MONITORING AND MODULATION OF RECIPIENT IMMUNE RESPONSIVENESS TO PREVENT KIDNEY GRAFT REJECTION IN THE EARLY POST-TRANSPLANT PERIOD

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Summary

In 41 consecutive living and cadaver donor renal transplant recipients, immunological monitoring was performed 2–3 times a week for the first two post-transplant months. Monitoring consisted of:

1) Circulating T and B cell levels (E-EAC Rosette assay)
2) T cell reactivity (PHA-Con A)
3) LMC and ADCC reactivity

Rejection was diagnosed by standard techniques including radioisotope renal scans and biopsy in some cases. Immunosuppression consisted of prednisone, imuran, cyclophosphamide and horse ALG. In 32 rejection episodes in the first two months, 22 (68%) were associated with a rise in T cell levels. Rejection activity also correlated with an augmented PHA mitogenesis count of 20 ± 5%. There was no positive correlation between Con A mitogenesis and rejection. There was also no correlation between rejection and circulating B cell levels. There was no significant correlation between a positive ADCC and graft rejection. Furthermore a positive ADCC in association with a negative LMC resulted in excellent long-term graft function. In conclusion, an excellent correlation of levels of circulating T cells and T cell reactivity with early in vivo rejection was shown.

Introduction

Immunological monitoring of renal allografting is a new and complex approach to the control of transplant recipients consisting of a battery of tests to assess both cellular and humoral immune response. These assays can be divided into four different groups:

i) In vitro response to phytomitogens¹,²,³

ii) Quantitation of circulating peripheral B and T cells⁴

iii) Study of cellular immunity by mixed lymphocyte culture
(MLC test)\textsuperscript{5}, direct cell-mediated lympholysis (LMC) and indirect cell-mediated lympholysis (CML)\textsuperscript{6,7}.

iv) Evaluation of humoral immunity by complement-dependent cytotoxicity (CDC) test, antibody-dependent cell-mediated toxicity (ADCC) test\textsuperscript{8}, and inhibition of the MLC by recipient serum\textsuperscript{9,10}.

Contradictory results are reported from different investigators\textsuperscript{11,12} concerning the in vitro behaviour of T and B cells and cytotoxic specificities in the recipients' sera during the post-operative clinical course\textsuperscript{13,14}. Such a disparity of results depends very often upon the different techniques employed, on the intervals intercurring between grafting and testing; on graft survival and on pre-existent immunological status.

This study concerns researches into the immune responsiveness of 41 patients during the first two months after grafting using Rosette E and EAC tests, reactivity to phytomitogens and the detection of cytotoxic antibody during rejection crises.

Methods

Immunological monitoring was performed in 41 recipients of kidney grafts: 28 from HLA haplo-identical related donors, and 13 from cadaver donors.

All recipients were transplanted and followed at the Transplant Unit of the II Ind Surgical Clinic, Rome, and were maintained on routine immunosuppressive (IS) therapy as previously described\textsuperscript{15}.

Rejection was diagnosed by creatinine clearance levels, changes in diuresis and standard techniques including radioisotope renal scans and kidney biopsy in some cases.

The isolation of lymphoid cells for all tests was made from sterile heparinised blood by means of gradient sedimentation in Ficoll-Hypaque according to the method of Böyum\textsuperscript{16}. Studies of T cell levels (A-RFC, T-RFC) were performed according to the Woody method\textsuperscript{17}.

T cell reactivity (PHA and ConA blastogenesis) determination has previously been described\textsuperscript{3}. The EAC Rosette determination was performed according to Jondal et al\textsuperscript{18}.

The Lymphocyte Mediated Cytotoxicity (LMC) assays were performed using, as effector cells, recipient lymphocytes (RL) in control heat-inactivated serum. The Target cells were PHA lymphoblasts from the kidney donors. The Effector/Target ratio was 50/1. \textsuperscript{51}Cr release was measured after 16h of incubation at 37°C and expressed as percent lysis according to the formula (ER-SR)/(MR-SR) x 100 where ER, SR and MR are experimental, spontaneous and maximum release, respectively.

The Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) was assayed by a microtest\textsuperscript{19} using a 16 hr assay procedure. The heat-inactivated serum of the patient was incubated for 4 hr with the target cells. The effector cells were obtained from recipients and healthy normal volunteers. The ratio of E/T was 50/1. The specific release was expressed as for the LMC test.
Results

Monitoring of thymus-dependent rosette forming-cells including both active (A-SRBC-RFC) and total (T-SRBC-RFC) was performed in 41 recipients.

Table I shows the data obtained from 32 rejection episodes occurring during the first two months. The T-RFC showed an average increase of 39.2% ± 7.6 SD in 68% of cases. The A-RFC showed an average increase of 28.5% ± 8.6 SD in 72% of cases. Even though the difference between the two percentages was not statistically significant, the A-RFC seemed to be more indicative

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Functioning graft</th>
<th>Rejecting graft</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>A-RFC (346)</td>
<td>25.1 ± 3.3</td>
<td>12.4 ± 7.1</td>
<td>28.5 ± 8.6</td>
<td>72</td>
</tr>
<tr>
<td>T-RFC (443)</td>
<td>60.7 ± 5.5</td>
<td>23.5 ± 6.2</td>
<td>39.2 ± 7.6</td>
<td>68</td>
</tr>
</tbody>
</table>

of the early diagnosis of rejection. In 32 rejection episodes, the T-RFC showed a significant increase in 22; in 10 episodes it was normal (23.5 ± 6.2 SD). In these 10 episodes the A-RFC showed a significant increase in 6.

