Relapse of Chronic Myeloid Leukemia After Allogeneic Bone Marrow Transplant: The Case for Giving Donor Leukocyte Transfusions Before the Onset of Hematologic Relapse

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Fourteen patients with chronic myeloid leukemia (CML) relapsing after allogeneic bone marrow transplant (BMT) were treated with leukocyte transfusions from the original marrow donor (sibling, n = 9; volunteer unrelated, n = 5). The relapse was defined at the molecular level in two cases, cytogenetically in five cases and hematologically in seven cases. Ten patients responded, seven of seven patients with cytogenetic/molecular relapse compared with three of seven with hematologic relapse (P < .03). All five recipients of cells from unrelated donors responded. Eight of the 10 responders have achieved polymerase chain reaction-negative status and this persisted in three patients for more than 2 years; no responder has shown sign of relapse. Reversible marrow aplasia occurred in two patients, both treated in hematologic relapse. Severe graft-versus-host disease occurred in four patients and was fatal in one. We confirm previous reports that donor leukocyte transfusions are effective in the management of CML in relapse after BMT. In this series, therapeutic intervention before the onset of hematologic relapse was associated with an increased likelihood of response and no marrow aplasia.

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Submitted November 24, 1993; accepted January 21, 1994.

Supported by Fellowships from the Leukaemia Research Fund (F.V., J.C., and A.S.), and by the Conselho Nacional de Pesquisa, Brazil (B.G.).

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0006-4971/94/8311-002733.00/0


Despite the Introduction of new therapies such as α-interferon (IFNa), allogeneic bone marrow transplantation (BMT) is the only treatment that results in long-term disease-free survival of chronic myeloid leukemia (CML). However, relapse after allogeneic BMT with unmanipulated donor marrow for CML in first chronic phase occurs in 10% to 20% of patients. The management of relapse is difficult because cytotoxic drugs and IFNa are not curative and a second BMT is associated with appreciable mortality. Recently, it has been reported that administration of IFNa together with leukocyte transfusions from sibling donors can restore complete cytogenetic remission in patients with hematologic relapse of CML after BMT. We report our experience with this approach in 14 patients in relapse after allogeneic BM transplant for CML. Seven patients had only cytogenetic or molecular evidence of relapse and were treated with donor leukocyte transfusions (DLT) before frank hematologic relapse. Five patients received leukocyte transfusions from their original volunteer unrelated marrow donor. Response to treatment was monitored hematologically and by cytogenetic analysis of the BM; residual disease was studied by reverse transcriptase/polymerase chain reaction (RT-PCR) for BCR-ABL transcripts.

Patients and Methods

Patients. Fourteen patients with relapsed CML after BMT received donor leukocytes from their original marrow donor (sibling, n = 9; volunteer, n = 5). Thirteen patients were treated for relapse of Philadelphia chromosome-positive (Ph+) CML. The fourteenth patient (unique patient number [UPN] 236) had been transplanted for Ph- CML, but leukemia-specific BCR-ABL mRNA was detected by RT-PCR. Twelve patients were in first chronic phase at the time of BMT, whereas the remaining two had accelerated phase disease. Sibling donors and recipients were HLA-identical and the volunteer donor-recipient pairs were serologically identical at HLA-A, -B, and -DR loci. Pretransplant conditioning consisted of cyclophosphamide and fractionated total body irradiation (TBI) as previously described. Three patients received donor marrow that was ex-vivo T-cell depleted using the monoclonal antibody Campath-1M. Further details of the original transplant procedures are reported in Table 1.

