Cellular and Humoral Immunity after Clinical Renal Transplantation

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Summary
Cellular and humoral responses against donor lymphocytes were studied in ten kidney-transplant patients, nine of whom had well functioning grafts.

No (T) cell-mediated cytotoxicity against donor cells was demonstrated. Specific anti-donor antibodies were found in two recipients with well-accepted grafts.

A single immunological factor responsible for a favourable clinical course was not demonstrated. Neither complete T nor B cell tolerance against donor cells had developed, and a well-tolerated graft could coexist with antibodies directed against donor cells.

Introduction
Long survival of allografted kidneys in patients receiving minimal doses of immunosuppressive drugs suggests immunological adaptation by the patient towards foreign antigens on the kidney. Whether the acceptance of the graft is partly due to enhancement or to a state of tolerance is still an open question (Suciu-Foca et al, 1974; Marcus et al, 1974; Quadracci et al, 1974; Falk et al, 1973), and our studies on a group of patients with functioning grafts represent an effort to elucidate these mechanisms.

The following in-vitro assays were used for cellular immunity: mixed lymphocyte-culture test (MLC) and cell-mediated lymphpolysis (CML) test and for humoral immunity: complement-dependent cytotoxicity (CDC) test, antibody-induced cell-mediated cytotoxicity (AICC) test and the inhibition of the mixed lymphocyte culture by recipient serum.

MATERIALS AND METHODS

Patient Material, Cells and Sera

Nine kidney-transplant patients with functioning grafts were studied together
with their living related donors. The recipients received minimal doses of azathioprine and prednisolone, which was not discontinued for the study. HL-A typing and clinical data are given in Table I. Included was one additional patient (EBT), who rejected a kidney from her mother after two weeks, and was successfully transplanted with a cadaver kidney 11 months later. The recipient is always designated A and the donor B. Control cells were sampled from two individuals for each donor/recipient pair selected to share (C) and not to share (D), the HL-A antigens of the donor being incompatible for the recipient. Donor, recipient, and control individuals were bled simultaneously, and lymphocytes separated with Ficoll-Isopaque flotation (Böyum, 1968). Serum was collected on the day of study and used without inactivation in CDC and after inactivation for 60 min at 56°C for AICC and MLC inhibition.

**Mixed lymphocyte-culture (MLC) test** was performed as a micro-test (Bondevik et al, 1974). Briefly, $0.15 \times 10^6$ responder and an equal number of mitomycin-treated stimulating cells were cultured in a volume of 0.2 ml in flat-bottomed microplates with a serum concentration of 20% either pooled normal AB—serum or a 1:1 mixture of recipient serum in normal serum. The results are expressed as stimulation index (SI), i.e. cpm in test combination/cpm in autologous control.

**Cell-mediated lympholysis (CML) test** was carried out as a microtest, modified from Lightbody and Bach (1972). Briefly, $0.24 \times 10^5$ Cr$^{51}$-labelled target cells were mixed with effector cells in a ratio of 1:30 and 1:100 in a volume of 0.2 ml, isotope release measured after 5 hr for the ratio 1:100 and 14–16 hr for the ratio 1:30. The specific release was expressed according to the formula $100(ER-SR)/(MR-SR)$ where ER, SR and MR are experimental release, spontaneous release and maximum release respectively.

**Complement-dependent cytotoxicity (CDC) test** was done with the Kissmeyer-Nielsen – Kjerbye technique (Kissmeyer-Nielsen and Kjerbye, 1967).

**Antibody-induced cell-mediated cytotoxicity (AICC) test** was performed as a microtest (Kaakinen et al, 1974). Briefly, antibody-treated Cr$^{51}$-labelled target cells were mixed with effector cells in a ratio of 1:30 in a volume of 0.20 ml in round-bottomed microplates. Isotope release was measured after 14–16 hr of incubation and the specific release expressed as for the CML test.

