Salvage Immunotherapy Using Donor Leukocyte Infusions as Treatment for Relapsed Chronic Myelogenous Leukemia After Allogeneic Bone Marrow Transplantation: Efficacy and Toxicity of a Defined T-Cell Dose

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Eight patients who had hematologic relapse of chronic myelogenous leukemia (CML) after undergoing allogeneic bone marrow transplantation (BMT) were treated with leukocyte infusions from the original bone marrow donors. All patients had previously received marrow grafts from HLA-identical siblings. Six patients were in the accelerated phase of their disease and two were in blast crisis. Each patient received a predetermined T-cell dose within a narrow range of 2.5 to 5.0 x 10^6 T cells/kg. Three patients also received short courses of therapy with α interferon to control elevated white blood cell counts within the first several weeks after leukocyte transusions. Seven of eight evaluable patients developed graft-versus-host disease (GVHD) at a median of 32 days after the initial infusion. One patient had fatal GVHD. A second patient had grade 3 acute GVHD, which has responded to immunosuppressive therapy. The remaining patients all had mild grade 1 GVHD. Six patients continue to require modest doses of prednisone more than 6 months after infusion.

A LLOGENEIC bone marrow transplantation (BMT) is the only known curative treatment for patients with Philadelphia chromosome (Ph) positive chronic myelogenous leukemia (CML).1 Despite successful outcomes in many patients, however, disease relapse remains a major cause of treatment failure, especially for those patients transplanted in the advanced phases of their disease. Treatment options for patients who do relapse are limited. Therapy with α interferon has resulted in suppression of the Ph chromosome in selected patients,2,3 but has not been shown to be curative and may be less effective in patients with hematologic relapse.4 Second marrow transplants have also resulted in clinical remissions in some patients, but are associated with significant treatment-related morbidity and mortality.5,6 Recently, the administration of donor leukocyte infusions has been reported to result in hematologic and cytogenetic remissions in several patients with relapsed CML7 and has emerged as another therapeutic option for this patient population. Although toxicity was minimal in the initial report, other preliminary studies have documented severe graft-versus-host disease (GVHD) and marrow aplasia in some patients.8,9 The infusion of immunocompetent donor T lymphocytes is thought to be primarily responsible for the antileukemic effect as well as for these adverse sequelae. However, no study to date has prospectively quantitated the number of infused T cells in individual patients receiving leukocyte infusions or correlated the T-cell dose with clinical response. In this report, we have administered a uniform T-cell dose to patients with relapsed CML after allogeneic BMT to determine the efficacy and toxicity of this immunotherapeutic approach.

MATERIALS AND METHODS

Patient Population

Eight patients who had hematologic relapse after undergoing allogeneic BMT for chronic myelogenous leukemia were enrolled in this study. All patients were treated on a protocol approved by the Internal Review Board of the Medical College of Wisconsin. Informed consent was obtained from all patients before enrollment. To be eligible for the protocol, patients had to have received HLA-matched sibling grafts and could not have active GVHD at the time of enrollment, a prior history of grade III or IV acute GVHD, or moderate to severe chronic GVHD. The disease status of patients was determined at the time of enrollment. All patients had been previously conditioned with cyclophosphamide, high-dose cytosine arabinoside, methylprednisolone, and 14 Gy of total body irradiation. Each patient received marrow grafts depleted of mature T cells by the αβ T-cell receptor antibody T10B4. Cyclosporine was also administered posttransplant to each patient for GVHD prophylaxis.

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DONOR LEUKOCYTES FOR RELAPSED CML AFTER BMT

TREATMENT SCHEDULE

Patients were taken off all immunosuppressive or cytotoxic agents (eg, prednisone, cyclosporine, hydroxyurea, or interferon) before administration of leukocyte infusions. Leukocyte collections from the original bone marrow donors were performed using a Cobe spectra apheresis system (Cobe Laboratories, Inc, Lakewood, CO). Infusions were administered every other day over a 1- to 2-week period immediately after collection from the donor. In donor/recipient pairs who were ABO-compatible, leukocytes were infused directly into the patient without further processing. In one patient who was ABO-incompatible with the donor, Ficol-Hypaque density gradient centrifugation was performed to remove red cells. No GVHD prophylaxis was administered to any of the patients.

