THE ROLE OF COMPLEMENT IN ACUTE ANTIBODY-MEDIATED REJECTION OF SKIN XENOGRAFTS IN THE MOUSE.

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Sint Radboudziekenhuis, University of Nijmegen.

Rat skin grafts, carried by immunosuppressed mice, can be acutely destroyed by intravenous administration of mouse antirat antibody. The velocity of the reaction and the histologic sequence of events depend on the amount of antibody administered: low doses give an Arthus-like reaction while at high doses a Swartsman-like pattern occurs. Depletion of C3 by Cobra venom factor treatment did not prevent acute rejection after intravenous injection of high doses of anti-C3 but changed the reaction from a Swartsman-like to an Arthus-like pattern. Conversely, supplementation of rabbit complement caused a violent Swartsman-like graft destruction after injection of low doses of antibody, that in complement-normal mice gave an Arthus-like reaction. The results show that complement can greatly amplify the antibody-mediated immune reactivity and can substantially modify its histologic pattern. It is, however, not an absolute requirement for the occurrence of the destructive process.

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

The work contained in this summary has been read at a scientific meeting, 10th. Int. Cong. of Immunology, Dec. 17, 1961, Amsterdam.

DINITROCHLOROBENZENE (DNCB) SKIN TESTING: NO CORRELATION WITH OUTCOME AFTER RENAL TRANSPLANTATION.


As a result of the increased interest in recent years in the role of host factors' (recipient - non-responder status) of transplant recipients a number of studies have attempted to evaluate host responsiveness using DNBC skin testing

This paper presents the findings of our study of DNBC responsiveness in patients on the dialysis/transplant programme, and also reports the findings in 49 patients who received 1 or more transplants post DNBC testing.

DNBC responsiveness in the total patient population showed a correlation with length of time on dialysis pre-testing, 62% of patients having a negative response.

For the transplanted patients, examination of DNBC scores of patients who had functioning grafts at 6 months as compared with patients whose grafts had failed within this period revealed that patients with failed grafts had a similar mean DNBC score (1.79) as a group than had patients with a functioning graft (3.60).

Actuarial analysis of graft survival for the two groups DNBC negative and DNBC positive revealed no difference in graft survival at 6, 12 and 18 months post transplantation.

We therefore conclude that on the figures available at present we cannot demonstrate a correlation between DNBC skin reactivity and outcome of renal transplantation.

REFERENCES:
A COMPARISON OF THE TEEM TEST TO LYMPHOCYTE TRANSFORMATION AND THE APPLICATION OF THESE TO EVALUATING LYMPHOCYTE DOSE RESPONSE TO IMMUNOSUPPRESSANTS.

PK Donnelly, BM Shepton, F Friedman, C Parker, RME Taylor. Departments of Surgery and Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne.

Although it has been shown to be clinically relevant, the immunological significance of the TEEM test has been disputed and its relationship to transformation assays has not been clearly defined. The aims of the study were:

1) to determine in the two test systems, the response of normal lymphocytes to a standard antigen after preincubation with matched doses of hydrocorisone.

11) to define lymphocyte sensitivity from normal and uraemic subjects, to immunosuppressive drugs in the TEEM test.

Lymphocytes from normal healthy volunteers were stimulated in both test systems with a recall antigen, purified protein derivative or mycobacterium tuberculosis PPD (33μg/ml).

1) A highly significant correlation (p<0.001) between lymphocyte inhibition and log cortisol concentration was found.

<table>
<thead>
<tr>
<th>Cortisol (ng/ml)</th>
<th>100</th>
<th>25</th>
<th>6.25</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEEM inhibition</td>
<td>92±3</td>
<td>75±12</td>
<td>52±6</td>
<td></td>
</tr>
<tr>
<td>Transformation</td>
<td>75±12</td>
<td>60±36</td>
<td>41±12</td>
<td>31±12</td>
</tr>
</tbody>
</table>

11) In the TEEM test, lymphocytes from healthy adult volunteers were compared to lymphocytes from potential renal transplant recipients in terms of the dose of immunosuppressive drugs required to produce 50% inhibition of response (ID50).

