal Transplant Unit, Charing Cross Hospital, London, U.K.

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 5 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.

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.

<table>
<thead>
<tr>
<th>Total ischaemic times</th>
<th>Function</th>
<th>No</th>
<th>&lt;480 Mins</th>
<th>480-960 Mins</th>
<th>960-1440 Mins</th>
<th>&gt;1440 Mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Machine</td>
<td>26</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Collin</td>
<td>81</td>
<td>8</td>
<td>2</td>
<td>27</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Hypertonic Citrate</td>
<td>66</td>
<td>2</td>
<td>0</td>
<td>16</td>
<td>7</td>
<td>30</td>
</tr>
</tbody>
</table>

\[ \chi^2 = p < 0.02 \]
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 regionally 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 lymphatics 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 passed from blood to lymph. The first population, having been passed through a syngeneic animal, includes a normal proportion of ASL. The second population, having been passed 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 ALLOGRRAFT REJECTION

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Synergistic functional cooperation between splenic T helper (Th) and 1 cytotoxic/suppressor (Ts) lymphocytes together with Interleukin 2 supplements has been demonstrated in Balb/c mice, by the ability of these adoptively transferred fractions to restore acute rejection in these T cell deprived hosts. These studies are further supported by Tc/s labelled lymphocytes migration experiments. Specifically, sensitized splenic T cells were separated by monoclonal antibody techniques into W3/25+OK8 (Th) and OK8+ W3/25 (Ts) subsets; these were subsequently labelled with 111 Indium oxine. 2 x 10^6 111 Indium labelled cells of one or other population were transferred to Balb/c mice 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 a percentage of total radioactivity recovered / gram of tissue sample. After 24 hours 111 Indium 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 “sensible” 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 10^6 labelled Th and 10^6 labelled Tc/s transfer resulted in much greater lymph node penetration (55% at 24 hours, 62% at 6 days) compared with either separated T cell subset (p<0.05), and concomitantly lower splenic penetration (42% at 24 hours, 36% at 6 days). Furthermore graft penetration was increased to 1.5% 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.
THE ROLE OF Fcγ 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 Fcγ receptor (Fcγ R)-blocking antibodies. 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 Fcγ R blocking activity by a rosette inhibition assay with chick erythrocyte-antibody complexes and normal human peripheral blood lymphocytes. Following transfusion significantly more non-uremic than uraemic subjects developed Fcγ R-blocking IgG (p < 0.001). Use of a donor lymphocyte panel showed HLA independent interdonor variation. IgG blocking lymphocyte Fcγ R also blocked polymorph Fcγ R and inhibited polymorph phagocytosis of IgG-coated latex beads. Monocyte Fcγ R were unaffected. No correlation was found between pretransplant Fcγ 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 Fcγ R blocking by the IgG peak. Fourteen patients with successful grafts showed maximal Fcγ R blocking by serum factors of molecular weight < 19 kDa > 7 kDa. Small circulating immune complexes rather than Fcγ R-blocking antibodies may preseage allograft survival.

Reference

MIgration Behaviour and Functional Activity of Adoptively Transferred Lymphoid Populations in B Rats

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The physiological significance of lymphocyte migration is to allow the full range of lymphocyte specificities 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 thioracic 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 (TsSL) and sTDL were labelled with 111In 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 (88%), as did TsSL (64%) when compared to sSL (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 TsSL (1.92) and lowest following infusion of sSL (1.1). sTDL recirculated much more vigorously than splenic populations with 10x the peripheral blood activity (F<0.001). Graft sequestration was equal and low for all inocula. Selective 111In-deoxy-2-deoxyuridine labelling of specifically sensitized T lymphoblasts and transfer to B recipients yielded different migration patterns when compared to 111In-labelled populations. sTDL lymphoblasts penetrated the graft in greater numbers than TsSL (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.
GENETICS OF THE HUMORAL IMMUNE RESPONSE TOWARDS NON-MHC ENDOTHELIAL ANTIGENS IN THE RAT

Renal Division, Department of Medicine, University Hospital Leiden, Leiden, The Netherlands.

When MAXX (RT1\sup{a}) 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\sup{b}) 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)\sup{F_2} and 10 (MAXX x ACI)\sup{F_1} x ACI animals. The sera were tested using kidneys from BN and BN.DA (RT1\sup{a}) strains which carry the endothelial antigen and are MHC-identical to the responding animals. Since none of the RT1\sup{b} homozygous animals responded and 30/32 animals that were RT1\sup{a/b} or RT1\sup{a} formed antibodies it appears that the immune responsiveness is encoded within the RT1\sup{b} 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\sup{a}) and BN.DA respectively. Since 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.

