Mixed Leucocyte Culture, Pre-Transplant Transfusions and Renal Allograft Rejection

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The mixed leucocyte culture (MLC) technique has found wide application in the study of both humoral and cellular aspects of the immune response in organ transplantation. The lymphocyte defined (LD) function of the major histocompatibility complex is assuming increasingly more importance, because the MLC (LD loci) match seems to correlate better with graft survival than does the serologically defined (SD) HL-A antigen match. We have undertaken a long term MLC study of renal transplant donor-recipient pairs in an effort to define the predictive value of the test, the possible mechanisms of immunologic adaptation, and the associated risk factors.

MATERIALS AND METHODS

Fifteen donor recipient pairs were selected on the basis of completeness of MLC data and length of follow up. MLC results, the number of pretransplant dialyses and transfusions, and the presence of cytotoxic antibodies in the recipient were correlated with the clinical course of the patients the length of which ranged from 264 to 515 days in patients with functioning kidneys. The blood transfusions were usually of white blood cell poor whole blood or packed cells. Fresh frozen washed red cells were used when available. Cytotoxic antibody assays were performed to test the pretransplant recipient's serum against cells of a standard panel of 18 donors. All patients received antihuman lymphoblast globuline, azathioprine and prednisone as immunosuppressive drugs. Rejection episodes were defined as a decrease in renal function which was treated by increasing immunosuppression with or without graft irradiation.

Mixed leucocyte cultures were prepared using a micro method (Shons et al, 1973). Responding cells, $2 \times 10^5$, were used in each 0.2 cc culture with $1 \times 10^5$, $2 \times 10^5$, or $4 \times 10^5$ stimulating cells incubated with 50 $\mu$g of mitomycin C/ml of cell suspension (Etheredge et al, 1973). Mixed cultures were
prepared using cells of the recipient, donor and an indifferent person. After five days of incubation the cultures were labelled with a μCi of radioactive thymidine of specific activity 16-18 Ci/m mole (Shons et al, 1972). The cultures were harvested 18 hours later by precipitation onto glass fibre filters which were counted in a Unilux-2A liquid scintillation counter. Means of triplicate culture counts/min values were determined.

RESULTS

The average number of pretransplant transfusions given to patients who did not reject their new kidney was 18 (Table I) and to those who did have rejection episodes was 36 (Table II). Patients who lost their kidneys due to rejection had an average of 52 pretransplant transfusions. Only one patient in

Table I. Patients with no rejections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Recip. Patient</th>
<th>Diagnosis</th>
<th>HL-A Match</th>
<th>Cytotoxic Antibodies</th>
<th>Dialyses</th>
<th>Number Transfusions</th>
<th>Rejections</th>
<th>MLC Reactivity</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>Diabetes</td>
<td>A</td>
<td>0</td>
<td>42</td>
<td>35</td>
<td>0</td>
<td>RH-DS</td>
<td>- 71 days</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>Diabetes</td>
<td>A</td>
<td>0</td>
<td>15</td>
<td>11</td>
<td>0</td>
<td>NRR</td>
<td>Dead - virus infection 274 days</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Diabetes</td>
<td>C</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>0</td>
<td>NRR</td>
<td>Cr 1.0 - 303 days</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>CPN</td>
<td>B</td>
<td>14/18</td>
<td>23</td>
<td>17</td>
<td>0</td>
<td>NRR</td>
<td>Cr 1.0 - 495 days</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>Polycystic</td>
<td>A</td>
<td>0</td>
<td>19</td>
<td>17</td>
<td>0</td>
<td>NRR</td>
<td>Cr 0.6 - 515 days</td>
<td></td>
</tr>
</tbody>
</table>

1CPN = Chronic Pyelonephritis; 2Rejections: Days after transplant; 3MLC Reactivity after transplant (a) NRR = Normal Recipient Response (b) RH-DS = Recipient Hyporespons - donor specific 4Transplant nephrectomy due to ureteral leak