<table>
<thead>
<tr>
<th>Immunosuppressant ID50 (μg/ml)</th>
<th>Normals ( n=5 )</th>
<th>Uraemics ( n=5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrcortisone</td>
<td>17.6±4.4</td>
<td>0.065±0.05</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>2.7±0.9</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1.4±0.4</td>
<td>p=0.08</td>
</tr>
</tbody>
</table>

We conclude that in both test systems, hydrocorisone in the same dose range induces significant suppression of lymphocyte response to PPD. The TEEM test measures a lymphocyte dose response to immunosuppressants which compares favourably with published data in other assay systems and demonstrates a significant difference between normal and uraemic subjects. It is hoped that the application of the TEEM test which takes 4 hours compared to 7 days for lymphocyte transformation, might provide a more satisfactory means of screening potential transplant recipients preoperatively for lymphocyte sensitivity to immunosuppressants.

Are HLA-Dw6 positive recipients high responders in renal transplantation?

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1. Eurotransplant Foundation, 2. Dept. of Immunohaematology, 3. Dept. of Nephrology, University Hospital, Leiden, Holland.

Of 183 HLA-A, -B and -DR typed Dutch cadaver kidney recipients who rejected their first renal allograft, 62 were found to be HLA-Dw6 positive, whereas on the basis of the frequency of HLA-Dw6 in the Dutch population only 42 were expected \( (p<0.01) \).

All patients received blood transfusions before transplantation and no difference is found in the average number of HLA-A, -B and -DR mismatches of both groups. When the interval between transplantation and transplantnecotomy is compared there is a striking difference between the two groups, 50 of the 55 HLA-Dw6 positive patients lost their graft within 3 months, versus only 62 of the 138 HLA-Dw6 negative patients \( (p<0.0001) \). From 18 Patients (31 HLA-Dw6 positive and 47 HLA-Dw6 negative) pretransplantation and posttransplantation sera were tested in a cytotoxicity assay for antibodies reactive with T-cells, B-cells and monocytes. Before transplantation no difference in sensitisation was found between the two groups. Both groups showed a similar frequency of antibodies reactive with T-cells, B-cells and monocytes.

After transplantation however there is a striking difference between the two groups. 83% of the HLA-Dw6 positive patients had antibodies (versus 45% before transplantation), while in the group of HLA-Dw6 negative patients the degree of sensitisation did not change (49%). The significant difference between the two groups was due to a higher frequency of antibodies to B-cells and monocytes in the HLA-Dw6 positive group.

The reactivity of the sera taken 3 weeks after transplantation, the period in which the patients immune system is still influenced by the corticosteroid treatment, was compared with the reactivity of the sera taken 3 months later period. Sera of the HLA-Dw6 negative patients show very low antibody reactivity when taken within 2 months after transplantation, whereas after two months the percentage of sensitized patients increased. In contrast the HLA-Dw6 positive patients show a strong humoral immune response whether or not corticosteroids were given. This phenomenon might be an explanation for the findings of DuBose et al. who found that corticosteroid resistant patients had a significant worse graft prognosis.

In conclusion we think that HLA-Dw6 positive recipients are high responders in renal transplantation because of:

1. A high incidence of HLA-Dw6 in patients who rejected their graft.
2. A faster rejection pattern.
3. In the HLA-Dw6 positive patients corticosteroid therapy seems to be less effective in reducing antibody production.

Not previously published
Not read at any scientific meeting
MECHANISMS OF CARDIAC ALLOGRAFT PROLIFERATION BY CYCLOSPORIN A.
IF Hutchinson, NL Tinley (Introduced by EMB Taylor). Department of
Surgery, Peter Bent Brigham Hospital, Boston, M.A.

Mechanisms of immunosuppression of the fungal peptide, Cyclosporin A
(CYA) have been studied using acardiac allograft rat model. The drug
(15mg/kg) was administered into 150 recipients for 7 days only from the
day of grafting. Despite major histocompatibility differences (WIFu + LEN
and LBN + LEN), all allografts functioned 7100 days without rejection
episodes in consistently healthy animals. To detect effect of cells with
suppressor characteristics, thymocytes or splenic lymphocytes (1x10^8)
from groups of LBN recipients bearing LBN hearts for 7, 14, 21 and 50 days,
were adoptively transferred into syngeneic untreated rats transplanted
with LBN hearts 24 hours later. Test grafts survived 12-16 days,
significantly p<0.001) longer than in unmodified animals (MST+SD= 7±0.5 days). Immunological activity of CYA treated grafted rats was then
tested. LMC mounted by lymphocytes from various host lymphoid compartments
including cells infiltrating the grafts, was reduced significantly (3-8x)
in untreated controls, LMC = 24-39 (p<0.0001). Anti-donor antibody
activity (CDC, ADCC and reverse indirect hemolytic plaque assay) were
also significantly decreased. Indefinite graft survival despite
discontinuing CYA suggests emergence of suppressor cells; these cells
in turn, may cause profound abrogation of cellular and humoral host
effector mechanisms.