Definition of relapse. Hematologic relapse was defined as peripheral blood leucocytosis with predominance of myelocytes and neutrophils in the differential count. This was accompanied by a hypercellular bone marrow and Ph positivity on cytogenetic analysis. Cytogenetic relapse was considered to be present if one or more Ph+ metaphases were detected without evidence of hematologic relapse. Molecular relapse was defined as more than 1,000 BCR-ABL transcripts/µg RNA detected in the blood by competitive PCR more than 4 months after transplant without evidence of cytogenetic or hematologic relapse. At the time of DLT, seven patients were in hematologic relapse. Two of the patients in hematologic relapse were deemed to have disease acceleration because both had multiple cytogenetic abnormalities associated with disease progression and a platelet count of more than 1,000 X 10^9/L. Five patients were in cytogenetic relapse at the time of leukocyte transfusion. Two patients (UPN 43 and 349) in complete cytogenetic remission had evidence of molecular relapse with high numbers of BCR-ABL transcripts detected by competitive PCR (5,000 and 3,000 transcripts/µg RNA, respectively). UPN 43 was in cytogenetic remission after previous IFN therapy.

Management of relapse. Treatment of relapse before DLT consisted of hydroxyurea (n = 3), IFNa (n = 3) and withdrawal of cyclosporin A (n = 4). The remaining 10 patients were not on cyclosporin A therapy at the time of relapse. Donor leukocytes were collected on a continuous flow cell separator (Cobe Spectra; Cobe Laboratories, Gloucester, UK) and immediately transfused to the recipient without plasma or red blood cell depletion. Informed consent was provided according to the Declaration of Helsinki. Donor leukocytes were obtained from the United Kingdom in 13 cases; in one case (UPN 334) leukocytes were obtained from a donor in the National Marrow Donor Program in the United States, transported to the United Kingdom at room temperature in acid citrate dextrose and administered to the recipient 36 hours after collection. Each donor was leukapheresed on an average of 2 occasions (range: 1 to 4) with a maximum interval of 16 days between collections. The nucleated cell and lymphocyte doses administered are shown in detail.
and short-term culture by G-banding for the presence of the Ph chromosome. At least 30 metaphases were examined in the majority.

Determination of T-lymphocyte chimerism before leukocyte transfusion. Cyto genetic analysis of the T-lymphocytes was performed to establish the donor or host origin. Peripheral blood samples of six recipients with a sex-mismatched donor were obtained before leukocyte transfusion and stimulated with phytohemagglutinin (PHA).

Qualitative and competitive PCR. Peripheral blood samples were studied for BCR-ABL transcripts by two-step RT-PCR with nested primers as previously described. More recently, PCR-positive and competitive PCR and a semiquantitative estimation of the number of BCR-ABL transcripts was made.

Definition of response. Hematologic remission was considered to be present if the peripheral blood counts had normalized with no morphologic evidence of CML in the BM. Cyto genetic remission was documented if no Ph+ metaphases were detected on cytogenetic analysis of at least 30 BM metaphases. Molecular remission was defined if BCR-ABL transcripts were not detected by two-step PCR with nested primers subject to satisfactory controls as described. Acute and chronic graft-versus-host disease (GVHD) were diagnosed using standard criteria. Chronic GVHD was diagnosed if GVHD occurred de novo or persisted more than 100 days after leukocyte transfusion. Marrow aplasia was defined by a hypoplastic BM with peripheral blood pancytopenia (hemoglobin <11 g/dL, neutrophils <0.5 x 10^9/L, and platelets <20 x 10^9/L).

RESULTS

Response. The median times from BMT to cyto genetic and hematologic relapse were 18 and 29 months, respectively (overall range: 3 to 108). Immunosuppressive therapy with cyclosporin A was discontinued with no response in 4 patients before transfusion of donor leukocytes. Three patients were treated with IFNα before DLT and a cyto genetic response was seen in only 1 patient (UPN 43). The median time from relapse to leukocyte transfusion was 13 months (range: 0 to 65). A complete response to transfusion of donor leukocytes with reversion to Ph or PCR negativity was observed in 10 of 14 (71%) patients. All 7 patients in cyto genetic or molecular relapse at the time of transfusion responded, but a complete cyto genetic response was noted in only 3 of 7 (43%) patients with hematologic relapse (P = .022, Fisher's exact test). One patient with hematologic relapse (UPN 202) responded to DLT only after addition of IFNα therapy. This patient had previously been treated for 13 months with IFNα with no cyto genetic response and IFNα had been discontinued 3 months before transfusion of donor leukocytes. The median follow-up after DLT was 8 months (range: 4 to 37). There was no significant difference in the numbers of nucleated cells administered to responders and nonresponders. Neither of the patients with relapsed CML in accelerated phase responded and a further nonresponder (UPN 263) developed disease acceleration 5 months after infusion of donor leukocytes. None of the three patients who received a second course of donor leukocytes responded.

