HLA-haploidentical blood progenitor cell transplantation in osteopetrosis

Ansgar S. Schulz, Carl Friedrich Classen, Walther A. Miňatsch, Michael Sigl-Kraetzig, Markus Wiesneth, Klaus-Michael Debatin, Wilhelm Friedrich, and Susanna M. Müller

Infantile osteopetrosis (OP) carries an extremely poor prognosis unless treated early by hematopoietic stem cell transplantation. We explored the use of purified blood progenitor cells from HLA-haploidentical parents in 7 patients lacking suitable matched donors. Blood progenitor cells were purified by positive selection and by additional T-cell depletion using rosette formation. For conditioning, patients received busulfan, thiotepa, and either cyclophosphamide (5 patients) or fludarabine (2 patients). Stable donor engraftment developed in 6 of 7 patients. Graft-versus-host disease was not observed. Three of the 7 patients had no major complications and 4 of 7 had both veno-occlusive disease and respiratory failure. Five of 7 patients survive with complete cure of OP at a median of 4 years. Patients with OP lacking HLA-matched donors can be successfully treated by transplantation of purified blood progenitor cells from HLA-haploidentical donors. (Blood. 2002;99:3458-3460)

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Introduction

Osteopetrosis (OP) represents a group of disorders characterized by excessive bone density due to defective osteoclast function. In recently patients with the severe autosomal recessive variant of the disorder (malignant infantile OP) mutations were described in genes expressed in osteoclasts affecting ion channel proteins. One gene, encoding the α3 subunit of the vacuolar H⁺-ATPase, was found to be affected in 10 of 19 patients with OP, including 5 of the 7 patients presented in this paper (2 patients were not analyzed). Osteoclasts are of hematopoietic origin, and hematopoietic stem cell transplantation (HSCT) represents an effective treatment. Because of the usually rapid development of complications such as hematologic failure and irreversible sensoneurologic impairment, treatment needs to be initiated expeditiously. The lack of a suitable, HLA-compatible donor and the low success rate using alternative donors have limited this strategy so far.

In the HLA-nonidentical setting, the use of T-cell–depleted transplants prevents complications of graft-versus-host disease (GVHD). This approach has been successful in particular in children with primary immunodeficiencies, whereas in patients with other congenital disorders including OP it has been complicated so far by high graft failure rates. Recently results of HLA-nonidentical HSCT improved markedly using large numbers of granulocyte colony-stimulating factor (G-CSF)–mobilized, T cell–depleted peripheral blood progenitor cells. In the current study, we explored the use of purified blood progenitor cells from HLA-haploidentical parents in the treatment of 7 infants with malignant OP.

Study design

Patients

Patients (6 girls and 1 boy) were treated at the Children’s Hospital of the University of Ulm between 1996 and 1999. Patients’ histories and clinical findings before transplantation are summarized in Table 1. In 6 of the 7 patients, OP was diagnosed by 2 months of age, either due to positive family histories (in 2 cases) or because of early hypocalcemic convulsions. The diagnosis was confirmed in each case by bone biopsies and by radiography studies. In each patient informed consent was obtained from parents before inclusion into the study, which was approved by the institutional ethical committee.

Transplantation procedure

Details of HLA-matching, of the preparative conditioning, and of the transplants used are shown in Table 2. Conditioning consisted of busulfan 4 to 5 mg/kg orally in divided doses daily for 4 days (total dose 16-20 mg/kg), thiotepa 10 mg/kg intravenously (IV) in 2 divided doses for 1 day, and either IVs cyclophosphamide 50 mg/kg once daily for 4 consecutive days (total dose 200 mg/kg; 4 patients) or cyclophosphamide 60 mg/kg once daily IV for 2 consecutive days (total dose 120 mg/kg; 1 patient) or fludarabine 40 mg/m² IV once daily for 5 consecutive days (total dose 200 mg/m²; 2 patients). For rejection prophylaxis, 6 patients received antihuman thymocyte globulin IV (ATG, Lymphoglobulin, Pasteur-Merieux, Leimen, Germany) for 3 or 4 consecutive days (total dose 20-30 mg/kg), and 1 patient received OKT-3 (Orthoclone OKT 3, Janssen-Cilag, Neuss, Germany) 0.1 mg/kg once daily IV for 10 consecutive days (total dose 1 mg/kg).