Each patient was assigned to receive a total T-cell dose of 2.5 to 5.0 x 10^7 T cells/kg. This dose was empirically derived from the estimated number of T cells administered to the initial patient in this study cohort (UPN 225) who has been previously reported.15 The T-cell dose was calculated according to the following formula: (total nucleated cell dose) x (% lymphocytes) x (% CD3+ T cells). The percentage of lymphocytes in each apheresis product (except those administered to the initial patient) was determined using a STKR Coulter Counter (Coulter Corp, Hialeah, FL). The percentage of CD3+ T cells in all collections was calculated by flow cytometric analysis. In the first patient in the study, the percentage of lymphocytes in each apheresis product was calculated by manual differentials from Wright-stained slide preparations. The proportion of T cells in the lymphocyte population was estimated by determining the percentage of CD3+ cells in a sample of peripheral blood obtained from the donor. A running total of the T-cell dose per kilogram administered to each patient was recorded. The infusion that placed each patient into the prescribed T-cell dosing range was the last infusion administered.

Peripheral blood and bone marrow specimens were obtained from all patients at least monthly for the first 3 months, and then at 2-month intervals for the next 4 months to assess chimerism and remission status. GVHD occurring within the first 100 days after infusion was operationally defined as acute according to the criteria of Thomas et al.12 GVHD appearing de novo or persisting beyond 100 days was deemed to be chronic and was classified as none, limited, or extensive.12 Marrow aplasia was defined by the presence of pancytopenia (absolute neutrophil count ≤500/μL, platelets ≤20,000/μL, and hemoglobin ≤10 g/dL) in the setting of a hypocellular bone marrow.

IMMUNOFLOUORESCENCE ASSAYS

Immunophenotyping was performed on each administered leukocyte product. An aliquot from each collection was obtained in an EDTA-anticoagulated tube. Using the Q-Prep whole blood lysis system (Coulter Corp) and phycocyanin or fluorescein isothiocyanate-labeled monoclonal antibodies, cells were stained with CD2/CD19, CD4/CD8, CD8/CD56, CD45/CD14, CD3 (Coulter Corp), and the appropriate isotypic controls. Analysis was performed using an Epics Profile II flow cytometer (Coulter Corp). Forward and side scatter properties were used to gate on the lymphocyte population. CD45/CD14 was used to remove red cell and monocyte contamination from the lymphocyte gate. The total number of CD4+, CD8+, CD56+, or CD19+ lymphocytes administered to each patient was determined using the same formula as that used to calculate the CD3+ T-cell dose. Data were expressed per kilogram of body weight.

CYTOGENETIC ANALYSIS OF BM ASPIRATES

Chromosome studies on bone marrow were performed using a direct technique for processing marrow aspirates.13 Slide preparations were stained by quinacrine mustard for fluorescence (QFQ) banding. In each case, whenever possible, at least 20 metaphases were examined microscopically for chromosomal aberrations and QFQ-banding polymorphisms. At least two karyotypes were prepared for each case.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) STUDIES

Chimerism and the extent of donor cell engraftment was assessed by RFLP analysis. High molecular weight DNA was extracted from EDTA-anticoagulated blood and marrow specimens and digested to completion with either Taq I, Pst I, or BamHI. The resulting DNA was fractionated on 0.8% agarose gels, blotted onto magnagraph (MSI), and subjected to Southern blot analysis.

The DNA probes were labeled by random priming (Amersham, Arlington Heights, IL) to a specific activity of 1 x 10^8 cpm/μg. After an overnight hybridization at 42°C (0.02 g/dL Ficoll, 0.02 g/dL polyvinylpyrrolidone, 0.02 g/dL bovine serum albumin, 0.5% sodium dodecyl sulfate [SDS], 5x standard saline citrate [SSC] [1 X SSC is 0.05 mol/l NaCl, 0.015 mol/l Na citrate, 0.02 mol/l NaPO4 [pH 6.3], 10% dextran sulfate, and 50% formamide), all filters were washed four times in 2x SSC/0.1% SDS for 15 minutes at 60°C. The washed filters were then subjected to autoradiography using XAR-5 film (Kodak, Rochester, NY) in the presence of intensifying screens (DuPont Lightning-Plus, Wilmington, DE).

The DNA probes/restriction enzyme combinations used in this study included: (1) YNH-24 (R White, University of Utah)/Taq I; (2) YHR (DJ Weatherall, Oxford University, UK)/Pst I; (3) YNZ22 (R White, University of Utah)/BamHI; and (4) PERT-25 (M Lalamde, Boston, MA)/Pst I. The probes identify DNA hypervariable regions detected following digestion by these restriction enzymes.

