MLR INHIBITORY ACTIVITY OF PRETRANSPLANT ANTI-DONOR B CELL ANTIBODIES, AND KIDNEY GRAFT SURVIVAL

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Summary

The prognostic significance of different types of pretransplant anti-donor B cell antibodies on graft outcome was studied in 193 cadaver kidney recipients. Additionally, in 84 combinations the MLR inhibitory activity of patient sera was determined, and the relationship of pretransplant anti-donor B cell antibodies, MLR inhibitory activity and graft outcome was analysed. Graft survival was high in recipients with cold B cell antibodies, whereas it was unfavourable in recipients positive for warm B cell antibodies. MLR inhibitory activity was highly correlated with the latter type of antibody. In patients with high pretransplant MLR inhibitory activity graft outcome was significantly impaired. In recipients with cold-reactive B cell antibodies who had excellent graft outcome, MLR inhibitory activity was not demonstrable.

Introduction

It is generally accepted that kidney transplantation can be performed in spite of a positive B cell crossmatch [1–3]. However, it is unclear whether preformed anti-donor B cell antibodies have a beneficial or adverse influence on graft outcome. In some clinical studies an enhancing effect of B cell antibodies was described [4, 5]; in addition, it could be shown in animal experiments that allograft survival can be prolonged by administration of anti-Ia serum [6, 7]. Others, however, reported on increased graft rejections in recipients with anti-donor B cell antibodies [8].

The evaluation of the significance of pretransplant B cell antibodies for the fate of a transplant is complicated because various types of antibodies may cause a positive B cell crossmatch: weak anti-A B C antibodies, Anti-DR antibodies, and non-HLA antibodies (frequently auto-reactive cold antibodies). The MLR inhibitory activity (MLR-IA) of sera from alloimmunised individuals appears to be largely determined by the presence of antibodies against HLA-D(DR) antigens
of the stimulating cells [9]. Whether MLR blocking antibodies have a protective or deleterious effect on graft survival is unclear. In this study, MLR inhibitory activity (MLR-IA) of different types of pretransplant B cell antibodies was analysed and correlated with kidney graft survival.

Patients and methods

The study group comprised 193 first cadaver kidney transplants, all of them with negative T cell crossmatches. Pretransplant serum samples were taken from each patient. Before use, aliquots of each serum were exhaustively absorbed on pooled human platelets. All sera were tested against specific donor B cells at 4°C and 37°C by the microcytotoxicity assay. Actuarial graft survival rates in recipients with no pretransplant B cell antibodies were compared with those in recipients with different types of anti-donor antibodies.

Pretransplant sera of 84 recipients were screened for inhibitory activity (IA) in donor-specific unidirectional MLR. Semi-micro MLR tests, described previously [10], were set up both with 20 per cent pooled AB serum and recipient serum obtained on the day of transplantation. MLR-IA was expressed as the fraction of mean cpm (mean counts per minute of triplicates) in recipient serum and mean cpm in control serum. MLR-IA was considered present when cpm in recipient serum was reduced to 25 per cent or less (fraction \( \leq 0.25 \)) as compared to cpm in control serum.

![Graph](image)

Figure 1. Actuarial survival rates of first cadaver kidney transplants in recipients with and without pretransplant anti-donor B cell antibodies (B-Ab). Graft survival in patients with cold B-Ab was significantly higher than in patients with no B-Ab.
Results

We examined retrospectively 193 first cadaver kidney recipients, all of them with negative T cell crossmatches. Fifty-one patients had pretransplant anti-donor B cell antibodies (B-Ab); 21 patient sera reacted only at 4°C (cold B-Ab), whereas 30 sera reacted optimally at 37°C (warm B-Ab). Hyperacute graft rejection was not observed in any case. Graft survival in recipients with cold B-Ab was significantly better (p = 0.03) than in patients with no detectable B-Ab (71 per cent versus 62 per cent at one year). The results in warm B-Ab positive recipients were unfavourable (54 per cent graft at one year). Possibly due to small numbers of cold and warm B-Ab positive recipients, the difference of graft survival rates in both groups was not statistically significant (Figure 1).

Since a positive B cell crossmatch may be caused by weak HLA-ABC antibodies, aliquots of all sera were exhaustively absorbed with pooled platelets and subsequently tested against specific donor B lymphocytes. In 10 sera, cold B-Ab were removed by platelet absorption indicating that they contained ABC antibodies; in 11 sera the lymphocytotoxic activity could not be absorbed. In the latter group, 82 per cent (9/11) of the grafts functioned at one year whereas graft survival was only 60 per cent (6/10) grafts) in recipients with cold B-Ab absorbable by platelets (Figure 2). In patients with warm B-Ab (whether absorbable by platelets or

![Figure 2. Actuarial graft survival rates in patients with different types of pretransplant anti-donor B cell antibodies (B-Ab). Graft outcome in recipients with cold B-Ab non-absorbable by platelets was significantly higher than in patients with warm B-Ab.](image-url)
or not) graft function was 54 per cent. Graft survival in recipients with pretransplant cold, non-absorbable B-Ab was significantly higher than in patients with warm B-Ab (p < 0.05) (Figure 2).