Stem cell preparation

To mobilize CD34⁺ progenitor cells, donors were treated with recombinant human G-CSF (rHuG-CSF; Lenograstim, Rhône-Poulenc, Cologne, Germany) at 10 μg/kg per day subcutaneously for 4 to 5 days. Donor leukocytes were obtained by apheresis using a COBE Spectra Cell Separator (COBE BCT, Heimstetten, Germany), CD34⁺ cells were isolated by positive selection using in 4 initial cases the CeprateSC device (Cellpro, Brussels, Belgium) and in 2 others by the CliniMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany). Residual T cells were further depleted by rosetting with human erythrocytes coated with anti-CD3 and anti-CD2 as described elsewhere. Numbers of CD34⁺ cells and CD3⁺ cells in the grafts were determined by flow cytometry. Transplanted stem
cell numbers ranged from 12 to 56 × 10^6/kg (median 30 × 10^6/kg) and transplanted T-cell numbers from 0.8 to 11.1 × 10^3/kg (median 5 × 10^3/kg). All patients except one received additional infusions of cryopreserved donor CD34⁺ cells as a boost. These cells were given early (between day 7 and 11) in 5 cases and late (day 42) in 2 cases, ranging in numbers from 0.8 to 11.1 × 10^3/kg (median 15 × 10^3/kg; Table 2). Recipients received rHuG-CSF until stable engraftment was achieved. Besides T-cell depletion, no further GVHD prophylaxis was administered.

### Analysis of engraftment

Engraftment of donor cells was analyzed by repeated HLA-typing of peripheral blood leukocytes and by group determination. Immune logic reconstitution was evaluated by monitoring the number of circulating T cells and B cells and by repeated determination of T- and B-cell functions as described.

### Results and discussion

Transplant courses were accompanied by a high rate of toxic and infectious complications (Table 2). Thus, severe respiratory problems requiring mechanical ventilation developed in 4 cases: UPN 275 and UPN 292 because of cytomegalovirus (CMV) pneumonitis, UPN 303 due to respiratory failure from severe airway

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**Table 1. Clinical findings and patient histories**

<table>
<thead>
<tr>
<th>UPN/sex</th>
<th>Genotype</th>
<th>Positive family history</th>
<th>Clinical abnormalities</th>
<th>Age at diagnosis</th>
<th>Age at transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>275/F</td>
<td>V-ATPase*</td>
<td>Yes</td>
<td>Hepatomegaly, anemia</td>
<td>1 mo</td>
<td>5.5 mo</td>
</tr>
<tr>
<td>292/F</td>
<td>V-ATPase*</td>
<td>No</td>
<td>Visual impairment, hypocalcemic convulsions, hepatosplenomegaly, anemia, thrombocytopenia</td>
<td>2 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>302/F</td>
<td>V-ATPase*</td>
<td>No</td>
<td>Visual impairment, hypocalcemic convulsions, hepatosplenomegaly, anemia</td>
<td>1 mo</td>
<td>3.5 mo</td>
</tr>
<tr>
<td>303/F</td>
<td>V-ATPase*</td>
<td>No</td>
<td>Visual impairment, hepatosplenomegaly, hypocalcemia, anemia</td>
<td>0.5 mo</td>
<td>2 mo</td>
</tr>
<tr>
<td>323/F</td>
<td>ND</td>
<td>Yes</td>
<td>Hypocalcemic convulsions, splenomegaly</td>
<td>At birth</td>
<td>1.5 mo</td>
</tr>
<tr>
<td>332/M</td>
<td>V-ATPase*</td>
<td>No</td>
<td>Hypocalcemic convulsions, splenomegaly</td>
<td>0.5 mo</td>
<td>5 mo</td>
</tr>
<tr>
<td>364/F</td>
<td>ND</td>
<td>No</td>
<td>Visual impairment, anemia, thrombocytopenia</td>
<td>5 mo</td>
<td>6 mo</td>
</tr>
</tbody>
</table>

ND indicates not determined.