RNA PREPARATION AND PCR

Bone marrow samples were obtained at defined intervals after infusion. Cells were either viably frozen or directly added to 4 mol/L guanidinium isothiocyanate and RNA prepared with minor modifications of previously described methods.14 RNA PCR was performed using abl sequence-specific reverse transcriptase primer and bcr/abl-specific PCR primers as previously reported.14 Second round PCR with nested internal bcr/abl primers was performed in all cases and the PCR products were evaluated for the bcr/abl transcript by direct visualization in ethidium bromide-stained agarose gels after electrophoresis. In reconstitution experiments, 1 CML cell per 10^6 normal mononuclear cells could be detected by this approach. The presence of intact RNA and adequate cDNA preparation was evaluated by a single round of PCR using abl sequence-specific PCR primers as previously described.14

CHIMERIC STATUS OF PREINFUSION T LYMPHOCYTES

The donor or host origin of T lymphocytes from preleukocyte infusion blood samples of sex mismatched donor/recipient pairs was determined by fluorescent in situ hybridization with Y chromosome minisatellite probes. Peripheral blood T cells were expanded by stimulation of 1 x 10^6 mononuclear cells/mL of culture medium containing a 1:100 dilution of phytohemagglutinin-M (PHA-M) and 20 U/mL recombinant IL-2. The cultures were incubated for 4 to 6 days at 37°C and 5% CO2. The proportion of CD3+ T cells as assessed by flow cytometry ranged from 80% to 95%. The presence of the Y chromosome was detected by in situ hybridization according to the manufacturers directions (Oncor, Inc, Gaithersburg, MD). Briefly, 1.5 x 10^6 of the PHA blasts were fixed with methanol/acetic acid (3:1 vol:vol) for 20 minutes, concentrated to a
volume of 0.5 mL, dropped onto clean glass slides, and allowed to
air dry. The hybridization of a combination of two biotinylated Y
chromosome probes (DY23 + DYZ1) was visualized by fluores-
cent microscopy after two series of sequential amplification with
fluorescein labeled avidin and anti-avidin. A control male sample
was tested with each assay. Background hybridization in female cell
slide preparations was 1% to 2%.

Cytogenetic analysis of peripheral blood samples was performed
by culturing 0.5 mL of blood in RPMI 1640 medium containing
15% fetal bovine serum and 2% phytohemagglutinin. Cultures were
incubated for 3 days in T25 flasks (Corning Science Products,
Corning, NY) at 37°C. Metaphase preparations were analyzed for
T-cell chimerism using informative polymorphisms between donor
and recipient.

Response Criteria

Hematologic remission was defined as normalization of all blood
counts with the patient off all other therapy (ie, cytotoxic agents or
biologic response modifiers) other than that needed for treatment of
GVHD. A cytogenetic remission was defined by disappearance of
the T-cell dose. The total nucleated cell dose administered
to the patient, cytogenetic analysis of PHA-stimulated peripheral
blood cells determined that

RESULTS

Patient Characteristics

The patient characteristics and disease status at the time
of transplant and before infusion are shown in Table 1. Six
patients were in the accelerated phase of their disease and
two were in lymphoid blast crisis at the time of enrollment.
Five patients initially transplanted in the chronic or acceler-
ated phase were deemed to be in the accelerated phase at
relapse by virtue of additional clonal abnormalities on cyto-
genetic analysis. The sixth patient had marked basophilia.
Five of six patients in the accelerated phase before infusion
had received treatment with either hydroxyurea or inter-
feron after relapse. The two patients in blast crisis were
given vincristine and prednisone after relapse in an effort to
control the blast cell count. However, these patients were
still in relapse and had not returned to a chronic phase at the
time leukocytes were administered. The median time be-
tween BMT and donor leukocyte infusions was 18.5
months (range, 4 to 61 months) and was greater than 1 year
in all but one patient (UPN 454). The median time from
hematologic relapse to infusion was 2.5 months (range, 0 to
18 months).

Preinfusion Chimerism Studies

RFLP studies were performed before infusion to deter-
mine the extent of donor/recipient chimerism in the periphe-
ral blood and bone marrow. These data are shown in Table
and demonstrate that all tested patients had predomi-
nantly recipient cells in the bone marrow. The majority of
patients also had a preponderance of host cells in the periphe-
ral blood.

