Successful HLA Nonidentical Bone Marrow Transplantation in Three Patients With the Leukocyte Adhesion Deficiency

By F. Le Deist, S. Blanche, H. Keable, C. Gaud, H. Pham, B. Descamp-Latscha, V. Vahm, C. Griscelli, and A. Fischer

Three consecutive patients with the severe phenotype of leukocyte adhesion deficiency characterized by a defective expression of LFA-1, Mac-1 (CR3), and p150.95 on leukocytes have received HLA partially incompatible bone marrow transplantation (BMT). The degree of HLA incompatibility between related donors and recipients was 2 HLA antigens in one and one full haplotype in the other cases. Three consecutive patients with the severe phenotype of leukocyte adhesion deficiency characterized by a defective expression of LFA-1, Mac-1 (CR3), and p150.95 on leukocytes have received HLA partially incompatible bone marrow transplantation (BMT). The degree of HLA incompatibility between related donors and recipients was 2 HLA antigens in one and one full haplotype in the other two. Graft-versus-host disease (GVHD) prophylaxis consisted in T-cell depletion of the bone marrow inoculum and a 60-day course of cyclosporin A. A first attempt led to autologous recovery in one patient. The second transplant in this patient and the first transplant in the two others led to stable partial engraftment of lymphocytes and phagocytic cells, as shown by expression of adhesion molecules (LFA-1, Mac-1) on leukocytes and by HLA typing and restriction fragment-length polymorphism studies using minisatellite probes. Although the level of mixed chimerism was lower in one patient (7% to 30% donor cells) and >50% in the two others, recovery of lymphocyte and phagocytic cell functions was sufficient enough to allow the patient to lead a normal life, infection free in the three cases. These patients, now 57, 32, and 19 months post-transplant, are in good condition without any therapy. These results lead us to propose that the LFA-1 molecule plays a role in HLA-incompatible graft rejection, probably by mediating adhesion of cytotoxic T and non-T lymphocytes to their targets.

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Until the age of 11 months she suffered from chronic otitis media and oral candidiasis. The severe LAD phenotype was detected by the complete absence of expression of leukocyte adhesion molecules (Table 2). She received T-cell-depleted BMT from her HLA-incompatible mother (Table 1) at the age of 11 months. The conditioning regimen and T-cell depletion method and GVHD prophylaxis were similar to those used in patient 2. 11.5 x 10^7/kg bone-marrow nucleated cells with 2.3 x 10^5/kg T lymphocytes were infused. Hematologic recovery occurred quickly (PMN > 500/µL day 12, last platelet transfusion day 16). A grade II acute GVHD with skin and gut involvement occurred at day 10. Enterovirus and adenovirus intestinal infections were found; GVHD gradually resolved within 3 months under steroids and cyclosporin A therapy. The patient is now 19 months post-transplant, doing well, free of major infections, and without any treatment.

**MATERIALS AND METHODS**

**Cell isolation.** Blood leukocytes were isolated from freshly drawn heparinized blood by Plasmagel (Roger Bellon Laboratories, Paris) sedimentation (for one hour at 37°C) and Ficoll-Hypaque (FH) density gradient centrifugation. The PMN were obtained from the pellet and the mononuclear cells (PBMC) from the interface. Rosette forming cells (E') and nonforming cells (E) were obtained by rosetting of cells with neuraminidase (Behring Werke, Marburg the pellet and the mononuclear cells (PBMC) from the interface.

**Immunofluorescence.** The following monoclonal antibodies (MoAbs) were used: IOM1 (Immunotech, Luminy, France) recognizes the CR3 chain (CD11b). 25-3 binds the chain of LFA-1 (CD11a) and TS1-18 (T Springer, Boston), the β chain (CD18) shared by these three proteins. Positive cells were enumerated by indirect immunofluorescence using a fluorescein-conjugated goat antimouse Ig (Nordic, Tilburg, The Netherlands). The percentage of positive cells was scored by microscope (Leitz, Wetzlar, Germany). In some experiences analysis was performed using a FACS scan analyzer (Becton Dickinson, Mountain View, CA).