*Mutations in the a3 subunit of the vacuolar H⁺-ATPase described in Kornak et al.*

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**Table 2. Transplant characteristics and outcome**

<table>
<thead>
<tr>
<th>UPN</th>
<th>Donor/HLA-mismatch</th>
<th>Preparative regimen</th>
<th>CD34⁺ cells (×10^6/kg)</th>
<th>T-cells (×10³/kg)</th>
<th>Neutro/μL</th>
<th>Reti</th>
<th>Platelets/μL</th>
<th>Major complications</th>
<th>Chimerism (last study)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>275</td>
<td>Mother/1 Ag (A)</td>
<td>Bu (16), Thio (10), Cy (200), ATG</td>
<td>25 40 (d &lt; 42)</td>
<td>2.8 2.6</td>
<td>27 44 45 49 75</td>
<td>Respiratory insufficiency, CMV-disease, VOD (liver)</td>
<td>Full donor (5 y) minor visual impairment</td>
<td>NE dead (d 34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>292 (1st H SCT)</td>
<td>Father/3 Ag (A, B, DR-B1)</td>
<td>Bu (20), Thio (10), Cy (120), ATG</td>
<td>36 —</td>
<td>1.1 —</td>
<td>— — — —</td>
<td>Rejection</td>
<td>NE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>292 (2nd H SCT)</td>
<td>Father/3 Ag (A, B, DR-B1)</td>
<td>5 Gy splenic radiation, OKT-3</td>
<td>56 —</td>
<td>9.1 —</td>
<td>— — — —</td>
<td>CMV-pneumonitis, ARDS, VOD (liver)</td>
<td>NE dead (d 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>302</td>
<td>Father/3 Ag (A, B, DR-B1)</td>
<td>Bu (16), Thio (10), Cy (200), ATG</td>
<td>20 10 (d + 11)</td>
<td>0.8 0.4</td>
<td>22 33 24 44 130</td>
<td>Respiratory insufficiency, VOD (liver)</td>
<td>Full donor (4 y) visual impairment</td>
<td>a/w (~48 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>303</td>
<td>Father/1 Ag (DR-B1)</td>
<td>Bu (20), Thio (10), Cy (200), ATG</td>
<td>36 24 (d + 10)</td>
<td>11.1 7.4</td>
<td>NE† 9 10 51 330</td>
<td>Respiratory insufficiency, VOD (liver)</td>
<td>Full donor (3 y) visual impairment</td>
<td>a/w (~47 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>Father/2 Ag (A, B, DR-B1)</td>
<td>Bu (20), Thio (10), Cy (200), ATG</td>
<td>12 12 (d + 10)</td>
<td>1.2 1.2</td>
<td>22 — — 70 —</td>
<td>Respiratory insufficiency, VOD (lung and liver)</td>
<td>Full donor (~37 d) mental retardation</td>
<td>dead (d 64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>332</td>
<td>Father/3 Ag (A, B, DR-B1)</td>
<td>Bu (20), Thio (10), Flu,* ATG</td>
<td>43 2 × 43 (d + 7, + 42)</td>
<td>1.7 2 × 1.7</td>
<td>17 68 50 53 57</td>
<td>—</td>
<td>Full donor (2 y)</td>
<td>a/w (~36 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>364</td>
<td>Aunt/1 Ag (DR-B1)</td>
<td>Bu (20), Thio (10), Flu,* OKT-3</td>
<td>15 15 (d + 10)</td>
<td>1.6 1.6</td>
<td>14 55 13 50 58</td>
<td>—</td>
<td>Full donor (1.5 y) visual impairment</td>
<td>a/w (~25 mo)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Bu: busulfan; Thio: thiopeta; Cy: cyclophosphamide (numbers in parenthesis indicate total doses in mg/kg body weight).

*Flu: fludarabine 200 mg/m² body surface. ATG: antithymocyte globulin. For details of application see text. Ag: HLA-antigen; NE: not evaluable; a/w: alive and well.