Chimerism of the recipient T-lymphocyte population in
the peripheral blood before leukocyte infusions was assessed
in five sex-mismatched donor/recipient pairs using in situ
hybridization (Table 2). In contrast to the RFLP data, this
analysis demonstrated that T cells in relapsed patients were
almost exclusively of donor origin. In an additional patient
(UPN 372) who received leukocytes from a same sex donor,
informative cytogenetic polymorphisms were present to dis-
tinguish the donor and recipient populations. In this pa-
tient, cytogenetic analysis of PHA-stimulated peripheral
blood cells determined that 29 of 30 metaphases were of
donor origin.

Immunophenotyping of Leukocyte Infusions

All patients received a T-cell dose within the assigned
range of 2.5 to 5.0 × 10^8 T cells/kg. The number of infusions
and total T-cell dose administered to each patient are shown
in Table 3. Three or four infusions were required to achieve
the T-cell dose. The total nucleated cell dose administered
to patients varied by approximately twofold. This was due
to the fact that some donor leukocyte collections had a
much lower percentage of lymphocytes (range, 36% to 97%) and/or CD3^+ T cells (range, 61.8% to 77%) than others.
These data show that the direct quantitation of T cells was
necessary to determine accurately the T-cell dose infused
into each patient. The total dose per kilogram of CD4^+,

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/Sex</th>
<th>Diagnosis at Time of BMT</th>
<th>Maximal GVHD Grade After BMT</th>
<th>Remission Duration (mo)</th>
<th>Post Relapse Therapy Before Leukocyte Infusions</th>
<th>Disease Status at Time of Enrollment</th>
<th>Time From BMT to Leukocyte Infusions (mo)</th>
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</thead>
<tbody>
<tr>
<td>225*</td>
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<td>CML, AP</td>
<td>/L</td>
<td>14</td>
<td>IFN, hydroxyurea</td>
<td>CML, AP</td>
<td>18†</td>
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<tr>
<td>364</td>
<td>58/F</td>
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<td>17</td>
<td>IFN, hydroxyurea</td>
<td>CML, AP</td>
<td>16</td>
</tr>
<tr>
<td>141</td>
<td>54/M</td>
<td>CML, CP</td>
<td>/L</td>
<td>9</td>
<td>IFN, hydroxyurea</td>
<td>CML, AP</td>
<td>61</td>
</tr>
<tr>
<td>251</td>
<td>24/F</td>
<td>CML, CP</td>
<td>/L</td>
<td>37</td>
<td>Vincristine, prednisone, IFN</td>
<td>CML, BC</td>
<td>40</td>
</tr>
<tr>
<td>372</td>
<td>33/M</td>
<td>CML, CP</td>
<td>None/none</td>
<td>12</td>
<td>IFN, hydroxyurea</td>
<td>CML, AP</td>
<td>17</td>
</tr>
<tr>
<td>357</td>
<td>47/F</td>
<td>CML, CP</td>
<td>/L</td>
<td>17</td>
<td>None</td>
<td>CML, AP</td>
<td>19</td>
</tr>
<tr>
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<td>/NE</td>
<td>4</td>
<td>Vincristine, prednisone</td>
<td>CML, BC</td>
<td>4</td>
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<tr>
<td>306</td>
<td>41/M</td>
<td>CML, AP</td>
<td>/L</td>
<td>13</td>
<td>IFN, hydroxyurea</td>
<td>CML, AP</td>
<td>31</td>
</tr>
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</table>

Abbreviations: L, limited; E, extensive; NE, not evaluable; IFN, interferon.

* Patient underwent two allogeneic BMTs before receiving leukocyte infusions.
† Time elapsed from second BMT to receipt of donor leukocytes.
and Methods.

GVHD, which was confined to the skin and was treated days) after the first infusion. Five patients developed grade toxiclastic vasculitis, which was successfully treated with prednisone.

DONOR LEUKOCYTES FOR RELAPSED CML AFTER BMT

CD8⁺, CD56⁺, and CD19⁺ lymphocytes was also determined in seven of the eight patients (Table 3). The number of infused CD4⁺ and CD8⁺ T cells differed between individuals with greater disparity observed in the CD8⁺ subset. The CD4:CD8 ratio varied from 1.4 to 3.6. Similarly, there was a sixfold difference in the dose of CD56⁺ cells (0.11 to 0.66 x 10⁸/kg). Although the percentage of CD3⁻ CD56⁺ cells was not quantitated, these data suggest that the number of NK cells infused into each patient was not uniform.

Toxicity

The first five patients in the cohort received their initial leukocyte infusion as inpatients to monitor them for any adverse reactions. All subsequent infusions were administered in the outpatient setting. No untoward transfusion reactions were observed. One patient (UPN 364) developed a rash 21 days postinfusion. A skin biopsy showed leukocytoclastic vasculitis, which was successfully treated with prednisone.