**Natural killer (NK) activity.** Natural killer (NK) activity was tested in a four-hour standard chromium release assay on K562 target cells as previously described. Positive cells were enumerated by the following formula:

\[
\text{Sample release} = \text{spontaneous release} \times 100
\]

\[
\text{Maximal release} = \text{spontaneous release} \times 100
\]

**Cytotoxicity T-lymphocyte activity.** Effector cells were prepared by coculturing 2 x 10^5 responder PBMC with 2 x 10^5 allogeneic PBMC in 200 µL of culture medium (RPMI 1640, Gibco, Scotland, supplemented of human AB[+] serum [10%]) in microtiter plates (Falcon 3040, Becton Dickinson, Sunnyvale, CA) for six days. The lytic capacity of the effector cells was measured in four-hour standard chromium release assay using PHA-M (Difco, Detroit; vol:vol = 1:100) three-day-stimulated allogeneic PBMC as target cells. Cytotoxic indexes were calculated as above.

**HLA typing.** HLA class I and class II typing was performed by standard serologic method in a microcytotoxic assay. Mixed leukocyte reaction was performed as previously published.

**Granulocyte function.** Adherence was tested by the use of a modification of the MacGregor et al method. One milliliter of

### Table 1. HLA Typing and Chimerism Study on Lymphocytes (E', E), PMN, and RBC After BMT

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>HLA Typing</th>
<th>MLR (cpm)</th>
<th>After BMT</th>
<th>RFLP Study</th>
<th>RBL Phenotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>A2 R15 DR4</td>
<td>A2 B15 DR4</td>
<td>19,000</td>
<td>D/R*</td>
<td>E'</td>
<td>D/R</td>
</tr>
<tr>
<td></td>
<td>A23B2 DR5</td>
<td>A2 B12 DR6</td>
<td>25,000</td>
<td>D/R*</td>
<td>E'</td>
<td>D/R</td>
</tr>
<tr>
<td>Patient 2</td>
<td>A1 B17 DR(-)</td>
<td>A1 B17 DR(-)</td>
<td>400†</td>
<td>R</td>
<td>E</td>
<td>D/R§</td>
</tr>
<tr>
<td></td>
<td>A28B17 DR7</td>
<td>A24B14 DR27</td>
<td>600</td>
<td>R</td>
<td>E</td>
<td>D/R</td>
</tr>
<tr>
<td>Patient 3</td>
<td>A24 B49 DR2</td>
<td>A24 B49 DR2</td>
<td>2,300‡</td>
<td>26,000</td>
<td>D/R</td>
<td>D/R</td>
</tr>
<tr>
<td></td>
<td>A24 B35 DR6</td>
<td>A1 B15 DRW13</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: D, donor type; R, recipient type; D/R, mixed chimerism; E', Erosette forming cells; E, Erosette nonforming cells.

† Patient's lymphocytes were responsive to unrelated control cells (8,000).
‡ Patient's lymphocytes were unresponsive to unrelated control cells.
§ RFLP study has been done on unseparated mononuclear leukocytes.

### Table 2. LFA-1 and Mac-1 Expression Before and After BMT

<table>
<thead>
<tr>
<th>Molecules Tested</th>
<th>Cells</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>36 Mos</td>
<td>After BMT</td>
<td>18 Mos</td>
</tr>
<tr>
<td>Subunit β</td>
<td>Lymphocytes</td>
<td>0</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>0</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PMN</td>
<td>0</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>LFA-1 subunit</td>
<td>Lymphocytes</td>
<td>0</td>
<td>68</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>0</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PMN</td>
<td>0</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Mac-1 subunit</td>
<td>Monocytes</td>
<td>0</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PMN</td>
<td>0</td>
<td>66</td>
<td>0</td>
</tr>
</tbody>
</table>
heparinized blood was filtered through a Pasteur pipette nylon wool column by gravity at room temperature. The percentage of granulocyte adherence was calculated as follows: (PMN in original sample – PMN in sample/PMN in original sample) x 100.