The work described in this summary has been published in "Transplantation"
September 1981 and read at the American Federation of Clinical Research
Washington, D.C.
Changes in human natural killer (NK) cell activity in the first month after renal transplantation

J.K. Hegarty, Carol F. Ramsden, C.P. Silk; and F.J. Guillou
University Department of Surgery, St. James’s University Hospital, Leeds

The cells which mediate natural cytotoxicity (NK cells) against certain cell lines are at least partially identical with those which mediate antibody dependent cellular cytotoxicity (ADCC). The role of ADCC in human renal allograft rejection is not known. Using the cytotoxic activity of peripheral blood lymphocytes against the myeloblastoid cell line K562 in a 4 hour 51 Chromium releasemessay we have examined NK cell activity before and at 48-72 hour intervals after transplantation in eight patients receiving immunosuppression with prednisolone and azathioprine. The change in NK activity over each 48-72 hour period was compared with the change in serum creatinine observed over the same period. Of 32 observations when NK activity rose, this was accompanied by a rise in the serum creatinine in 19, and in 29 instances when NK activity fell the serum creatinine fell in 23 ($X^2 = 8.2; p<0.01$). When changes in NK activity in one 48-72 hour period were compared with the change in serum creatinine in the following period it was found that of 35 occasions when NK activity rose, this was followed by a rise in serum creatinine in 18, and of 33 observations when NK activity fell, this was followed by a fall in serum creatinine in 27 instances ($X^2 = 8.2; p<0.01$).

Since the NK activity of 24 haemodialysis patients was not significantly different from that of 29 healthy control subjects (means 54.5% ± 14.2% and 59.9% ± 10% respectively at 100:1 effector:target ratio) it seems unlikely that the change in serum creatinine influences NK reactivity. These observations suggest that NK cells, and thus the cells available for mediating ADCC, are generated during the in vivo allo-immune response in man and that their activity may be modified by immunosuppression.

This work has not been read at a scientific meeting.

The work described in the summary is currently in press.
The optimum dose of steroids for maintenance immunosuppression in clinical kidney transplantation has not been established. We have examined the merits of high and low dose steroid therapy in inbred rats given heterogenic cardiac allografts.

In untreated AK recipients, living hearts were rejected in a median time of 5 days (Group 1). Groups of rats were treated with different immunosuppressive protocols and the results are shown in the table.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MAINTENANCE THERAPY*</th>
<th>BULLIS THERAPY**</th>
<th>%</th>
<th>GRAFT SURVIVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NIL</td>
<td>NIL</td>
<td>0</td>
<td>0, 2, 4, 8, 16, 32, 64</td>
</tr>
<tr>
<td>2</td>
<td>A 4mg/Kg</td>
<td>NIL</td>
<td>2</td>
<td>0, 2, 4, 8, 16, 32, 64, 100</td>
</tr>
<tr>
<td>3</td>
<td>A 4mg/Kg</td>
<td>P 4mg/Kg</td>
<td>10</td>
<td>6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24</td>
</tr>
<tr>
<td>4</td>
<td>A 4mg/Kg</td>
<td>P 0.5mg/Kg</td>
<td>YES</td>
<td>3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100</td>
</tr>
<tr>
<td>5</td>
<td>A 4mg/Kg</td>
<td>P 0.5mg/Kg</td>
<td>YES</td>
<td>10, 20, 30, 40, 50, 60, 70, 80, 90, 100</td>
</tr>
<tr>
<td>6</td>
<td>A 4mg/Kg</td>
<td>P 0.5 mg/Kg</td>
<td>NO</td>
<td>115, 130, 145, 160, 175, 190, 200, 210, 220, 230, 240</td>
</tr>
</tbody>
</table>

* A - Azathioprine t/p, P - Prednisolone t/p.
** Sulfas - Methyiprednisolone 16 mg/kg on day 5 & 6.

Neither high dose (4mg/Kg) nor low dose (0.5mg/Kg) of Prednisolone prolonged graft survival when given daily with Azathioprine (Groups 2 & 3). Significant prolongation was achieved however (p<.005) when Methylprednisolone 16mg/Kg was administered in addition on days 5 & 6 following transplantation (Groups 4 & 5). Changing from a low dose maintenance therapy to a high dose on day 5 had the same effect as giving Methylprednisolone (Group 6). It would seem from these results that high doses of maintenance steroids are no more effective than low doses, and their administration during the first post transplant week fails to delay rejection. However when high doses are given for the first time at the onset of rejection, graft survival can be greatly prolonged.