Table II. Patients with rejections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Recip. Patient</th>
<th>Diagnosis</th>
<th>HL-A Match</th>
<th>Cytotoxic Antibodies</th>
<th>Dialyses</th>
<th>Number Transfusions</th>
<th>Rejections</th>
<th>MLC Reactivity</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>36</td>
<td>CGN</td>
<td>A</td>
<td>0</td>
<td>17</td>
<td>9</td>
<td>7</td>
<td>RH-NS</td>
<td>Tx nephrectomy - 14 days</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>CPN</td>
<td>B</td>
<td>0</td>
<td>24</td>
<td>22</td>
<td>36,49</td>
<td>RH-NS</td>
<td>Tx nephrectomy - 65 days</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>CGN</td>
<td>C</td>
<td>0</td>
<td>56</td>
<td>75</td>
<td>40, 63, 73</td>
<td>NRR</td>
<td>Tx nephrectomy - 85 days</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>HU</td>
<td>A</td>
<td>0</td>
<td>97</td>
<td>84</td>
<td>14, 28, 50</td>
<td>RH-NS</td>
<td>Tx nephrectomy - 113 days</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>Diabetes</td>
<td>C</td>
<td>6/18</td>
<td>18</td>
<td>27</td>
<td>90</td>
<td>RH-DS</td>
<td>Cr 1.2 - 264 days</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>Polycystic</td>
<td>C</td>
<td>0</td>
<td>50</td>
<td>55</td>
<td>21</td>
<td>RH-NS</td>
<td>Cr 1.4 - 264 days</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>Diabetes</td>
<td>C</td>
<td>0</td>
<td>17</td>
<td>22</td>
<td>55</td>
<td>RH-NS</td>
<td>Cr 1.3 - 306 days</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>19</td>
<td>CGN</td>
<td>D</td>
<td>0</td>
<td>52</td>
<td>25</td>
<td>60</td>
<td>RH-NS</td>
<td>Cr 1.4 - 364 days</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>33</td>
<td>Diabetes</td>
<td>A</td>
<td>0</td>
<td>7</td>
<td>13</td>
<td>150</td>
<td>RH-NS</td>
<td>Cr 1.6 - 386 days</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>Diabetes</td>
<td>C</td>
<td>0</td>
<td>11</td>
<td>13</td>
<td>80</td>
<td>NRR</td>
<td>Cr 1.2 - 408 days</td>
<td></td>
</tr>
</tbody>
</table>

1CPN = Chronic Pyelonephritis; CGN = Chronic Glomerulonephritis; HU = Haemolytic Uraemic 2Rejections: Days after transplant 3MLC Reactivity after transplant (a) NRR = Normal Recipient Response (b) RH-DS = Recipient Hyporespons - donor specific (c) RH-NS = Recipient Hyporespons - non-specific 4Transplant nephrectomy due to rejection and ureteral leak 5Transplant nephrectomy due to rejection
Figure 1. Preoperative MLC studies of 7 HL-A A match patients. The response of $2 \times 10^5$ recipient cells/culture stimulated by $1 \times 10^5$ mitomycin C treated cells/culture of the donor is shown. The response in the autologous controls is also shown. The means of triplicate culture counts per minute values plus or minus the standard deviation are shown. Patients 1, 2, 5 had no rejections and patients 6, 9, 14, 15 had rejections.

Each group had cytotoxic antibodies. Seven patients had diabetes mellitus, and the one death and the one kidney loss due solely to technical problems occurred in these seven recipients.

Seven patients were HL-A identical matches with their donors (Figure 1). Three patients (1, 2 and 5) had stimulation indices ($\text{SI} = \frac{\text{test CPM}}{\text{control CPM}}$) of 2.1, 1.1 and 0.92 (respectively) and had no rejection episodes. Another four patients (6, 9, 14 and 15) had SI's of 0.87, 4.2, 2.8 and 5.1 and these had rejection episodes. One patient (6, SI=0.87) had a combined rejection-ureteral leak problem which resulted in the loss of the kidney.

Three patterns of post operative MLC response can be determined. A pattern of recipient responsiveness we now call 'normal', as shown in Figure 2, was seen in 4 of the 5 patients with no rejection and in 2 of the 10 patients who had rejections. This type of response is characterised by
marked stimulation of cells of the recipient by both cells of the donor and
cells of an indifferent person before and after transplantation. There was a
generally decreased response in autologous plasma compared to the response
in pooled plasma in both preoperative and post operative cultures.

Two patterns of postoperative recipient cell hyporesponsiveness were
determined (Figure 3). The donor specific recipient cell hyporesponsiveness
that was noted in two patients was characterised on postoperative day 1 by a
loss of donor cell stimulation of recipient cells in both pooled and autologous
plasma; a normal recipient cell response to indifferent cells was maintained.
A pattern of nonspecific recipient cell hyporesponsiveness was seen in 7 of
the 10 patients who had rejection episodes; it was not seen in any of the
patients without rejection episodes. Cells of the recipient were hyporespon-
sive to both donor and indifferent cells in both pooled and autologous plasma
at postoperative days 1-2; recipient cell responsiveness returned by post-
operative days 4-6.