**RESULTS**

**Mixed Lymphocyte Culture**

Two of the donor/recipient pairs showed non-stimulation reciprocally, probably due to sharing of the MLC locus alleles (Table II). The other eight showed a positive MLC response when stimulated with donor cells (SI > 2). Thus no donor-specific tolerance could be demonstrated with the MLC test in these recipients of long-standing grafts. Cells from all ten recipients responded to
<table>
<thead>
<tr>
<th>Recipient</th>
<th>Renal disease</th>
<th>HL-A Recipient</th>
<th>HL-A Donor</th>
<th>Fam. rel.</th>
<th>Hapl. diff.</th>
<th>Mismatched antigens</th>
<th>Transf. prior to transpl.</th>
<th>Obs. time (months)</th>
<th>Rejections</th>
<th>Present graft funct. creat. mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>EÖ 9</td>
<td>Glom.nephr.</td>
<td>3,W19/5,W15</td>
<td>3,W19/5,W15</td>
<td>sib.</td>
<td>0</td>
<td>0</td>
<td>0^1</td>
<td>19</td>
<td>+</td>
<td>1.1</td>
</tr>
<tr>
<td>OKC 6</td>
<td>Tb. kidney</td>
<td>1,8/1,8</td>
<td>1,8/---</td>
<td>par.</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>120</td>
<td>+</td>
<td>1.3</td>
</tr>
<tr>
<td>NH 6</td>
<td>Glom.nephr.</td>
<td>2,9,27</td>
<td>2,27</td>
<td>sib.</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>55</td>
<td>+</td>
<td>1.1</td>
</tr>
<tr>
<td>BÖ 6</td>
<td>Glom.nephr.</td>
<td>1,12/3,7</td>
<td>3,7/3,W5</td>
<td>par.</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>54</td>
<td>++</td>
<td>1.5</td>
</tr>
<tr>
<td>IS 6</td>
<td>Glom.nephr.</td>
<td>--/2,12</td>
<td>W19,12/2,12</td>
<td>sib.</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>66</td>
<td>-</td>
<td>1.3</td>
</tr>
<tr>
<td>OMB 6</td>
<td>Glom.nephr.</td>
<td>3,W5/3,W15</td>
<td>3,W5/3,7</td>
<td>par.</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>67</td>
<td>+</td>
<td>1.6</td>
</tr>
<tr>
<td>TFO 6</td>
<td>Pyelonephr.</td>
<td>W19,12/2,W10</td>
<td>3,14/2,W10</td>
<td>par.</td>
<td>1</td>
<td>2</td>
<td>15</td>
<td>59</td>
<td>+</td>
<td>1.7</td>
</tr>
<tr>
<td>BS 6</td>
<td>Glom.nephr.</td>
<td>1,8/3,W5</td>
<td>1,8/2,7</td>
<td>par.</td>
<td>1</td>
<td>2</td>
<td>38</td>
<td>77</td>
<td>+++</td>
<td>2.0</td>
</tr>
<tr>
<td>LH 6</td>
<td>Glom.nephr.</td>
<td>2,5/9,12</td>
<td>3,7/2,12</td>
<td>sib.</td>
<td>2</td>
<td>2</td>
<td>15</td>
<td>80</td>
<td>+</td>
<td>1.2</td>
</tr>
<tr>
<td>EBT 9</td>
<td>Trauma</td>
<td>1,9,W15</td>
<td>3,7/9,W15</td>
<td>par.</td>
<td>1</td>
<td>2</td>
<td>8^2</td>
<td>19^5</td>
<td>50^6</td>
<td>++++</td>
</tr>
</tbody>
</table>

1 None has been transfused since transplantation.
2 Degree of severity indicated.
3 Two pregnancies, but not transfused.
4 Living donor kidney rejected, later successfully transplanted with necro-kidney.
5 Transfusions before and after first transplantation.
6 Observation time from the first transplantation.
third-party cells, but usually to a lesser degree than responding cells from healthy individuals.

### TABLE II. Cellular and Humoral Immunity

<table>
<thead>
<tr>
<th>Recipient</th>
<th>MLC¹ AB-serum Eff./Targ. 30:1</th>
<th>CML² Eff./Targ. 100:1</th>
<th>CDC Donor Panel</th>
<th>AICC² Gen.inhib.</th>
<th>MLC Recipient serum Spec.inhib.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EÖ</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>–</td>
</tr>
<tr>
<td>OKC</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>–</td>
</tr>
<tr>
<td>NH</td>
<td>4.0</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>+</td>
</tr>
<tr>
<td>BO</td>
<td>14.7</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>+</td>
</tr>
<tr>
<td>IS</td>
<td>3.3</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>–</td>
</tr>
<tr>
<td>OMB</td>
<td>7.1</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>+</td>
</tr>
<tr>
<td>TFO</td>
<td>4.7</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>+</td>
</tr>
<tr>
<td>BS</td>
<td>35.4</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>–</td>
</tr>
<tr>
<td>LH</td>
<td>4.4</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>+</td>
</tr>
<tr>
<td>EBT</td>
<td>15.9</td>
<td>–</td>
<td>–</td>
<td>0/18</td>
<td>+</td>
</tr>
</tbody>
</table>

1 Stimulation index (SI) above 2.0 is considered a positive result (Bondevik et al, 1974)
2 Specific release less than 10% is considered negative (Kaakinen et al, 1974)

**Cell-mediated Lympholysis.**

Direct CML with unconditioned effector cells in an effector/target ratio of 30:1 was done in all ten experiments. For seven of the experiments a ratio of 100:1 was used. No positive CML could be demonstrated (Table II).

**Complement-dependent Cytotoxicity.**

As a routine cross-match, sera from all recipients had been tested for CDC activity against their respective donor cells before transplantation. All these tests were negative. When tested again at the time of this study, no recipient serum displayed CDC activity, neither against donor cells nor against a selected panel of 18 different cells (Table II).