GVHD. Seven of eight patients developed acute GVHD (Table 4). One patient (UPN 454) who died 2 weeks after receiving leukocyte infusions was not evaluable. The median day of onset of GVHD was 32 days (range, 28 to 88 days) after the first infusion. Five patients developed grade I GVHD, which was confined to the skin and treated with modest doses of prednisone (10 to 40 mg/d). One patient had fatal grade IV GVHD. The remaining patient developed grade III GVHD involving the liver and skin. This patient had marked jaundice and has required prolonged treatment with steroids, cyclosporine, XomaZyme, and thalidomide. He is presently on prednisone and thalidomide and his serum bilirubin level has normalized. Three patients (UPN 225, 251, and 364) who had responded to steroids subsequently had a flaring of GVHD. GVHD involved the skin and mouth in two, and the skin and liver in the remaining patient. These patients continue to require steroid therapy and were classified as having extensive chronic GVHD. All six patients who have been observed for longer than 6 months continue to require treatment with tapering doses of prednisone. One patient is also receiving cyclosporine therapy.

Marrow aplasia. Four patients who received infusions in accelerated phase developed pancytopenia at a median of 130 days (range, 49 to 200 days) after infusion. In each case, markedly hypercellular marrows became profoundly hypocellular as leukemia was eradicated. Serial biopsies from one representative patient (UPN 364) are shown in Fig 1. All four patients required platelet and/or red cell transfusions to maintain a platelet count ≥20,000/µL and hemoglobin level ≥10 g/dL. Each patient also received treatment with granulocyte-colony stimulating factor to augment the absolute neutrophil count. One of these four patients had resolution of neutropenia and eventually became transfusion independent without any further interventions. The remaining three patients had persistent marrow aplasia. All received marrow boosts from the original leukocyte donors. In two cases, the marrow was depleted of mature T cells with the T₁₀B₉ antibody, and in one case marrow was reinfused without T-cell depletion. Two patients had sustained engraftment and are now transfusion and growth factor therapy independent. One recipient of T-cell depleted marrow had transient engraftment and required a second marrow boost that was not depleted of T cells. This patient remains transfusion and growth factor therapy dependent with a persistently hypocellular marrow 3 months after the second boost. In three of the four patients, pancytopenia developed 2 to 5 months after the initial clinical manifestations of GVHD. In only one patient (UPN 225) who had early deval-

Table 2. Preinfusion Donor/Recipient Chimerism in Peripheral Blood and BM

<table>
<thead>
<tr>
<th>UPN</th>
<th>BM</th>
<th>Peripheral Blood</th>
<th>RFLP Studies</th>
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<td>225</td>
<td>ND</td>
<td>ND</td>
<td>M/F</td>
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<tr>
<td>364</td>
<td>95% Recipient</td>
<td>95% Recipient</td>
<td>F/M</td>
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<tr>
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<td>90% Recipient</td>
<td>10% Recipient</td>
<td>ND</td>
</tr>
<tr>
<td>251</td>
<td>&gt;99% Recipient</td>
<td>95% Recipient</td>
<td>ND</td>
</tr>
<tr>
<td>372</td>
<td>98% Recipient</td>
<td>ND</td>
<td>M/F</td>
</tr>
<tr>
<td>367</td>
<td>90% Recipient</td>
<td>80% Recipient</td>
<td>ND</td>
</tr>
<tr>
<td>454</td>
<td>90% Recipient</td>
<td>80% Recipient</td>
<td>ND</td>
</tr>
<tr>
<td>306</td>
<td>90% Recipient</td>
<td>80% Recipient</td>
<td>ND</td>
</tr>
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Table 3. Schedule and Content of Leukocyte Infusions

<table>
<thead>
<tr>
<th>UPN</th>
<th>Infusions (N)</th>
<th>Total Nucleated Cell Dose (×10¹⁰/kg)</th>
<th>Total Cell Dose (×10⁵)</th>
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<tr>
<td>225</td>
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<td>372</td>
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<td>6.84</td>
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<td>6.05</td>
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<td>454</td>
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<td>3.96</td>
<td>2.9</td>
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<td>306</td>
<td>4</td>
<td>3.09</td>
<td>2.9</td>
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Abbreviation: ND, not done.