The work described in this summary has not been previously published nor read at a previous scientific meeting.

Classification of Centres According to their Post-Transplant Performance

B.A. BRADLE, N.H. BELLWOOD, S. VAN LANGHOLZ & O.T. LAUNDY.

UK Transplant Service, GM Regional Transfusion Centre, Southend Rd, Bristol.

Thirty-one centres in the UK and Ireland were compared for post-operative results. Death with a functioning transplant, non-immunological failure and graft rejection were examined. The method was applied to discrete post-operative time intervals. For example, rejection had a bimodal distribution with two intervals; 0 to 25 days and 26 to 130 days. Each centre was compared with every other centre using the logrank test. Groups which were not significantly different from each other were clustered into HIGH survival, LOW survival and INTERMEDIATE. This method provides a sliding scale which depends on the existence of a significant difference between HIGH and LOW. If all centres performed equally well, no significant differences would exist between them and variations in rank order would be attributable to sampling error.

That such an utopian state of affairs is still far off, is illustrated by the fact that wide differences exist between centres and the probability that they occur by chance is, in many cases, less than 1 in 1,000. Some rank HIGH by all parameters whereas others rank HIGH for deaths and LOW for rejection and vice versa. A clear difference exists when performance between 0 and 25 days is compared to 26 to 130 days, presumably reflecting environmental risks.

This method provides a rational basis for the future investigation of the "centre effect".

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

The work contained in this summary has not been read at a previous scientific meeting.
Cytotoxic T lymphocytes. Both arterial and venous endothelial cells have been cultured from veins and arteries obtained by surgery from otherwise healthy dogs. Both venous and arterial endothelial cells appeared to be very sensitive targets in a 51Cr release test; CTL generated in a mixed lymphocyte culture specifically lysed stimulator type target endothelial cells. Lymphocyte stimulation by endothelial cells appeared to induce lymphocyte proliferation and generation of CTL. The cytotoxic potency of CTL generated with allogeneic venous endothelial cells was higher for venous endothelial than for arterial endothelial cells and for PHA stimulated lymphoblasts. In accordance CTL generated with allogeneic arterial cells showed preference for arterial targets. Moreover cold target inhibition experiments showed that endothelial cells carry other target antigens than PHA stimulated lymphoblasts. These data would favor the use of 51Cr labelled endothelial cells instead of PHA stimulated lymphoblasts for monitoring of graft rejection. Since damage of endothelium by CTL might cause intravascular thrombosis and so irreversibly damage the grafted organ, information on the presence of CTL directed versus endothelium may be important.

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

The work contained in this summary has not been read at a scientific meeting.
A CORRELATION OF RENAL FUNCTION PRIOR TO CARDIAC ARREST AND AFTER TRANSPLANTATION IN 105 NON HEART BEATING CADAVERIC DONORS.

TA Geoghegan, KR Harris, S Wood, W Slapak
Wessex Regional Transplant Unit, St. Mary's Hospital, Portsmouth, Hants.

The function of kidneys removed from non heart beating cadaveric donors for renal transplantation was studied immediately prior to nephrectomy and serially post-transplanted for a period of 1 to 32 months. A non-smash technique using an intracortic balloon and hyperosmolar citrate was employed. In 18 recent donors creatinine clearance studies were performed approximately one hour prior to cardiac arrest. Of 130 kidneys transplanted locally with mean warm ischaemic time of 4.3 ± 2.6 minutes and a mean cold ischaemic time of 10.7 ± 4.4 hours, 53% had immediate life supporting function. In 47% of patients a mean of 3.4 ± 2.1 haemodialysis were required. Their overall one year graft survival rate is 48.9%.

From 18 donors in whom agonal creatinine clearances were performed 34 kidneys were transplanted, 2 being discarded due to anatomical abnormalities. Twenty nine of these kidneys (85%) assumed immediate life-supporting function following transplantation. The mean donor creatinine clearance in this group was 46.8 ± 28.0 ml/min. (range 6-95 ml/min). Five recipients required a mean number of 4.8 ± 3.4 haemodialysis. The mean creatinine clearance in these kidneys was 58.1 ± 24.5 ml/min. (range 23-83 ml/min), not statistically different from the previous group.

It is of particular interest that an 81 year old donor with a creatinine clearance of 4 ml/min. in the last hour prior to cardiac arrest had immediate life-supporting function, the recipient being discharged from hospital with a serum creatinine of 200 mmols/l at 14 days.

We conclude that although impaired renal function prior to cardiac arrest is of importance, poor renal function in the hour prior to death, particularly in young patients, has no relevance and does not correlate with post transplantation function.