**Antibody-induced Cell-mediated Cytotoxicity.**

In each experiment, cells from the kidney recipient, as well as cells from an individual not possessing the incompatible HL—A antigens present on donor cells, were used as effector cells. Cells from the donor and the two controls, C and D, were used as target cells. Sera known to give a positive AICC result were included in all experiments. The results are summarised in Table II. Seven of the sera were completely negative in AICC. One serum, EO, was AICC positive against control cell D, but not against the donor. Two sera, TFO (Figure 1) and LH, showed
specific AICC activity against the donor, while sera obtained before transplantation were negative.

Figure 1. Antibody-induced cell-mediated cytotoxicity test with recipient TFO serum on donor and control target cells. Specific release (ordinate) expressed as (ER - SR)/(MR - SR). Serum dilutions are given on the figure. Anti-HL-A3 serum included as control serum.

The release from control target cells sharing the incompatible HL-A antigens (C) was as high as that from donor cells, while there was no release from the other control (D). HL-A specificity of the antibodies causing AICC has previously been demonstrated (Kaakinen et al., 1974). Figure 1 demonstrates a lack of effector function in AICC for the recipient cells. This finding needs further confirmation with other positive sera.
MLC Inhibition by the Recipient Serum

No inhibition of MLC response was found in three of the sera (EO, OKC, BS) (Table II). Six sera (NH, BO, IS, OMB, TFO and EBT) showed a different degree of general inhibition of all responses regardless of cell origin. Two of the sera, TFO (Figure 2) and LH, demonstrated a specific inhibition of responding B (donor) and C (control) cells sharing the incompatible HL–A antigens. In addition, TFO possibly showed slight general inhibition. Clear inhibition of stimulating cells was not observed.

![Figure 2. Effect of recipient TFO serum on MLC response. A, B, C and D designate cells from recipient, donor and control individuals sharing and not sharing the incompatible HL–A antigens with donor. The results are given as the median value of triplicates, the range is also indicated. Autologous control AA_m, BB_m, etc.](image)

DISCUSSION

This study was undertaken to determine whether any cellular or humoral response against donor cells could be detected in recipients with well accepted and tolerated kidney grafts.

No direct (T) cell-mediated cytotoxicity could be demonstrated using the CML test with donor cells as target cells. The fact that other investigators (Quadracci et al, 1974), using another test system, could demonstrate such cytotoxicity in a considerable percentage of their patients, might indicate lower sensitivity in our test system. Interestingly, however, they found that the
percentage of recipients expressing cell-mediated cytotoxicity decreased with increasing intervals after transplantation, being less than 30% after 12 months. Our patients, with a mean interval after transplantation of 65 months, the shortest being 19 months, might be just beyond the stage of detectable cytotoxicity.

No complete donor-specific tolerance of recipient T cells did exist, however, since it was possible to initiate an MLC response in recipient lymphocytes by confronting them with stimulating cells from the donor in MLC tests in all cases where MLC locus disparity was most likely. Recent studies have indicated that different sub-populations of T cells are responsible for the MLC response and the cytotoxicity, at least when cytotoxic cells are generated in vitro (Bach et al, 1973; Thorsby, 1974). Thus a state of tolerance involving the latter sub-populations cannot be excluded by our studies.

Two of the recipients exhibited donor-specific antibodies which were able to induce cell-mediated cytotoxicity in the AICC test and gave specific inhibition of MLC reactions. Thus, B-cell tolerance did not exist, at least in these two cases. Several reports (Quadracci et al, 1974; Falk et al, 1973; Hattler & Miller, 1973) have postulated a state of enhancement in recipients of well-functioning kidneys, with anti-donor antibodies blocking the specific cellular response. However, we were not able in all our patients to demonstrate antibodies which inhibited MLC tests or induced AICC involving donor target cells. Specific antibodies may not be a prerequisite for a well-accepted graft, although more sensitive assays are needed to completely exclude this.

In general, lymphocytes from the recipients showed a below-average response in MLC towards third-party cells. Also recipient serum exhibited different degrees of general inhibition of the MLC reaction. Both these findings may be attributed to the immunosuppressive regime, which was not discontinued before the tests were performed. This could also be the reason why recipient lymphocytes showed a reduced ability to function as effector cells in AICC. This latter finding, if confirmed, might possibly also explain why in-vivo AICC reactions are not destroying grafts in our patients.

Our studies do not point to any single immunological factor as being responsible for the stable clinical course in these patients. They prove that neither complete T nor B cell-tolerance against donor cells has developed. A well-accepted graft may coexist with antibodies directed against donor cells. Whether or not the antibodies are, in fact, advantageous for graft acceptance can so far only be hypothesised. A larger patient material, also including patients rejecting their grafts, would be needed. Perhaps the existence of donor-specific antibodies showing specific inhibition in MLC as well as being active in AICC, are correlated with good function of a renal graft (Descamps, Sengar, personal communications).

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