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

Alan Ting and Peter J. Morris.
Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford.

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%, 54%, 39%. 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.
EXPERIENCE WITH DR MATCHING IN 1002 FIRST CADAVER RENAL TRANSPLANTS REPORTED TO THE UK TRANSPLANT SERVICE

P.T. Klauda (1), W.R. Gilks (2), B.A. Bradley (1).
(1) UK Transplant Service, Bristol.
(2) MRC Biostatistics Unit, Cambridge.

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 month graft function in the 1002 recipients was 77.7%. In 541 transplants two DR antigens were detected in both donor and recipient.

The three months graft survival in these patients was:

- 87 transplants with 2 DR mismatches: 73.5%
- 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 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 DR52, 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.

CYCLOSPORIN PHARMACO-KINETICS AFTER RENAL TRANSPLANTATION

The Nuffield Department of Anaesthetics and Surgery, John Radcliffe Hospital, Oxford, OX3 9DU.

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 points 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 the 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.
Clinical trial of cyclosporin (C.Y.) 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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Renal Transplant Unit, Royal Liverpool Hospital.
(1) University Dept. of Immunology.
(2) Director Transfusion Centre for Merseyside.

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 C.Y. 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 200 ml 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 4 and 8 weeks) were not significantly altered. The one way MLC in Group I 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 C.Y. is associated with reduced sensitisation following D.S.T.

A Prospective study comparing cyclosporin alone with cyclosporin plus steroids in cadaver renal transplantation

University Departments of Surgery and Medical Genetics and the Department of Nephrology, Manchester Royal Infirmary.

The effectiveness of C.Y.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 C.Y.A alone against C.Y.A + steroids (prednisolone 0.5 mg/kg), in 80 patients 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 intraoperatively; immunosuppressive therapy was commenced 6 hours post, when diuresis was established. C.Y.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 C.Y.A were the two most troublesome problems resulting in a forced change to conventional therapy for 6 patients in the C.Y.A alone group and 8 patients in the C.Y.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 C.Y.A with oral steroids.
LOW DOSE STEROIDS DO NOT AUGMENT CYCLOSPORIN IMMUNOSUPPRESSION, BUT DO DIMINISH CYCLOSPORIN NEPHROTOXICITY

J.R. Salaman and P.J.A. Griffin
Renal Transplant Unit, Royal Infirmary, Cardiff.

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 (0.5 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.

<table>
<thead>
<tr>
<th></th>
<th>Cyclosporin alone</th>
<th>Cyclosporin + Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>First/second grafts</td>
<td>23/6</td>
<td>25/6</td>
</tr>
<tr>
<td>Kidneys rejected</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Graft survival (6 months)</td>
<td>24(83%)</td>
<td>22(60%); N.S.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Cyclosporin alone</th>
<th>Cyclosporin + Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients having toxicity</td>
<td>81%</td>
<td>48%; p &lt; .01</td>
</tr>
<tr>
<td>Toxic episodes/patients</td>
<td>1.73</td>
<td>0.77; p &lt; .001</td>
</tr>
<tr>
<td>% whole blood levels &gt;1000 ng/ml</td>
<td>30%</td>
<td>38%</td>
</tr>
<tr>
<td>% levels &gt;1000 ng/ml that were toxic</td>
<td>38%</td>
<td>16%; p &lt; .01</td>
</tr>
</tbody>
</table>

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

J.R. Hobbs for The Westminster Bone Marrow Team, Westminster Hospital, London

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 Cycothalamide (CY) 400-500 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 6% of those 1,000 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 number of circulating cells (e.g. 50-300 g leucocytes daily); (7) release of active component; (8) transfer of active component into recipient 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-temalental assessments.

Seid - Can get defective host cells to respond to mitogen - D.BTT
- Tapper for graft cells - say 40% cells not affected and by soft cell donation in future can occur.

Need to eliminate host BM cells to allow graft cells to take - displace clumped cells first

Don't relate WBTI - cancer can cause.

Use Bumelphan + Cy - Donor leukocytes grow pre-Cy to ablate anti-graft 1 Recipient

we now engage - then graft marrow - hard tissue for new enzyme.
SOY BEAN LECTIN SEPARATION OF ALLOGENIC BONE MARROW IN ADULTS: T CELL DEPLETION AND STEM CELL RECOVERY

G.W. Slocombe (1), A.C. Newland (1), N.W.J. Yatesman (2) and H.M. Jones (3).

