Influence of Mixed Chimerism on the Results of Allogeneic Bone Marrow Transplantation for Leukemia


Serial cytogenetic studies were performed on 60 leukemic recipients of HLA-matched bone marrow transplants (BMT) who were prepared by high doses of alkylating agents and fractionated total body irradiation (TBI). Forty-three patients were recipients of untreated BMT and 17 were recipients of T-depleted BMT. Donor or host mitoses were identified by examination of sex chromosomes in 54 patients or by evaluation of the polymorphism of other chromosomes after specific banding in six patients. Mixed lymphoid chimerism (MLC) was identified in 29 patients and full donor lymphoid chimerism (FDLC) in 29 patients. Complete donor hematopoiesis was recovered in most patients after 12 months, but two T-depleted patients had persistent host cells at 46 and 52 months after the graft. Acute graft-versus-host disease was significantly less frequent in patients with MLC, especially when more than 10% of residual lymphoid cells were detected. The probability of relapse and survival was identical in patients with MLC and FDLC, except in patients with chronic myeloid leukemia where MLC was significantly associated with an increased risk of relapse.

DURING THE last decade, it became evident that "supralethal" radiochemotherapy followed by allogeneic bone marrow transplantation (BMT) could eradicate severe hematologic malignancies and lead to long-term survival. It was generally accepted that hematologic reconstitution was achieved exclusively by donor cells.

There have been several reports showing recovery of recipient hematopoiesis after BMT. When the residual recipient cells are normal, they can become tolerant to donor cells and contribute to the establishment of mixed chimerism. It is well known that recipients of T-cell-depleted transplants had a high incidence of mixed chimerism after BMT for leukemia. In animal models, stable mixed chimeras have been associated with a decreased incidence of graft-versus-host disease (GVHD). It can be postulated that if mixed lympho-hematopoietic chimeras are tolerant to the host (decreased incidence of GVHD), they can be tolerant to the residual leukemia of the host as well (increased incidence of relapse).

To clarify the clinical implication of mixed chimerism, we used serial cytogenetic analyses in 60 leukemic recipients of allogeneic BMT. The objectives were to quantify the patterns of lymphoid and myeloid engraftment after standard and T-depleted BMT and to analyze the impact of mixed chimerism on GVHD, leukemic relapse, and BMT outcome.

MATERIALS AND METHODS

Patients

From March 1984 until December 1989, 99 consecutive HLA-matched allogeneic BMT were performed in patients with hematologic malignancies. Thirty-nine patients were not evaluable (absence of cytogenetic markers, early deaths) and were excluded from this analysis. Of the 60 evaluable patients, 32 had acute lymphoblastic leukemia (ALL) in remission, 15 chronic myeloid leukemia (CML) in chronic phase, and 13 acute myeloid leukemia (AML) (12 in first remission, one in early relapse). There were 36 male and 24 female patients. The conditioning regimen consisted of cyclophosphamide (120 mg/kg, 44 patients) or high doses of phenylalanine mustard (140 mg/m², 16 patients) followed by fractionated total body irradiation (TBI) (11 Gy) as described. Seventeen patients received a T-cell-depleted marrow graft. In vitro procedures using T monoclonal antibodies to remove T cells have been previously described. Postgraft immunosuppression was administered to all patients and consisted of methotrexate (12 patients), cyclosporine A (21 patients), or both (27 patients).

Methods

Cytogenetic analyses were performed for each patient on phytohemagglutinin or PHA/interleukin-2 (IL-2)-stimulated peripheral blood (PB) lymphocytes (IL-2, 10 U/mL; Lymphocult-T-LF, Biotest Diagnostics, Dreieich, Germany) and on bone marrow (BM) short-term cultures without growth factors or with 10% conditioned medium from the human bladder carcinoma cell line 5637 that contains several myeloid growth factors. Whenever it was possible, at least 50 mitoses were analyzed in each sample and the patients were studied at 1, 2, 3, 6, 9, 12, and 24 months post-BMT and then twice a year. In 54 patients, sex chromosome determination allowed differentiation of donor and host cells. Autosomal heteromorphisms were used in five patients with sex-matched donors. One patient showed a constitutional reciprocal translocation t(8;16), Philadelphia (Ph1)-positive CML patients without any discriminating sex chromosome or heteromorphism were excluded from this analysis.

Lymphoid chimerism was evaluated from 193 samples of peripheral blood (from 58 patients before relapse) and 11,041 mitoses were analyzed (mean 57 mitoses per sample). According to Hook, this average number of metaphases allows the detection of a level of 6% mosaicism with a confidence limit of 95%. More precisely, 10 to 25 mitoses were analyzed in 22 samples, 26 to 50 mitoses in 94 samples, and 51 to 100 mitoses in 77 samples.

Definition of Mixed and Full Donor Chimerism

Patients who showed 100% donor mitoses at all times were considered to have full donor chimerism. Patients who demonstrated the presence of at least 1% recipient mitoses in the BM or PB at any time were considered to have mixed chimerism (MC). MC was also stratified between patients with more or less than 10%
host mitoses. Evaluation of chimerism was not continued when a patient did relapse, to exclude cases with leukemic recipient cells from the group of mixed chimeras.

Statistical Analysis
Relapse, survival, and disease-free survival from BMT were analyzed using the life table of Kaplan and Meyer.2 For patients who relapsed, chimerism status was evaluated from the time of the transplant until relapse occurred, and survival was estimated until the last follow-up. Log-rank test for significance was applied in making comparisons between the different groups. Proportions were compared by the chi-squared test or Fisher's test when either group included less than 10 patients.