Chemotaxis was studied by a modified agarose technique. The chemoattractant used was zymosan-activated normal serum (ZAS). Incubations were performed in 5% CO2 atmosphere at 37°C and were terminated after 180 minutes. O2 consumption by PMN stimulated with opsonized zymosan (OPZ; 1 mg/mL) was measured continuously with a Clark electrode (Yellow Springs Instrument Co, Yellow Springs, OH) and recorded with a Gilson oxygraph (Gilson Electronics, Inc, Middleton, WI). Chemiluminescence was performed as previously described. DNA restriction fragment-length polymorphism (RFLP) analysis was performed as previously described. In brief, high molecular weight DNA was isolated from the cell nuclei. The purified DNA was digested with restriction endonucleases. Hybridization was then carried out using the appropriate probes.

Serum antibodies. Antibodies to tetanus toxoid and to influenza virus were measured by an enzyme-linked immunosorbent assay (ELISA) assay and to poliovirus by complement fixation.

RESULTS

Engraftment. As shown in Table 1 and Fig 1, leukocyte adhesion molecules LFA-1 and Mac-1 were detectable on leukocytes following BMT in all three patients. In patients 1 and 3, both positive and negative cells were detected with an approximately equal percentage on the different cell populations. The percentage of positive cells in patient 2 remained relatively low (7% to 34%) but stable over time. As indicated in Table 3, some variations were observed in the expression of LFA-1 and Mac-1 on leukocytes in patient 1, since 10 months post-transplant the percentage of positive cells had transiently dropped off. In patient 3, virtually all PMN, monocytes, and lymphocytes express LFA-1 and Mac-1.

As shown in Table 1, engraftment of donor cells was confirmed by HLA typing and RFLP analysis using minisatellite probes in patient 1; by RFLP and by the study of red cell ABO antigen expression in patient 2; and by the three methods in patient 3 in whom there is a nearly full chimerism. Failure to detect donor cells by HLA typing in patient 2 remained relatively low (0.62%) but stable over time. As indicated in Table 3, some variations were observed in the expression of LFA-1 and Mac-1 on leukocytes in patient 1, since 10 months post-transplant the percentage of positive cells had transiently dropped off. In patient 3, virtually all PMN, monocytes, and lymphocytes express LFA-1 and Mac-1.

Correction of leukocyte functions associated with leukocyte adhesion molecules. Adhesion of PMN that was defective prior to transplantation in the three patients tested was partially corrected after BMT (Table 4). Similarly, ZAS-induced migration of PMN improved in patients 1 and 3, but not in patient 2. OPZ-induced oxidative metabolism of PMN was also significantly improved in patients 1 and 3 and marginally in patient 2.

NK activity was found defective in all three patients prior to BMT. Post-BMT NK activity became normal (Table 4). Cytotoxic T-lymphocyte (CTL) activity was also entirely corrected in patient 1 and partially in patient 2.

Antibody production toward immunization antigens was absent in patient 1 despite normal serum immunoglobulin levels. Following BMT and a new series of immunizations, patient 1 developed detectable serum antibodies to polioviruses (16 titer x 10–3), tetanus toxoid (1 U/mL), and influenza virus (4 µg/mL anti-H3N2 influenza virus). A normal antibody response was also found post-BMT in patient 2; however, because of her age (4 months), antibody response could not be evaluated prior to BMT. In patient 3, antibody responses to tetanus toxoid and polioviruses were present both before and after BMT. T- and B-lymphocyte numbers as well as lectin and antigen-induced T-cell prolif-
eration were within the normal range both prior to and after transplantation (>6 months post-BMT) in all three patients (data not shown).