References:

The use of routine urine cytology as a detector of virus reactivation in renal allograft recipients.

E.F.D. Mackenzie, C.R. Smith, S.D. Gardner
Southend Hospital, Bristol. Virus Reference Laboratory, Colindale, London.

In an initial study 57 transplanted patients' urines were routinely screened for virus inclusions, of these 7 were positive. Serial virus antibody titres showed these to be due to Human Polyoma Virus (HPV) infection. Four of these 7 patients had ureteric stenosis.

We will report on a prospective study on a further 48 patients. Of these 25 showed a rise in antibody titre to HPV (8K and JC) and in 19 this was preceded by the appearance of virus inclusions. In 29 there was a rise in antibody titre to Cytomegalovirus (CMV) preceded by inclusions in only 7 cases. Fourteen patients with viral infection had associated leukopenia and pyrexia, 9 leukopenia only, 4 pyrexia only and 14 were asymptomatic. One patient in this study had a transient episode of ureteric obstruction proven by retrograde pyelography.

8 patients were CMV seronegative pre-transplant and later developed primary infections. All had leukopenia and pyrexia. Five had clinical chest infection, one with acute pancreatitis. Virus inclusions were detected in urine in 4. All had functioning allografts during their infections.

This prospective study confirms the value of cytological screening of transplant urines in providing early warning of virus infection.
POST CYCLOSPORIN A INDUCE DONOR SPECIFIC NON-RESPONsIVENESS?

D.J.G. WHITE, T. NAGAO and H.F.S. DAVIES

Department of Surgery, Addenbrooke's Hospital, Cambridge.

It has been demonstrated in several different animal species that cessation of Cyclosporin A treatment does not inevitably lead to the loss of the allograft which was being protected from rejection by the Cyclosporin A immunosuppression. The establishment of this graft acceptance depends on the duration of therapy and the type of graft implanted. We have studied this graft acceptance state to ascertain its stability and specificity.

P388 (RTI) rats were induced to accept heterotopic DA (RTI) allografts by two weeks' i.m. treatment with 15 mg/kg Cyclosporin A per day. Measurement of Cyclosporin A blood levels failed to detect any significant depot of the drug in these animals. The stability and specificity of graft acceptance was tested by challenging these (P388) recipients at 1, 2, 4, 8, or 16 weeks post-transplant with either DA (donor specific) or MAC (RTI, third party) skin grafts. The results showed that the heart graft acceptance went through three phases. The first was stable but non-specific. The second was both unstable and non-specific, and the final phase was both stable and specific. A study of this final stage showed a specific deficit in the immunological competence of peripheral blood lymphocytes, lymph node lymphocytes from the same rats were fully competent. Serum failed to transfer this graft acceptance state to naive recipients.

Part of this work has been presented previously at the XVI International Course on Transplantation and Clinical Immunology and published in the Proceedings. Part has never been presented or published.

IDENTIFICATION OF LYMPHOCYTE SUBPOPULATIONS IN RENAL ALLOGRAFT RECIPIENTS USING MONOCLONAL ANTIBODIES AND A CELL-SORTER.


Nuffield Department of Surgery, University of Oxford, Oxford.

The monoclonal antibodies OKT3, OKT4 and OKT8 were used to define lymphocyte subpopulations in the peripheral blood of 22 laboratory workers and 13 renal allograft recipients. OKT3 is reactive with all T lymphocytes, OKT4 with helper-inducer T cells and OKT8 with suppressor-cytotoxic T cells. The proportion of lymphocytes reacting with each of these antibodies was analysed using an Ortho Cytofluorograf cell sorter. The procedure used to identify and count labelled lymphocytes using this instrument will be described.

The mean values (± S.D.) for OKT3, OKT4 and OKT8 in the 22 laboratory workers were 77±(27), 49±(10) and 20±(6) of lymphocytes respectively. The mean ratio (± S.D.) of helper-inducer T cells to suppressor-cytotoxic T cells (T4/T8 ratio) was 1.9±(4.8). The T4/T8 ratio was measured repeatedly over a five week period in 4 of these laboratory workers. Little fluctuation in the ratio was observed, the greatest variation being between 2.3 and 3.2 in one individual.

The T4/T8 ratio was determined at least five times weekly in 13 patients during the first three weeks after transplantation, and at less frequent intervals thereafter. No patient maintained a constant ratio. Results from this preliminary clinical study will be presented and the use of the T4/T8 ratio in the diagnosis of rejection will be discussed.