(1) Department of Haematology, The London Hospital
(2) Department of Immunology, The London Hospital
(3) University College Hospital, London

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 ml; containing a mean of 0.2 x 10^6 nucleated cells/kg with 2.6 x 10^4 CFUGM/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 x 10^9/l 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 CFUGM 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


Departments of Immunology and Haematology, Royal Free Hospital and School of Medicine, London.

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 or reduced either by the removal of immunocompetent (IC) T lymphocytes from the donor marrow inoculum. In our study a variety 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 + OKT11 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 reagents (RFT2), preferentially 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.

993 de Velos & RFT2 (Gallic) 

Regenerade delayed a few days. 

MBG6 clone difficult to grow (997 Ab)
DEOXYADENOSINE AND 2'-DEOXYCOFORMYCIN SELECTIVELY KILL
CLONOGENIC MALIGNANT T LYMPHOCYTES FROM HUMAN
BONE MARROW

N.H. Russell and A.J. Bellingham. (Introduced by J. Goldman),
University Department of Haematology, Royal Liverpool Hospital, Liverpool.

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 chemotherapy of
transplant 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 AdDr. Incubation of Molt 4 cells with
dCF (10^-5M) and AdDr (5 x 10^-5M) 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
AdDr proved less toxic. Similarly, when studying growth of Molt 4 cells in suspension
culture a 4 hour pre-incubation with dCF (10^-5M) and AdDr (5 x 10^-5M) proved
toxic to cell growth as continuous exposure for 72 hours. In contrast at these
concentrations of dCF and AdDr normal marrow CFUs 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 AdDr 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.

T lymphocytes have high levels of deoxynucleoside 3'-kinase
which convert dATP into dADP and feed back to deoxynucleoside 5'-kinase;
deoxynucleoside nucleotides are metabolised via the salvage pathway.

ARE ISLET ALLOGRAFTS ABLE TO MAINTAIN A STATE OF
UNRESPONSIVENESS?

The Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford.

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
auxiliary 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
verified using PVP and then B1N islets. The results show that LEW islets given to
rats bearing long-surviving auxiliary 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.
TRANSLANTATION OF CRYOPRESERVED ISLET GRAFTS IN DOGS

T.N. Walsh, D. Alderson and J.R. Farmdon.
Department of Surgery, University of Newcastle upon Tyne.

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 parathyroidectomy 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:

<table>
<thead>
<tr>
<th></th>
<th>Success (Euglycaemic No insulin)</th>
<th>Partial Success (250% reduction in insulin demands)</th>
<th>Failure (50% reduction in insulin demands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Group 3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Group 4</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

All fresh graft recipients were euaglycaemic at one month. All grafts failed in Group 2. Of grafts cooled at 0.5°C/min, 3 of 7 that 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.


THE USE OF CYPLOSPORIN IN PARATHYROID TRANSPLANTATION IN RATS

A.R. De Bolla and A.D. Barnes.
Surgical Immunology Unit, Queen Elizabeth Hospital, Birmingham.

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) 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) donors, implanted into a groin muscle. Forty seven grafts were technically successful. In Group 1, 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 17 mg/kg cyclosporin daily, but not 8 mg/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.

Ref:


"very few people could branch / get this approach"  
that will be 0% with V+ D
CHYLO-OESOPHAGEAL FISTULA: A NEW APPROACH TO THORACIC DUCT DRAINAGE

E.P.M. Williamson and R.A. Sells.
Renal Transplant Unit, Royal Liverpool Hospital, Liverpool.

Thoracic duct drainage, although an effective immunosuppressive 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 16 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 90% from 8.4 x 10^9/1 pre-operatively, to 3.7 x 10^9/1 at 1 week, 4.1 x 10^9/1 at 2 weeks, and 4.3 x 10^9/1 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.

RED CELL SURVIVAL AND THE TIMING OF THE BLOOD TRANSFUSION EFFECT

Department of Surgery, University of Newcastle upon Tyne, Newcastle upon Tyne.

The blood transfusion effect (BTE) may be attributable to the development of high plasma suppressive activity (PSA) (1) associated with the reticulocyte 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 red blood group compatible DA rat blood developed high PSA (maximum 7.14 days post transfusion). (ii) High PSA coincided with maximum red cell destruction (1½ 7 days). Transfusing 2ml syngeneic WAG blood caused no significant change in PSA and red cells were cleared at normal rate (½ 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 (azathioprine, 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)?