**DISCUSSION**

We herein report the successful partially incompatible BMT of three patients with the severe phenotype of the leukocyte adhesion deficiency. In all cases patients received a T-cell-depleted BMT and a short course of cyclosporin A. The first patient required two transplants, an observation already made in a similar patient having received unmanipulated HLA-compatible BMT. Engraftment can require an aggressive myeloablative conditioning regimen in the LAD disease because of bone marrow hyperactivity, as in other inborn errors of the myeloid-macrophage lineage.

In two patients (1 and 3), a majority of leukocytes of donor origin gave rise to normal leukocyte functions. In patient 2 only a minority of detectable donor leukocytes (and erythrocytes) yielded a partial correction of lymphocyte and phagocytic cell functions. One has to stress, however, that the mixed chimeraism has been stable in this patient over a 32-month period and that leukocyte biological functions were present enough to avoid clinical infectious problems. This is not surprising, since patients with the moderate phenotype of the LAD disease expressing 1% to 10% of normal levels of leukocyte adhesion molecules show less severe and less frequent infections. It is thus conceivable that the presence of 7% to 30% leukocytes with a normal expression of adhesion molecules and presumably normal functions is enough to ensure in vivo protection. The occurrence in these patients of a stable mixed chimeraism is comparable with observations made in patients with similar or other inborn errors having received HLA-matched or mismatched BMT.

So far in only one group of diseases (ie, the severe combined immunodeficiencies that are characterized by an absence of T lymphocytes in which HLA is partially incompatible), T-cell-depleted bone marrow grafts are not usually rejected. This is presumably due to the lack of T-cell effectors able to mediate graft rejection. The fact that in three consecutive patients with LAD no rejection occurred following HLA incompatible T-cell–depleted BMT suggests that the absence of the LFA-1 molecule on T (or non-T) lymphocytes precludes graft rejection. Indeed, graft failure of HLA-incompatible, T-cell-depleted bone marrow occurred in 40% to 90% of the cases in patients treated for other inborn errors or leukemia, including patients having similarly received T cells and cyclosporin A; engraftment occurred in only one out of seven patients. Patients with the LAD deficiency have low non–T- and T-lymphocyte activities. Since both CTL and NK-like cells appear to be involved in graft rejection, it is possible that LFA-1(−) lymphocytes are unable to be activated by and to kill HLA-incompatible marrow cells. However, one cannot rule out that the addition of VP16 in the conditioning regimen of patients 2 and 3 has facilitated engraftment, although the very same conditioning regimen was unsuccessful in allowing engraftment of T-cell–depleted, HLA-nonidentical bone marrow in two patients with other inherited leukocyte disorders (unpublished data).

Although based on a small number of patients, these results have led to the proposal of blocking in vivo the LFA-1 molecule functions by a specific MoAb to prevent graft rejection. Ferrara et al have demonstrated that the infusion of 0.1 mg of an anti-LFA-1 subunit antibody daily from day +1 to +5 following a T-cell–depleted fully H2-incompatible BMT allows sustained engraftment in most cases. In humans the IV infusion of an antisubunit antibody has also been proven to prevent graft rejection in children receiving HLA partially incompatible T-cell–depleted bone marrow because of partial immunodeficiency or other inborn errors.

**REFERENCES**


<table>
<thead>
<tr>
<th>Table 4. PMN Functions and Cytotoxic Activities</th>
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<tr>
<td><strong>Function Tested</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PMN functions*</td>
</tr>
<tr>
<td>Adherence</td>
</tr>
<tr>
<td>ZAS-induced migration</td>
</tr>
<tr>
<td>OPZ-induced O2 consumption</td>
</tr>
<tr>
<td>OPZ-induced chemiluminescence</td>
</tr>
<tr>
<td>Cytotoxic activities (% cytotoxic activity)</td>
</tr>
<tr>
<td>NK activity effector/target ratio = 50:1</td>
</tr>
<tr>
<td>CTL activity effector/target ratio = 100:1</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not done.
*Results are expressed as the ratio, patient:control values.


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