†Patient 4 (UPN 303) received granulocyte transfusions on day + 5, + 7, + 10.
obstruction, and UPN 323 due to late veno-occlusive disease (VOD) of the lung. Each of these 4 patients also developed hepatic VOD, representing an unusually high incidence of this complication. The use of intensive conditioning, the young age of our patients at transplantation as well as disease-specific problems related to the underlying disorder may account for this increased risk to develop transplant-related complications. In an attempt to lower this risk, in particular of VOD, an alternative conditioning was evaluated in 2 more recent cases, replacing 1 of the 2 alkylating agents, namely cyclophosphamide, by fludarabine. This regimen led to markedly reduced toxicity with absence of VOD, and although limited, warrants further exploration in additional patients.

In 5 of the 7 cases sustained engraftment of donor cells was observed. Two patients developed graft failures: in patient UPN 275 graft function deteriorated at 1 month in the context of CMV disease, which was reversible by a second graft on day 42 from the same donor without further conditioning. This complication may reflect the vulnerability of the reconstituting hematopoietic system after purified blood progenitor cell transplantation. In the other patient (UPN 292) the graft was rejected on day +15, following conditioning with a lowered total dose of cyclophosphamide (120 mg/kg instead of 200 mg/kg). This child died from CMV pneumonia shortly after a second transplant from the opposite parent. Graft rejection in this case may emphasize the critical role of the intensity of conditioning, even if high numbers of stem cells are used for transplantation. It should also be pointed out that in the other patients complete hematologic recovery with development of normal blood cell counts was delayed (Table 2), this delay may be related to the occlusion of marrow space characteristic for the disease. In an attempt to shorten the time interval to hematologic recovery, additional donor CD34+ cells were infused at around 1 week after HSCT in 5 of the 7 patients. Although no effect on the slow kinetics of hematologic recovery was evident, regardless of the number of CD34+ cells infused, this approach may have led to stabilization of engraftment, because none of the patients developed graft failures. Two patients received late boosts of CD34+ cells at day 42 because of persistent marked tricipitenia in one (UPN 332) and because of secondary graft failure in the other (UPN 275 mentioned above). In both patients rapid normalization of blood cell counts was observed, suggesting that delayed boosts may improve hematopoietic functions after HSCT in OP. T-cell immunity by donor cells developed with a delay of 3 to 6 months, regardless of the total number of CD34+ cells infused (data not shown). No patient developed acute or chronic GVHD.

Five of 7 patients survive for 25 to 60 months (median, 47 months). In these patients, the disease regressed as demonstrated by normalization of hematopoietic function, regular calcium homeostasis, and reversal of hepatosplenomegaly. One patient (UPN 303) developed portal vein thrombosis, possibly as a late complication of hepatic VOD and also shows developmental retardation of unknown origin at 4 years after transplantation. Visual impairment due to optical nerve atrophy present at the time of transplantation in 4 of the surviving patients remained unchanged. Other serious neurologic sequelae are absent. As demonstrated by repeat bone marrow biopsies and by serial skeletal radiograms, structural abnormalities of the skeletal system improved gradually, normalizing at around 1 year after transplantation. Repeat chimerism analysis revealed stable donor cell engraftment in all surviving patients.

The outcome of HLA-haplotype mismatched SCT for treatment of OP has been disappointingly poor so far, with survival rates of only 10% (data of the European Bone Marrow Transplantation study, the International Bone Marrow Transplant Registry and the National Marrow Donor Program, reviewed by Fasth and Porras6 and Gerritsen et al16). In this study we explored a novel approach using high numbers of purified CD34+ blood progenitor cells from related family donors. In addition we applied an intensive triple-drug conditioning regimen incorporating also anti-T-cell antibodies for additional immunosuppression. It is not possible to dissect the respective roles of the hematopoietic grafts and the intensive immunosuppression used for the observed low rate of graft failure and the improved survival rate. Nevertheless, our finding of stable donor cell engraftment in 6 of 7 cases and of cure of disease in 5 of 7 patients is encouraging. Because of the usually rapid progression of the disease, HSCT needs to be performed as early as possible after diagnosis, and the approach used in our study makes this feasible.

References

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