* T-cell dose in this patient was estimated as described in Materials and Methods.
Table 4. Chimerism, Remission, and Toxicity Data

<table>
<thead>
<tr>
<th>UNP</th>
<th>Duration of Follow-Up (wk)</th>
<th>GVHD* (acute/chronic)</th>
<th>Marrow Aplasia</th>
<th>RFLP† Studies</th>
<th>Cytogenetic† Remission</th>
<th>Detection of BCR/ABL Transcript</th>
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<td>92</td>
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<td>364</td>
<td>43</td>
<td>I/E</td>
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<tr>
<td>141</td>
<td>8</td>
<td>IV/NE</td>
<td>NE</td>
<td>98% Donor</td>
<td>2% Recipient</td>
<td>Positive</td>
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<tr>
<td>251</td>
<td>38</td>
<td>I/E</td>
<td>No</td>
<td>&gt;99% Donor</td>
<td>Yes</td>
<td>Negative</td>
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<tr>
<td>372</td>
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<td>No</td>
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<tr>
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</table>

Abbreviations: L, limited; E, extensive; NE, not evaluable; ND, not done.

* Acute GVHD was defined as that occurring within the first 100 days after infusion.
† Studies were performed at time of last follow-up.

Clinical Response

The white cell counts of seven patients rose after leukocyte infusions due to the discontinuation of cytotoxic therapy. Three patients in accelerated phase whose WBC exceeded 100,000/μL were treated with alpha interferon for a median of 3 weeks (range, 2.5 to 7 weeks). The dose of interferon was 5 to 10 × 10^6 U/d in two patients and 5 × 10^6 U three times a week in the last individual. One patient treated in lymphoid blast crisis (UPN 454) received adjunctive therapy with interferon and prednisone. The first manifestation of a clinical response was typically a decline in the white cell count, which occurred 3 to 7 weeks after leukocyte infusions in the majority of patients. Interferon was discontinued in patients once the WBC count began to decline.

Two patients were treated in blast crisis. One (UPN 141) died of grade IV GVHD within 6 weeks of leukocyte infusion. Before demise, a bone marrow study had demonstrated cytogenetic remission; however, residual tumor was seen at autopsy. The second patient (UPN 454) died of progressive disease within 2 weeks post-infusion. All six patients treated in accelerated phase are in hematologic and cytogenetic remission at a median of 42 weeks after infusion (range, 34 to 92 weeks). The onset of cytogenetic remission in these patients was variable (Table 5). No responses were observed by 8 weeks after infusion. By 12 weeks, three patients were in cytogenetic remission, whereas two additional patients were found to be in remission at weeks 20 and 28, respectively. The last patient required 34 weeks to enter remission. Mixed chimerism was observed in several patients before they eventually remitted. Five of six cytogenetic responders are also now in molecular remission as determined by absence of the bcr/abl RNA transcript (Table 4). These patients all have evidence of complete donor cell engraftment by RFLP analysis. Eradication of leukemia as determined by PCR studies occurred slowly. Only one patient (UPN 225) entered into a sustained molecular remission within the first 7 months after infusion. The remaining patients required 7 to 10 months before becoming PCR negative. Two of the three patients (UPN 364 and 357) who required marrow boosts were PCR positive but in cyto-

Fig 1. (A) BM biopsy (UPN 364) obtained 4 weeks after leukocyte infusions demonstrating a hypercellular marrow with granulocytic hyperplasia consistent with persisting morphologic relapse. (B) Biopsy obtained 7 months after infusion shows a markedly hypocellular marrow with eradication of leukemia.
DISCUSSION

In this study, leukocyte infusions that administered a defined T-cell dose to each recipient were able to mediate a potent antileukemic effect in patients with overt hematologic relapse. Although in most patients an incipient antileukemic response was observed within the first 3 to 7 weeks after infusion, eradication of the leukemic cell population occurred gradually and the median time to a molecular remission before the marrow infusions was not overly aggressive and still responsive to therapy with hydroxyurea or interferon. This was evidenced by the fact that all six patients treated in accelerated phase are now in cytogenetic remission. In contrast, the rapid proliferation of leukemic cells appeared to overwhelm the nascent GVL effect. Prior studies have shown a correlation between the number of T cells administered to patients with leukemia at time of BMT and the subsequent incidence and severity of GVHD. Moreover, T-cell depletion of the donor graft has been associated with increased relapse rates in patients with CML. Whether this was due strictly to a quantitative deficiency in the number of T cells, or to qualitative abnormalities in T-cell function (eg, defective cytokine production or cytolytic activity) or absence of other cell populations with potential antileukemic activity (eg, natural killer and lymphokine-activated killer [LAK] cells) is unknown. Truitt et al have speculated that some leukemic relapses may result from suboptimal GVL reactions due to the developments of donor/host tolerance or suppression of the GVH reaction. These data support the concept that the number of T cells administered to the recipient is a biologically relevant variable that impacts on clinical outcome.