Cyto genetic and PCR data. Eight of 12 patients with hematologic or cyto genetic evidence of relapse went into complete cyto genetic remission. Five patients were in cyto genetic relapse and had a mean of 58% Ph+ metaphases in the marrow at the time of treatment (range: 16.7% to 83%). The median time to complete cyto genetic remission in the responders was 4 months (range: 2.5 to 11). Complete molec-
DONOR LYMPHOCYTES AFTER BMT

Cellular remissions with no detectable BCR-ABL transcripts were documented in 8 of 10 responders, but the remaining 2 both have reducing transcripts levels and may become PCR negative on longer follow-up. The median time to achieve PCR negativity was 6 months (range: 2.5 to 15). The remissions in the responder group appear to be durable and no second relapses have been seen. The three longest responders remained PCR negative at the time of DLT. The remaining nonresponder (UPN 321) who was in cytogenetic remission after previous therapy, but high numbers of BCR-ABL transcripts were still detectable by competitive PCR. After responding to DLT, a reduction in numbers of BCR-ABL transcripts was noted and this patient subsequently became PCR negative (Fig 1B).

The type of BCR-ABL transcript (b3a2, n = 10; b2a2, n = 4) expressed did not correlate with response (data not shown).

### T-lymphocyte chimerism before leukocyte transfusion

Cytogenetic evaluation of PHA-blasts in all sex-mismatched donor/recipient pairs showed that in all cases, the T lymphocytes were 100% of donor origin at the time of relapse.

**GVHD.** Acute GVHD occurred in 7 of the 10 responders and was confined to skin and gut (grade 1) in 4. Two patients developed acute liver GVHD (grade 4) that responded to therapy with systemic corticosteroids. One of these patients (UPN 202) initially showed no response to transfusion of donor leukocytes and was treated with IFNalpha 6 months later. This patient developed severe liver GVHD 1 month after commencing IFNalpha and was later documented to be in complete cytogenetic remission. Extensive chronic GVHD occurred in 3 responders and proved steroid responsive in 2.

GVHD was observed in two nonresponders. One patient (UPN 112) developed skin GVHD (grade 1) after which a transient reduction in peripheral white blood cells was observed. The remaining nonresponder (UPN 321) who was in accelerated phase at the time of relapse developed pneumocystis carinii pneumonia 4 weeks post-DLT, and responded to appropriate therapy. No response was observed to transfu-
sion of donor leukocytes and IFNα therapy was commenced 2 months later after which severe acute liver GVHD occurred. This patient died after 7 months because of liver failure, sepsis, and hemorrhage from a liver biopsy site. Histology of the liver showed the typical features of GVHD with complete obliteration of small bile ducts.

There was no correlation between cell dose transfused and the occurrence of GVHD or between GVHD after DLT and after the original transplant.

Marrow aplasia. Two patients with hematologic relapse developed severe pancytopenia requiring blood support and marrow aplasia that was confirmed by trephine biopsy. One
patient (UPN 239) developed pancytopenia 2 months after leukocyte transfusion and GVHD was not observed. He recovered normal hemopoiesis shortly after a second marrow infusion from his sibling donor. The other patient (UPN 236) had received a sex-mismatched transplant from a volunteer donor, and pancytopenia occurred 4 months after treatment with donor leukocytes. BM myeloid cells obtained during pancytopenia were shown to be of donor origin by fluorescence in situ hybridisation (FISH) with Y-chromosome and BCR-ABL probes. This patient was managed with granulocyte colony-stimulating factor and marrow function recovered slowly over several months. No aplasia was observed in any of the patients who were in cytogenetic or molecular relapse at the time of DLT.