In five cases the T-RFC reached high levels without clinical rejection, but in the same cases the A-RFC remained normal. Nine patients with a normal post-operative course had low levels of both T-RFC and A-RFC.

The EAC Rosettes showed no relation to rejection.

The Con A reactivity reached, in the functioning grafts, a mean value of 26,250 cpm ± 13,750 SD and in the rejecting grafts 28,920 cpm ± 14,560 SD. The difference is not statistically significant.

In the PHA assay the difference between the functioning grafts (42,560 cpm ± 19,450 SD) and rejecting grafts (54,380 cpm ± 22,260 SD) is significant (p < 0.05). All the transplanted patients showed a negative pre-transplant ADCC. In the post-transplant ADCC assay there was no correlation between this test and the occurrence of rejection (p = 0.05). We found some cases with a positive ADCC without a rejection crisis and in the case illustrated in Figure 1 there was, vice versa, rejection with negative LMC and positive ADCC.

LMC reactivity has been studied in 15 patients: all were negative prior to transplantation. Nine of these patients developed a positive donor-specific LMC reaction with a peak of cytotoxicity (48% of $^{51}$Cr release) between days 7 and 12 after transplantation. All these had clinical signs of acute rejection. Five more cases without rejection remained LMC negative post-transplant.

Finally, two cases developed a positive LMC with a mean of 14% of $^{51}$Cr release. These patients showed signs of a very mild rejection.
Discussion

Several investigators have examined the percentage variation of T-RFC in the post-operative course of kidney transplant patients, and looked for any correlation with rejection crises. The T-RFC are conditioned by the immunosuppressive therapy, but whereas the immunosuppressive therapy was similar for the 41 patients tested, we found an increase of T-RFC over the mean value in 68% of cases in whom rejection occurred in the first two post-operative months.
Relatively little attention has been given to the A-RFC subpopulation of lymphocytes, perhaps due in part to the technical difficulties of the test. Significantly reduced levels of A-RFC have been observed in patients with cell-mediated immunodeficiencies, although the T-RFC may be in the normal range. These results also confirm the findings of Wybrand et al. and Yu that the A-RFC, although a subpopulation of T-RFC, may vary independently of T-RFC. Surface membrane differences between A-RFC and T-RFC support the thesis that A-RFC is a distinct subpopulation of T lymphocytes. Our results suggest that the increase in circulating A-RFC may represent a subpopulation of the most immunologically active T cells. A low number of A-RFC might imply a functional T cell deficiency, whereas a larger number of A-RFC would suggest augmented T cell reactivity. In fact the percentage variation of A-RFC is correlated closely with clinical rejection episodes (72%). In five cases without rejection A-RFC was stable at low levels, whereas the T-RFC increased. ADCC represents an interface of humoral and cell-mediated immunity and the resulting complement-independent cytolysis of specific target cells can be considered a product of the synergistic activity of these two components of the immune system. Different opinions have been expressed concerning the importance of this test in immunological monitoring. Jeannet et al. have suggested that the presence of ADCC for donor target cells before and after transplantation is associated with early acute rejection. Thomas et al. demonstrated a correlation between proteinuria and some other signs of chronic rejection. Our experience of ADCC and LMC is limited, but suggests the importance of a negative ADCC before grafting and the relevance of LMC as a promising diagnostic aid in predicting acute allograft rejection.

Both ADCC and LMC are immunological phenomena which may be controlled with appropriate immunosuppressive therapy directed against recipient K-cell activity. Employing these immunological tests twice a week it may be possible to assess the intensity of therapy and to modify it according to immunological reactivity.

References

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17 Woody, JM (1975) J. Immunol. Methods, 8, 331
22 Yu, DTY (1975) J. Immunol., 115, 91
25 Thomas, JM, Thomas, FT, Kaplan, AM and Lee, HM (1975) Transplantation, 22, 94

Open Discussion

HABERAL (Ankera) Did you use Con A or phyto mitogen in immunosuppressive treatment clinically?

FAMULARI No.

HARY (Helsinki) You monitored the E-rosettes as per cent of all lymphocytes. We know that after institution of immunosuppressive therapy, the number of lymphocytes, leucocytes being normal, is reduced to approximately 1/10 or 1/5 of the original. To me at least it seems unlikely that small differences in the percentage distribution would make any difference if you think that total number of lymphocytes is just a fraction of normal.

Secondly, as regards the PHA and Con A responses, you probably agree that you read the results so late that they have no bearing on therapy. The final point you made is interesting. You have found, like the group of Kristensen, cytotoxic cells in the blood. I think that this really might be a possibility for future monitoring of graft rejection in a very rapid way. Unfortunately we are not ready for that yet because it seems that it is difficult to detect the killer cells prior to rejection.