Pretransplant sera from 84 recipients were tested for MLR-IA (Figure 3). Forty sera showed a positive B cell crossmatch (26 warm B-Ab and 14 cold B-Ab), whereas 44 sera were negative. In the warm antibody group (26 patients), 12 sera contained multispecific anti-DR antibodies, 8 sera weak anti-ABC antibodies, and in 6 sera the antibody specificities could not be defined. In this group the mean MLR-IA was 0.49, while the mean MLR-IA of B-Ab negative sera was 0.96 (p < 0.05); mean MLR-IA of cold B-Ab positive sera was 1.18. According to our criteria, 14/26 warm B-Ab positive sera exhibited strong MLR-IA (Index < 0.25) whereas only 3/44 in the antibody negative group displayed MLR-IA (χ² with Yates' correction = 9.8, p < 0.005).

Clinical follow-up was at least one year in all patients. In Table I the relationship of pretransplant MLR-IA, B-Ab and kidney graft survival rates is shown. One
TABLE I. Relationship between pretransplant MLR inhibitory activity (MLR-IA), anti-donor B-cell antibodies (B-Ab) and kidney graft survival

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number of patients</th>
<th>Graft outcome failure/success</th>
<th>% Graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>84</td>
<td>32/52</td>
<td>61.9</td>
</tr>
<tr>
<td>MLR-IA+</td>
<td>17</td>
<td>11/6</td>
<td>35.3 p* &lt; 0.05</td>
</tr>
<tr>
<td>MLR-IA−</td>
<td>67</td>
<td>21/46</td>
<td>68.7 p* = n.s.</td>
</tr>
<tr>
<td>Warm B-Ab+</td>
<td>26</td>
<td>12/14</td>
<td>53.8 p* = n.s.</td>
</tr>
<tr>
<td>Warm B-Ab−</td>
<td>58</td>
<td>20/38</td>
<td>65.5</td>
</tr>
<tr>
<td>Warm B-Ab+</td>
<td>26</td>
<td>12/14</td>
<td>53.8 p* = n.s.</td>
</tr>
<tr>
<td>Cold B-Ab+</td>
<td>14</td>
<td>3/11</td>
<td>78.6</td>
</tr>
<tr>
<td>MLR-IA+, B-Ab+</td>
<td>14</td>
<td>10/4</td>
<td>28.6 p* &lt; 0.05</td>
</tr>
<tr>
<td>MLR-IA−, B-Ab−</td>
<td>41</td>
<td>15/26</td>
<td>63.4</td>
</tr>
</tbody>
</table>

* Chi-square test

year graft survival in the total group was 61.9 per cent. In 17 patients selected for strong MLR-IA one year graft survival was only 35.3 per cent as compared to 68.7 per cent in patients with weak or no pretransplant MLR-IA (p < 0.05). Comparing the graft survival rates in MLR-IA and B-Ab positive recipients to those in MLR-IA and B-Ab negative recipients, a significant difference was found (28.6 per cent versus 63.4 per cent at one year, p < 0.05). Pretransplant warm anti-donor B-Ab seemed to be unfavourable (one year graft survival in B-Ab recipients: 53.8 per cent, in B-Ab negative recipients: 65.5 per cent, p = n.s.), whereas in cold B-Ab patients most of the grafts (78.6 per cent) functioned.

Discussion

The influence of pretransplant anti-donor B cell antibodies is a controversial topic. They have been correlated with unfavourable, indifferent, or improved graft outcome [1–5, 8]. Our results extend previous observations that warm B-Ab are associated rather with an unfavourable graft prognosis [11]. In agreement with data of Iwaki et al [12] and Ayoub et al [13] we observed better graft survival rates in patients with pretransplant cold-reactive antibodies.

Our data demonstrate that the presence of warm B-Ab is associated with strong MLR-IA. MLR-IA was, however, also present in a few B-Ab negative patients. None of the cold-reactive B-Ab positive sera exhibited MLR-IA. Strong anti-donor MLR-IA was significantly correlated with poor graft outcome. These results are in agreement with those of Cochrum et al [14], Suciu-Foca et al [15] and Hardy et al [16]; they contradict findings of Sengar et al [17] and Etterger et al [18]. The discrepant findings might possibly be explained by different
criteria on which MLR-IA was defined. In addition, in previous reports the MLR-IA was not differentiated according to the different types of B-Ab. Further studies are needed to find which role cold- and warm-reactive B cell antibodies play in graft rejection or protective mechanisms.

References

5. D’Apice AJF, Tait BD. *Transplantation* 1979; 27: 324

Open Discussion

BACH (Chairman) What is the correlation between DR and graft survival? Your data suggest a correlation between the presence of anti-DR antibodies and the outcome of the graft. There have been reports by various groups that graft survival was also very much dependent on DR compatibility. Now how do these two types of correlation match with each other? Is production of antibodies against DR antigen essentially a matter of incompatibility between donor and recipients for DR antigens or is it related to other kinds of immunological background?

LENHARD The first question with respect to the DR matching – we have analysed this and we didn’t find any significant difference between the different groups. The DR match as well as the HLA ABC match in the groups was not statistically different. The second question was what is the immunological stimulus for production of cold reactive antibodies, I guess. The cold reactive autoantibodies and the cold reactive antibodies are mostly autoactive in our hands and are presumably not produced after blood transfusions, but by viral infections, I think. The warm antibodies are mostly produced after blood transfusions.