**Leukocyte transfusion from volunteer unrelated donors.** Five patients were treated with leukocytes from their original volunteer marrow donor (hematologic relapse n = 1, cytogenetic relapse n = 3 and molecular relapse n = 1). A complete cytogenetic or molecular response was seen in all five patients compared with five of nine (44%) patients in the sibling donor group (P = .10 Fisher’s exact test). Prophylactic cyclosporin A was administered for 4 to 12 weeks to four patients receiving cells from a volunteer donor and a response was only observed after cyclosporin A had been withdrawn (Fig 1, B C). GVHD was seen in all five patients. One patient (UPN 349) developed acute GVHD (grade 4) of the liver that resolved with systemic steroid therapy. Extensive chronic GVHD of the skin was observed in 2 patients and was poorly responsive to immunosuppressive measures in one (UPN 197). Severe GVHD was seen in two of nine patients in the sibling donor group, both of whom had received additional therapy with IFNa after transfusion of donor leukocytes.

**DISCUSSION**

Relapse of CML after allogeneic marrow transplant is an important cause of treatment failure and the optimum strategy for the management of relapse remains to be defined. Administration of IFNa can improve short-term survival by inducing a cytogenetic remission in 10% to 15% of patients that delays disease progression. A second transplant may salvage a minority of patients and result in long-term disease-free survival, but at the cost of increased morbidity and mortality. Several reports have now described the use of DLTs from the original marrow donor as an alternative to a second transplant. We report here cytogenetic or molecular responses in 10 of 14 patients (71%) treated for recurrence of CML after transplant. The median time to restoration of Ph negativity was 4 months. However, reducing numbers of BCR-ABL transcripts could still be shown by competitive PCR in four responders in cytogenetic remission, indicating that the elimination of the leukemic clone after donor cell infusion was gradual. Eight of 10 responders eventually achieved complete molecular remissions and long-term follow-up in 3 shows that these remissions are durable.

The evolution of relapse of CML posttransplant is in general slow, and hematologic relapse is often preceded by a gradual reappearance of Ph+ cells in the marrow that is referred to as cytogenetic relapse. We have previously reported that the detection of BCR-ABL transcripts more than 1 year after BMT identifies a group of patients at increased risk of relapse, but is not predictive for any particular individual. Serial measurement of residual disease by competitive PCR can identify patients with increasing numbers of leukemia-specific transcripts who may be progressing to clinical relapse. However, it has not yet been established whether administration of donor leukocytes before overt hematologic relapse is desirable. In this series, patients with only cytogenetic or molecular evidence of disease recurrence responded better to transfusion of donor leukocytes than those who were in hematologic relapse. Molecular data were used to initiate therapy in three patients with high levels of BCR-ABL transcripts, all of whom responded to therapy. However, it must be remembered that Ph+ cells identified in the marrow may disappear spontaneously, but such reversal is rare if the number of leukemic cells in the marrow exceeds 40%. Thus early treatment has the disadvantage that it may unnecessarily expose some patients with transient chromosomal relapse to the adverse effects associated with transfusion of donor leukocytes.

Marrow aplasia is a potentially fatal complication of DLT and a recent review of published data suggests that pancytopenia develops in 54% of patients treated in hematologic relapse. Persistent marrow aplasia could occur if the residual donor stem cell pool were insufficient to repopulate the BM after elimination of the leukemic cell population. A second stem cell infusion from the donor would then be required for full hematopoietic reconstitution. We observed marrow aplasia only in patients treated in hematologic relapse; no aplasia was seen in the seven responders who were transfused with donor leukocytes during cytogenetic or molecular relapse. Therefore, it is reasonable to speculate that administration of donor leukocytes before progression to frank hematologic relapse may prevent aplasia. We showed in one patient residual donor hematopoiesis in the myeloid compartment during pancytopenia using FISH analysis that appeared to predict for recovery from aplasia.