Before receiving leukocyte infusions, each patient was studied to determine chimerism by RFLP analysis. All patients had informative cytogenetic polymorphisms, we were also able to determine the chimeric status of pre-infusion T cells. In each patient, essentially all T cells were of donor origin, indicating that complete donor chimerism in the T-cell compartment was not sufficient to prevent disease relapse. From this study, leukocyte infusions that administered a predetermined T-cell dose as adoptive immunotherapy to BMT recipients. This approach was taken so that the efficacy and toxicity of leukocyte infusions could be analyzed in a cohort of patients who had received a similar biologic dose. Although multiple effector populations are thought to contribute to GVL and GVH reactivity after allogeneic marrow transplantation for CML, donor T cells appear to play a major role. Prior studies have shown a correlation between the number of T cells administered to patients with leukemia at time of BMT and the subsequent incidence and severity of GVHD. Moreover, T-cell depletion of the donor graft has been associated with increased relapse rates in patients with CML. These data support the concept that the number of T cells administered to the recipient is a biologically relevant variable that impacts on clinical outcome.

Table 5. Serial Cytogenetic Studies Performed on BM Aspirates Before and After Leukocyte Infusions

<table>
<thead>
<tr>
<th>UPN</th>
<th>PRE</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>Week 20</th>
<th>Week 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>225</td>
<td>46,XX,t (7;9;22)</td>
<td>46,XX,t (7;9;22)</td>
<td>46,XY [17]</td>
<td>46,XY [20]</td>
<td>46,XY [20]</td>
<td>ND</td>
</tr>
</tbody>
</table>

The term "others" is used to denote other clonal and nonclonal abnormalities that have not been included in order to simplify analysis of the karyotypic progression. Numbers in brackets refers to the number of metaphases analyzed.

Abbreviations: NE, not evaluable; ND, not done.
before elimination of the leukemic clone. Although the mechanism of disease relapse remains unclear, what is apparent from this study is that an effective antileukemic response could be restored by administration of exogenous leukocytes.

Because T cells constituted the major mononuclear cell population in the leukocyte products, this suggests that these cells played an important role in the GVL response either by eliciting a GVH reaction or exerting an antileukemia effect independent of GVHD. Because all evaluable patients developed GVHD, it was not possible to dissociate these effects, although Kolb et al have shown that GVHD need not occur for an effective GVL response. The relative contributions of CD4+ and CD8+ T cells or NK cells to the GVL effect could not be assessed. Both T cells and NK cells have, however, been shown to play an important role in preserving antileukemia reactivity in murine models, and it is likely that the GVL response was mediated by multiple effector populations. Finally, we cannot exclude the possibility that α interferon may have augmented the antileukemia effect in some patients, even though most of the treated patients received only short courses of therapy.

One of the complications observed after leukocyte infusions was the development of GVHD, which was fatal in one patient and severe in a second, although the latter has had an excellent response to immunosuppressive therapy. In the remaining patients, GVHD has been mild and manageable with single agent immunosuppression in all but one patient. Several animal studies have shown that the delayed infusion of immunocompetent donor spleen cells or lymphocytes into allogeneic chimeras is associated with substantially less GVHD than if the same inoculum is administered at the time of transplant. Our results are generally supportive of these data. At our institution, the mean T-cell dose, administered to patients receiving T-cell depleted HLA-matched sibling grafts, is approximately $1.7 \times 10^9$ T cells/kg (unpublished results). Using this T-depletion strategy plus posttransplant cyclosporine, the incidence of grade II-IV acute GVHD has been approximately 15% to 20%. In the present study, the number of infused T cells exceeded that given to patients at the time of transplant by approximately 3 logs. That most of these patients developed only mild GVHD in the absence of post-infusion GVHD prophylaxis suggests that the severity of GVHD will be less in humans when the administration of T cells is delayed. It should also be emphasized that most patients received leukocytes a year or more after relapse. This differs from a previous study where the early administration of donor buffy coat cells within the first 5 days after BMT was shown to exacerbate GVHD significantly. Whether the apparent amelioration of GVHD with delayed leukocyte infusions is due to the interval recovery of the recipient from the effects of the conditioning regimen, the antecedent development of donor/host tolerance, or other factors is unknown. Future studies will be of interest to determine if leukocytes can be administered earlier with acceptable toxicity.