DISCUSSION AND CONCLUSIONS
A possible beneficial effect of blood transfusions on renal transplant recipi-
ents has been suggested (Opelz et al, 1972). There have also been studies
Figure 3: Pre and post operative MLC studies of patients 10 and 11. The cross hatched bars indicate the response of 2 x 10^5 recipient cells/culture stimulated by 2 x 10^5 mitomycin C treated donor cells/culture; the stippled bars indicate the response of 2 x 10^5 recipient cells/culture stimulated by 2 x 10^5 mitomycin C treated indifferent cells/culture. The means of triplicate culture counts/min values plus or minus the standard deviation are shown. A=25% autologous (recipient) plasma culture supplement; P=25% pooled plasma culture supplement. The dashed line at the bottom of each bar represents the autologous control response.

showing a correlation between a good clinical course of multiple transfused renal transplant recipients and the presence of serum blocking activity as measured by pretransplant MLC. In our small group of patients, however, those who had received the most pretransplant transfusions had more rejection episodes. This is consistent with previous hypotheses; sensitization by whatever method will adversely effect the outcome of a kidney transplant (Terasaki et al., 1971).

The only two complications other than rejection episodes - death from overwhelming virus infection and loss of kidney due to ureteral leak - both occurred in patients with diabetes. This is indicative of our experience in the much larger group of diabetics we have transplanted.

The significant MLC stimulation in 3 of 7 HL-A A-match donor-recipient pairs is interesting. It is a far higher incidence of MLC nonidentity - HL-A
identity than has previously been reported (Segal et al., 1973; Seigler et al., 1972). We attribute this increased incidence of MLC stimulation to our sensitive MLC technique. The correlation of rejection episodes with MLC stimulation in the HL-A identical patients would indicate that the high incidence of MLC reactivity is not simply a laboratory artefact. In a larger study of HL-A identical donor-recipient pairs we found a significant stimulation (SI = 2.8 or greater) in 7 of 18 pairs, 4 of 7 of which suffered rejections while only 1 of 11 with SI of less than 2.8 had a rejection (Etheredge et al., 1973).

In an earlier report we described patterns of MLC response in the immediate postoperative period and at random points months to years after transplant (Shons et al., 1973). The clinical significance of the MLC reactivity was not known because of the short follow up. We still cannot ascertain the importance of cellular hyporeactivity or specific serum blocking factors first found in patients months to years after transplantation. All rejection episodes in our patients occurred within the first 150 days. It would seem that acute rejection which develops more than a year after transplant, or chronic rejection, represents a more complex problem than rejection episodes of the type seen by us. Longer follow up and MLC correlation in these patients is needed. However, we can now see the importance of the MLC response patterns noted within the first week after grafting. There is a positive correlation between the loss of recipient cell reactivity on postoperative day 1 or 2 and subsequent rejections. This loss of reactivity was donor specific or, what may represent simply a more extreme form of the same process, nonspecific. This immediate loss of recipient cell reactivity has previously been noted in a study of unmodified canine renal allografts (Miller et al., 1971). This may represent a trapping of reactive lymphocytes, the first step in a process which then led to rejection. 7-150 days later in our patients. It is possible that MLC identity or adequate immunosuppression prevented the initial lymphocyte trapping in patients 2 - 5 who suffered no rejections.

Among those donor recipient pairs which stimulate in MLC we are, as yet, unable to correlate the level of preoperative MLC stimulation with incidence or severity of rejection. Only the postoperative loss of reactivity seems significant so far.

In this study recipient plasma both before and after transplantation was generally inhibitory when compared with pooled plasma in MLC. This inhibition is probably caused by preoperative uraemia and postoperative immunosuppressive therapy. The possibility exists, however, that the inhibition may also be due to serum blocking factors.
REFERENCES

Shons, A. R., Etheredge, E. E. and Najarian, J. S. (1972) Clinical and Experimental Immunology, 12, 351
Shons, A. R., Kromrey, C. and Najarian, J. S. (1973) Cellular Immunology, 6, 420

OPEN DISCUSSION

J SYBESMA (Utrecht): In the group that did not respond to MLC I should like to know whether the patients who have had transfusions did or did not develop lymphocyte antibodies.

SHONS: The group was small and therefore there was not a good correlation. To obtain this information we shall have to study more patients.