The high response rate to donor leukocytes underlines the powerful antileukemic effect mediated by allogeneic lymphocytes and is strong support for the existence of a graft-versus-leukemia effect (GVL) after allogeneic marrow transplant. It is notable that two patients who did not develop GVHD after DLT achieved complete remission, suggesting that GVL is not always dependent on clinically manifest GVHD. It is not clear why GVL may fail to eliminate small numbers of residual leukemic cells after the initial transplant and yet transfusion of further donor cells is a highly effective treatment of relapse in patients in whom complete donor T-cell chimerism can be shown. There has been considerable variation in the number of mononuclear cells transfused in various studies and although more lymphocytes are present in a leucapheresis collection compared with a marrow harvest, a clear relation between cell dose transfused and thus far, response has not been apparent. Three nonresponders in our series did not benefit from a second course of DLTs. However, we did observe a complete response to a single transfusion of only 0.6 x 10^6 nucleated cells/kg and the minimum cell dose required for the induction of GVL may be lower than has hitherto been assumed. Qualitative factors
other than cell dose such as expression of minor histocompatibility antigens on leukemic cells, and therefore, GVHD prophylaxis could also be important determinants of GVL. GVHD prophylaxis after transplant could predispose some patients to relapse by deleting or suppressing lymphocyte populations mediating GVL. Withdrawal of cyclosporin A has been reported to restore remission in a few patients with relapsed CML after transplant. We treated four patients who received donor leukocytes from volunteer donors with prophylactic cyclosporin A and a response was only observed after cessation of cyclosporin A therapy. These observations suggest that immunosuppressive therapy can indeed abrogate GVL.

Five patients received leukocytes from a volunteer donor and all responded to therapy. This has important implications for volunteer donor registries because requests for leukocyte donation are likely to increase and may obviate the need for a second marrow harvest in case of relapse. Severe acute or extensive chronic GVHD occurred in three patients in the volunteer donor group and was only seen in the sibling group in patients treated with IFNα after transfusion of donor leukocytes. Despite close phenotypic matching volunteer donor–recipient pairs may differ for both major and minor histocompatibility antigens; this could not only increase the incidence of GVHD, but also enhance GVL. However, it should be emphasized that in this series there was no significant difference in response between patients receiving cells from volunteer donors compared with those transfused with cells from HLA-identical siblings. The observation that the use of IFNα may be associated with severe GVHD after DLT can be explained by upregulation of class I expression that may increase presentation of minor histocompatibility antigens. Most investigators have combined the use of IFNα with DLTs, but in our series only one responder required additional administration of IFNα. These findings suggest that concomitant IFNα therapy is not mandatory. Therefore, it seems prudent to reserve the combination of IFNα and leukocyte transfusions to nonresponders.

Adoptive immunotherapy for relapse of CML is an important new development and offers the prospect of using donor leukocytes to restore GVL after T-cell-depleted BMT. The introduction of a reliable competitive PCR assay for BCR-ABL transcripts permits the early prediction of relapse and could be used to time the transfusion of a targeted, small T-cell dose that would minimize GVHD and prevent marrow aplasia. However, ultimately, new treatment strategies will probably be required to separate the lymphocyte populations causing GVHD from those mediating GVL. GVL reactivity does not appear to be confined to a particular lymphocyte subset and recent experimental work suggests that it may be feasible to achieve a functional separation of GVL and GVHD by selectively deleting donor lymphocytes responding to host-specific minor histocompatibility antigens presented by patient PHA-lymphoblasts.

ACKNOWLEDGMENT

We thank the nursing staff of the hematology clinic at the Hamnesmith Hospital (London, UK) for their assistance in collecting leukocytes from the donors.

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Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse

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