RAPID COMMUNICATION

Donor Leukocyte Transfusions for Treatment of Recurrent Chronic Myelogenous Leukemia in Marrow Transplant Patients

By H. J. Kolb, J. Mittermüller, Ch. Clemm, E. Holler, G. Ledderose, G. Brehm, M. Heim, and W. Wilmanns

Three patients with hematologic relapse after bone marrow transplantation for chronic myelogenous leukemia were treated with interferon α and transfusion of viable donor buffy coat. All had complete hematologic and cytogenetic remission, which persisted 32 to 91 weeks after treatment. In two patients graft-versus-host disease developed and was treated by immunosuppression. These results are an example of adoptive immunotherapy without cytoreductive chemotherapy or radiotherapy in human chimeras.

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A LLLOGENEIC bone marrow transplantation (BMT) is currently the only curative treatment for chronic myelogenous leukemia (CML). Among patients with hematologic relapse after BMT, cytogenetic remissions are rare and second transplants are performed.

We report here the successful treatment of hematologic relapse with interferon α (IFNα) and transfusion of viable buffy coat cells from the marrow donor without additional chemotherapy or radiotherapy in three patients.

MATERIALS AND METHODS

Patients were grafted in chronic phase with BM from an HLA-identical sibling following conditioning with total body irradiation and cyclophosphamide as previously described. The combination of cyclosporine A (CSA) and methotrexate was administered for prophylaxis of graft-versus-host disease (GVHD).

Chimerism was studied by the use of restriction fragment length polymorphism (RFLP) and polymorphic isoenzymes (phosphoglucomutase 1 [PGM1] and acid phosphatase 1 [ACP1]) in separated marrow and blood cells, typing of the blood group with an immunoperoxidase method, and cytogenetic analysis of marrow cells.

Heparinized blood and marrow samples were spun repeatedly by centrifugation (800g and 1,100g for 10 minutes) for the separation of platelets, buffy coat, and red blood cells (RBCs). Buffy coat was further separated into mononuclear cells (MNC) and polymorphonuclear cells (PMN) by density gradient centrifugation using Ficoll-Hypaque of a density of 1.077 g/L. RBCs were lysed using 0.84% ammonium chloride. Cell fractions were studied from contamination by microscopy.

Polyomorph ic isoenzymes were determined in cell lysates by isoelectric focusing as previously described with modifications. In mix experiments the sensitivity was 1%.

Minisatellite probes 33.15 and 33.06 (Fa. ICI, Abingdon, England) labeled with [α32P]dCTP (Fa. Amersham Buchler, Braunschweig, Germany) were used for the determination of DNA polymorphism. DNA was extracted, digested with Hinf1 (Fa. Biozym, Hameln, Germany), and separated in agarose gel electrophoresis (0.8%) by routine methods. It was transferred to a nylon membrane (Geen Screen Plus, Fa. Dupont, Dreieich, Germany) as described by Southern and hybridized with the minisatellite probes under highly stringent conditions. The sensitivity of this method is 5% according to mixing experiments.

Cytogenetic preparations were obtained from 2-hour cultures without stimulation. G-banding was performed according to the method of Seabright with some modifications. Analyses were done on at least 20 metaphases, whenever possible. Markers of chimerism are summarized in Table 1.

IFNα 2b (Intron A, Fa. Essex Pharma, München, Germany) was administered daily in a dose of $5 \times 10^8$ U/m² subcutaneously. The dose was decreased in cases of cytopenia.

Buffy coat was prepared from the marrow donor by a continuous flow cell separator (Fa. Cobe, Denver, CO, model 5000). Between 250 mL and 365 mL were collected per session, and between three and five sessions were performed within 5 to 9 days.

RESULTS

Patient unique patient number (UPN) 105, a 22-year-old man, was transplanted in chronic phase on April 4, 1985 with marrow from his HLA-identical brother with $4.2 \times 10^6$ nucleated cells. The patient had blood group A and his donor B. He did not develop clinically significant GVHD. CML recurred 3 years later in chronic phase. The degree of chimerism decreased as shown by PGM 1, a1,2 positive cells and the reappearance of RBCs of blood group A (Table 2). He was treated with IFNα from June through September 1988 with a decrease of leukocyte counts from 60 g/L and 13.8 g/L. The lactate dehydrogenase (LDH) remained elevated and the karyotype of all marrow cells had the Philadelphia (Ph1) chromosome. In addition, a new clone characterized by a 14q+ chromosome evolved and expanded.