RESULTS
Evolution of Chimerism
Documentation was possible in 60 cases who underwent allogeneic BMT from HLA-identical sibling donors. Chimerism differed strikingly between the non-T-depleted and T-depleted patients (Table 1). In the group of T-depleted patients, mixed chimeras were significantly more frequent and prolonged in both PB and BM cultures.

Within the control group of 43 patients, 16 patients (37%) had residual lymphoid cells within the first 3 months after the graft, but only five patients (12%) had these cells after 3 months. Complete donor hematopoiesis was found in every patient after 12 months.

Among the 17 T-depleted patients, residual lymphoid cells were found in 13 cases (76%) within the first 3 months and in seven cases (41%) after 3 months. Five of these patients were full donor lymphoid chimera after 12 months and two patients had persistent lymphoid host cells at 46 and 52 months.

Influence of Lymphoid Chimerism on BMT Outcome
When considering the 58 patients evaluated for lymphoid chimerism, 29 were found to be mixed lymphoid chimeras (MLC) (at least 1% host cells) and 29 were full donor lymphoid chimeras (FDLC) with a complete donor hematopoiesis (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Evolution of Chimerism After BMT</th>
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<td>Origin of Mitoses in:</td>
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<tr>
<td>Months Post-BMT</td>
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<td>Un-treated BMT</td>
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<td>Abbreviation: D/R, number of mitoses of donor/recipient origin.</td>
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<td>*P &lt; .01 between untreated and T-depleted BMT.</td>
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Lymphoid chimerism and GVHD. Acute GVHD (a-GVHD) (grade ≥ 2) developed in 20% of patients with MLC and in 55% of patients with FDLC. In fact, the amount of residual host mitoses (more or less than 10%) was critical to influence the occurrence of a-GVHD: patients with ≥10% residual host lymphocytes had a statistically significant decrease in their incidence of a-GVHD when compared with patients who had FDLC or less than 10% residual host lymphocytes (P < .01).

The incidence of chronic GVHD (c-GVHD) did not differ significantly whether or not there was an MLC, and c-GVHD was not influenced either by the amount of recipient cells.

Lymphoid chimerism: Relapse and survival. Fourteen patients relapsed (seven within the two groups); there was no difference between MLC and FDLC. Neither the date of detection of MLC (before or after 3 months), the duration of MLC (more or less than 3 months), or the proportion of recipient cells (more or less than 10%) could help to predict for the risk of relapse. Overall, with a median follow-up of 27 months for patients with MLC, survival and disease-free survival did not differ between the two groups (Fig 1).

Lymphoid chimerism and BMT outcome in chronic myeloid leukemia versus acute leukemia. The type of leukemia did influence the relationship between the results of BMT and status of chimerism (Table 3). When the 14 CML patients were analyzed, a significant correlation was found between chimerism and leukemia relapse: five of seven CML pa-
patients with MLC relapsed instead of one of seven CML patients with FDLC (P < .01). For patients with acute leukemias, no correlation was found between chimerism and relapse; only 2 of 22 patients with MLC relapsed, which is statistically different from five of seven CML patients with MLC (P < .01).

**Lymphoid chimerism and BMT outcome in the group of non-T-depleted patients.** In the aim to eliminate the influence of T-cell depletion on BMT outcome, we analyzed separately 42 non-T-depleted patients. In this selected group, MLC was also associated with lower incidence of a-GVHD but without a clear statistical value (P = .08) (Table 4).

Survival and relapse-free survival were not statistically influenced by chimerism.

**DISCUSSION**

We confirmed in this study that residual host cells are frequently identified after chemotherapy-TBI followed by BMT, and that MC is both more frequent and more prolonged after T-depleted grafts.4,15

Compared with molecular techniques,10,20-31 the use of cytogenetics has a major limitation in sensitivity and it is likely that low levels of chimerism have been missed in this analysis.34 However, the use of cytogenetics had the advantages of studying only cells that retain proliferative capacity and of separating myeloid and lymphoid engraftment. Thus, this semi-quantitative evaluation of lymphoid chimerism allows us to address the potential role of recipient lymphocytes in mediating tolerance to the host and the leukemia. Our results showed that, as expected from animal models and previously observed in aplastic anemias, MLC are associated with a lessened incidence of GVHD.5,16 Such an association was stronger if more than 10% of host lymphocytes were identified in PB cultures postgraft.

However, a more precise correlation between the quantity of host lymphocytes and the induction of tolerance remains to be determined, considering the limited confidence interval allowed by examination of limited numbers of mitoses.35

There are conflicting data in the literature concerning the influence of mixed chimerism on the risk of leukemic relapse.1,9,10,12,14,15,21,30,32

In this study, MLC was not statistically associated with the recurrence of leukemia, and some patients could have long-term MC without recurrence of leukemic cells up to 5 years post-BMT. In fact, prolonged MC without relapse was only found in patients receiving T-depleted grafts for AML or ALL. In our patients with CML, prolonged lymphoid MC was almost always associated with relapse. Similarly, Offit et al15 suggested that MC with greater than 25% normal host cells had a higher probability of clinical relapse in CML. However, in our study the small number of patients with CML and the confounding effects of T-depletion invites caution in the interpretation for these observations. The reasons of such a finding could be various: either residual disease of CML is more sensitive to immune control by the graft than other leukemias, or some patients with acute leukemia undergo allogeneic BMT at a stage where their residual disease is extremely low, if present, and could need less immune control by the graft.

Finally, this study of 60 leukemic patients showed with a long follow-up that the persistence of normal recipient cells was not systematically associated with a negative prognostic significance. Therefore, it is possible that, in some cases of leukemias, tolerance to transplant antigens of the host is not associated with tolerance to the residual leukemias and that graft-versus-leukemia could be achieved without GVHD.

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