The other major complication observed in this patient population was marrow aplasia, which occurred in four of six evaluable patients. The development of marrow aplasia after leukocyte infusions was similar to that which occurs in transfusion-associated GVHD. The temporal progression to pancytopenia and marrow aplasia after donor leukocyte infusions, however, occurred more slowly than in transfusion-associated GVHD where most deaths attributable to pancytopenia occur within the first month. This disparity may be due to the fact that the leukemia cell target population was substantially larger and therefore required longer to eradicate. The mechanism of aplasia in transfusion-associated GVHD is thought to be due to the destruction of recipient hematopoietic cells by naïve donor immunocompetent cells. A similar mechanism appeared to be operative in these patients. Prior studies in allogeneic marrow transplant recipients have shown that donor-derived effector populations can suppress and lyse recipient hematopoietic cells in vitro. Mackinnon et al demonstrated that interleukin-2 activated peripheral blood mononuclear cells obtained from CML patients after allogeneic BMT inhibited CFU-GM proliferation of recipient leukemic cells. Furthermore, in a majority of patients, LAK cells were able to kill CML cells in chromium release assays. Unfortunately, in three of the patients in this study, eradication of leukemia was not accompanied by the restoration of normal donor hematopoiesis. These patients ultimately required marrow boosts and one of them remains transfusion dependent. We speculate that the paucity of residual donor hematopoietic cells may have been insufficient to reconstitute hematopoiesis in these hosts. In the latter patient, however, we cannot exclude the possibility that transfused leukocytes may have damaged the host microenvironment and thus impaired hematopoietic function even after the boost was given. Marrow aplasia may be better averted in the future if patients are treated when there are a greater number of donor myeloid cells present. This would probably require that some patients be treated with leukocyte infusions when they were in cytogenetic relapse only. This premise is supported by data from Bar et al who observed marrow aplasia only in the two patients who had exclusively recipient cells by cytogenetic analysis before leukocyte infusions. None of the remaining patients who exhibited mixed chimerism developed this complication. In the present study, in contrast, all six evaluable patients were complete recipient chimeras by marrow cytogenetic studies, which may partially explain the higher incidence of aplasia.

The optimal T-cell dose for the treatment of relapsed CML after allogeneic BMT is not known. The T-cell dose we used was effective in eradicating leukemia, but was also associated with some adverse effects. In the report by Bar et al, the total T-cell dose administered to patients was more variable and somewhat lower, although there was overlap with the dosing range used in this study. All of their patients, except one nonresponder, developed clinical GVHD. The observed response rate was similar to that of this study; however, the disease status of patients in hematologic relapse at the time of infusion was not specified. We would infer from our data and that of Bar et al that the administration of T-cell doses that are higher than were administered in the present study may not significantly improve efficacy, but might adversely affect the therapeutic index by escalating
toxicity. It should be emphasized that all patients in this study were treated in hematologic relapse, had received T-cell-depleted grafts, and had received leukocyte infusions from HLA-matched siblings. Direct extrapolation of these data to other patient populations should therefore be made with caution. Patients treated in cytogenetic relapse might require fewer T cells for a GVL effect because of the lower leukemia cell burden. Similarly, patients who receive equivalent T-cell doses from unrelated donors may have enhanced GVL/GVH reactivity due to the greater HLA disparity between donor and host. Recipients of unmodified grafts might also respond differently than patients given T-cell-depleted marrow grafts. This study provides data that may be useful in addressing these issues and in designing future clinical trials to determine the optimal T-cell dose for patients in various stages of relapse and for those who have received more HLA-disparate grafts.

In summary, we have examined the therapeutic efficacy and toxicity of leukocyte infusions using a predetermined T-cell dosing range. This T-cell dose was able to provide an effective antileukemic response albeit at the expense of some toxicity. While assessment of the durability of cytogenetic and molecular remissions will require a longer follow-up, the fact that the remission duration after leukocyte transfusions of the initial patient in the cohort has now exceeded either of the first two remissions after BMT is promising. This study further demonstrates that leukocyte infusions can be effectively used as salvage immunotherapy in patients with overt relapse and may be superior to existing immunomodulatory and chemotherapeutic strategies for the treatment of relapsed CML. Additional studies are needed to determine the optimal timing of infusions, once relapse is detected, and the minimal number of T cells that will reduce toxicity yet preserve antileukemia reactivity.

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Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T-cell dose

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