He received four viable buffy coat transfusions containing $4.4 \times 10^6$ nucleated cells from his marrow donor. IFNα was discontinued. Seven weeks later, 6 of 18 marrow cells were Ph1-negative. At the same time he presented with a generalized skin rash and increased liver enzymes. Immunosuppressive therapy with CSA, prednisolone, and azathioprine was started 10 weeks later because of rising bilirubin (peak value: 8.3 mg/dL) and deteriorating skin changes. GVHD responded promptly and immunosuppressive medication was decreased and discontinued. Nine weeks after buffy coat transfusion all marrow cells were Ph1-negative. Repeated examinations at 15, 24, 46, 52, 60, and 91 weeks showed only Ph1-negative cells in his marrow (Table 3). Chimerism is

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Patient UPN 162, a 30-year-old woman, was transplanted in October 29, 1986 in chronic phase with \(5.4 \times 10^8\)/kg nucleated marrow cells from her brother. In January 1988, 45% of her marrow cells were female and Phl-positive. CSA was discontinued and on repeated examination in March and June 1988 all cells were male and Phl-negative. In October CML recurred in chronic phase with immature cells in the blood. Again 5 of 11 marrow cells were female and Phl-positive. In February 1989 her platelet count increased to 704 g/L; all marrow cells were Phl-positive. Minisatellite probes showed only host type DNA in marrow cells and a mixture of host and donor type in blood granulocytes. The proportion of platelets and granulocytes with donor-type isoenzyme decreased in the peripheral blood and mononuclear cells remained of donor type. The patient was started on IFNa and responded with normalization of platelet counts and disappearance of immature cells, but her marrow cells remained Phl-positive in May 1989. She was given five transfusions of buffy coat from her marrow donor containing \(5.1 \times 10^8\)/kg nucleated cells in May 1989. She did not develop symptoms of GVHD. IFNa was discontinued. Cyto genetic analyses were done 18, 22, 35, and 49 weeks after the transfusion and all marrow cells were male and Phl-negative (Table 3). Granulocytes and platelets were again of donor type.

**DISCUSSION**

In these patients with hematologic relapse after allogeneic BMT we observed unmaintained cytogenetic remissions after treatment with IFNa and transfusions of buffy coat cells from their BM donor. Treatment with IFNa has induced clinical remissions in about 75% and cytogenetic remissions in about 20% of untransplanted patients.13 In patients with hematologic relapse after marrow transplantation treatment with IFNa did not induce cytogenetic

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**Table 1. Markers of Chimerism**

<table>
<thead>
<tr>
<th>UPN</th>
<th>Isoenzyme</th>
<th>RFLP*</th>
<th>Blood Group</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>105</td>
<td>R</td>
<td>12.3; 9.5</td>
<td>A1</td>
<td>46 XY (Phl+)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>10.4</td>
<td>B</td>
<td>46 XY</td>
</tr>
<tr>
<td>132</td>
<td>R</td>
<td>7.5</td>
<td>O</td>
<td>46 XX (Phl+)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>12.4</td>
<td>O</td>
<td>46 XY</td>
</tr>
<tr>
<td>169</td>
<td>R</td>
<td>8.5</td>
<td>B</td>
<td>46 XX (Phl+)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>12.5</td>
<td>B</td>
<td>46 XY</td>
</tr>
</tbody>
</table>

Abbreviations: R, recipient; D, donor; ND, not different.

*Numbers indicate kilobases of DNA fragments marked by minisatellite probes 33.15 and 33.06.

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**Table 2. Response of Leukemia and Chimerism in UPN 105**

<table>
<thead>
<tr>
<th>Time (wk posttransplant)</th>
<th>Clinical Condition</th>
<th>Treatment</th>
<th>Blood</th>
<th>Chimerism</th>
<th>Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MNC</td>
<td>PMN</td>
<td>RBC</td>
</tr>
<tr>
<td>49</td>
<td>Remission</td>
<td>None</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>121</td>
<td>Remission</td>
<td>None</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>166</td>
<td>HR</td>
<td>None</td>
<td>M</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>170</td>
<td>HR</td>
<td>IFNa</td>
<td>M</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>177</td>
<td>HR</td>
<td>IFNa</td>
<td>M</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>183</td>
<td>HR</td>
<td>IFNa + buffy coat</td>
<td>—</td>
<td>—</td>
<td>R</td>
</tr>
<tr>
<td>187</td>
<td>HR</td>
<td>IFNa discontinued</td>
<td>M</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>191</td>
<td>GVHD</td>
<td>—</td>
<td>M</td>
<td>R</td>
<td>—</td>
</tr>
<tr>
<td>193</td>
<td>GVHD</td>
<td>CSA, Pred, Aza</td>
<td>D</td>
<td>M</td>
<td>—</td>
</tr>
<tr>
<td>199</td>
<td>GVHD</td>
<td>CSA</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>208</td>
<td>GVHD decrease</td>
<td>CSA discontinued</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>230</td>
<td>Remission</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>236</td>
<td>Remission</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>244</td>
<td>Remission</td>
<td>None</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>275</td>
<td>Remission</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hematologic relapse; D, donor type; R, recipient type; M, mixed; Pred, prednisolone; Aza, azathioprine.
remissions.\textsuperscript{5} Cytogenetic examinations in two of our patients after 11 and 14 weeks of IFN therapy showed persistent Ph1-positive chromosome. A new clone evolved in one. In contrast, complete cytogenetic responses were seen 9 and more weeks after buffy coat transfusions. Although none of these patients developed GVHD after transplantation, two developed clinically significant GVHD after donor buffy coat transfusion.

In canine chimeras transfusion of donor buffy coat did not induce GVHD unless donors were sensitized,\textsuperscript{15} suggesting active mechanisms of GVH tolerance. Donors of our patients were males who had not been transfused previously. Mechanisms of tolerance may differ with GVHD prophylaxis, ie, CSA instead of methotrexate, and tolerance may be disturbed by treatment with IFNa instead of methotrexate, and tolerance may be disturbed by treatment with IFNa. Alternatively, the reappearance of host-type hematopoietic cells, particularly monocytes with the leukemic clone may be a precondition for the development of acute GVHD after buffy coat transfusions. The role of host-type monocytes and tumor necrosis factor \( \alpha \) has been previously discussed.\textsuperscript{15} In both patients GVHD resolved under immunosuppressive treatment.

Neurologic complications as in our patient UPN 139 have recently been described by others in patients with CML treated with IFNa for recurrence after transplantation.\textsuperscript{18} In our patient symptoms disappeared after immunosuppressive treatment.

GVHD is associated with an antileukemic effect,\textsuperscript{17} and patients with chronic GVHD may have improved survival.\textsuperscript{18} This antileukemic effect of GVHD is well documented in both acute leukemia and CML.\textsuperscript{17,18} Depletion of T cells from the marrow graft decreases the incidence of GVHD and increases the risk of recurrent leukemia.\textsuperscript{4,17,20} However, depletion of T cells also increases the risk of recurrent leukemia in patients who develop GVHD.\textsuperscript{17} Therefore, neither does the presence of GVHD guarantee a strong antileukemic effect, nor does the absence of GVHD indicate the absence of an antileukemic effect. In our patient UPN 169 the malignant clone disappeared without development of GVHD.

The treatment of our patients is an example of successful adoptive immunotherapy. Several aspects differ from a prior study of adoptive immunotherapy reported by Sullivan et al.\textsuperscript{23} Their patients had acute leukemia and were transfused with buffy coat early after transplant. In our patients, chimerism was already established for a long period of time before buffy coat transfusion. In addition, our patients did not receive prophylactic immunosuppressive therapy, but they did receive IFN. IFN may have supported cytotoxic activity of the transfused cells. IFN has differential effects on the expression of class I and class II antigens.\textsuperscript{22} Increased expression of class I HLA antigens as restricting elements of minor histocompatibility antigens (HA) may support cell-mediated cytotoxicity of transfused buffy coat cells against host-type hematopoietic cells. Minor HA are differentially expressed on hematopoietic progenitor cells\textsuperscript{23} and the cytotoxic reaction against minor HA is restricted by class I HLA antigens.\textsuperscript{24} A GVH reaction against minor HA of the host appears more likely than a reaction against potential leukemia-specific antigens. With the disappearance of Ph1-positive metaphases all hematopoietic cells of host type disappeared. We don't know whether all hematopoietic cells of host type were produced by the Ph1-positive clone or whether some were produced by residual normal host hematopoiesis. However, specificity for minor HA on hematopoietic cells of the host may be sufficient for an antileukemic effect without the risk of severe clinical GVHD.

\textbf{REFERENCES}

CML Clinical Studies


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