Persistence of Host-Type Hematopoiesis After Allogeneic Bone Marrow Transplantation for Leukemia Is Significantly Related to the Recipient's Age and/or the Conditioning Regimen, But it Is Not Associated With an Increased Risk of Relapse


We investigated the chimerism pattern within flow-sorted peripheral blood- or bone marrow-derived cell populations after allogeneic bone marrow transplantation (BMT) for the treatment of leukemia in children. This study was performed to define the identity of persistent host-type cells, to identify prognostic variables for the persistence of host-type hematopoiesis, and to determine the prognostic significance of the chimerism pattern on the duration of the leukemia-free interval, the overall survival, and the leukemia-free survival. The patients received either HLA-identical non-T-cell-depleted (n = 46) or HLA nonidentical T-cell-depleted (n = 7) BMT. In the peripheral blood, the children showed either stable mixed chimerism (SMC); ie, persistent host-type hematopoiesis; n = 14), (transient) mixed T-lymphoid chimerism (MTLC; n = 9), or complete chimerism (CC; n = 30). In the bone marrow, only donor-type cells were found in children with either CC (n = 8) or MTLC (n = 2), and a mixture of donor- and recipient-type cells was found in children with SMC (n = 7). The persistence of host-type hematopoiesis (SMC) was significantly related to a lower age of the recipient, the type of conditioning regimen, a lower total body irradiation dose, T-cell depletion of the bone marrow graft, and the use of cyclosporine A for acute graft-versus-host disease prophylaxis. No significant differences were found between patients with (SMC) or without (CC/MTLC) persistent host-type hematopoiesis with respect to the duration of the leukemia-free interval, the overall survival, or the leukemia-free survival. We conclude that ablation of host-type hematopoiesis is not compulsory for long-term leukemia-free survival after allogeneic BMT for various hematologic malignancies.

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Relapse remains a major obstacle for success after allogeneic bone marrow transplantation (BMT) for the treatment of hematologic malignancies. Therefore, it is important to identify transplant recipients at high risk of relapse, who might benefit from additional therapy. Important clinical events such as engraftment, graft rejection, and leukemia relapse are frequently documented by chimerism analysis. It remains controversial whether a relationship exists between mixed chimerism and leukemia relapse. In this regard, several aspects are worthy of consideration. First, it seems important to distinguish between the persistence of nonmalignant and malignant host-type cells. Thus, it is unlikely that the controversy can be resolved by chimerism analysis of unfractionated peripheral blood (PB) or bone marrow (BM) samples. Second, it may be important to distinguish between the persistence of mature host-type lymphocytes with limited or no self-renewal capacity and the persistence of host-type hematopoiesis, because these patterns of chimerism may differ with respect to the establishment of donor-host tolerance. Third, if a relation between the presence of mixed chimerism and leukemia relapse is found, then it may be due to the confounding influence of other variables. For instance, several investigators suggested a relation between the chimerism pattern and the conditioning regimen, or T-cell depletion of the BM graft, or the development of acute graft-versus-host disease (GVHD). These same variables may also affect the risk of leukemia relapse. Thus, it is important to exclude or adjust for the confounding influence of such variables.

We previously reported a new methodology for the analysis of the chimerism pattern in fractionated PB or BM samples using the fluorescence-activated cell sorter (FACS) and amplification of highly polymorphic variable number of tandem repeats (VNTRs) by the polymerase chain reaction (PCR), so-called FACS/PCR-VNTR (FPV) analysis. Our preliminary results obtained by FPV chimerism analysis in 25 children after allogeneic BMT for leukemia suggested that persistence of host T cells is not associated with a significantly increased risk of relapse or with decreased survival. Here, we present the results obtained in a larger group of patients (n = 53). The median follow-up of survivors in continuous complete remission was 22 months (range, 8 to 142 months). We identified several clinical variables associated with the persistence of host-type hematopoiesis and determined the prognostic significance of the persistence of host-type hematopoiesis on the duration of the leukemia-free interval (LI), the overall survival, and the leukemia-free survival (LFS).
with the panel of 5 VNTRs (n = 1), or hepatitis B virus infection of the patient (n = 1). The results of the remaining 53 cases are presented in this study.

Twenty-six patients were considered to be at low risk of leukemia relapse, including patients with adult-type Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) in chronic phase (n = 4), acute undifferentiated leukemia (AUL) in first complete remission (CR) (n = 1), acute myeloid leukemia (AML) in first CR (n = 11), acute lymphoid leukemia (ALL) in first CR (n = 4), myelodysplastic syndrome (MDS); either refractory anemia [RA] or refractory anemia with excess of blasts [RAEB]; n = 3), or MDS progressing to overt leukemia blast crisis followed by remission induction (MDS/AML in first CR; n = 3). The remaining 27 patients were considered to be at high risk of leukemia relapse, including those with juvenile CML (n = 2), Ph+ ALL in second CR (n = 1), AML in second CR (n = 3), ALL in second CR (n = 16), RAEB in transformation (RAEBT; n = 1), non-Hodgkin lymphoma (NHL) in second CR with BM involvement (n = 2), or secondary AML (sAML) in first CR (n = 2).

Transplant procedure. All patients transplanted with BM from an HLA-identical sibling donor were conditioned with cyclophosphamide (Cy) intravenously (IV) at 60 mg/kg body weight (BW) × 2, followed by total body irradiation (TBI) using a linear accelerator in a single dose of 5.0 Gy for one infant 10 months of age and three children 1 to 2 years of age, 7.0 Gy for children 2 to 4 years of age, 7.5 Gy for children 4 to 10 years of age, and 8.0 Gy for children more than 10 years of age. From unique patient number (UPN) 134 (July 1989) onwards, children more than 2 years old received a more intensive conditioning regimen with additional cytosine arabinoside (AraC) 2 g/m² × 2 on days −9 and −8 (CML/AML/MDS) or etoposide (VP16) 350 mg/m² × 2 on days −9 and −8 (ALL/NHL). Also, from UPN 134 onwards, children more than 10 years of age received fractionated TBI at 6.0 Gy × 2 on days −1 and 0 instead of single-dose TBI at 8.0 Gy. The instant TBI dose rate was ≥23 cGy/min. Infants less than 2 years of age and children with a short stature received TBI at a higher instant dose rate (up to 100 cGy/min).

The exceptions to this general conditioning regimen were UPN 119 and 170, who received fractionated TBI at 3.0 Gy × 4 (cumulative lung dose: 10 Gy) following VP16 at 40 mg/kg BW × 1,² because of preexisting cardiomyopathy. Furthermore, 4 patients were prepared with the BuCy4 regimen,² with varying doses of busulfan (Bu) depending on the age of the patients: 4 mg/kg BW × 4 (UPN 161), 5 mg/kg BW × 4 (UPN 184), or 6 mg/kg BW × 4 (UPN 194 and 196) (days −9 through −6) plus Cy at 50 mg/kg BW × 4 (days −5 through −2).

Seven patients were graftedin with more than “2 log” allogeneic/ESR-罗斯ette T-cell–depleted (TCD) BM² from an other than HLA-identical sibling donor. Two patients (UPN 143 and 188) received BM from an HLA haplo-identical, mixed lymphocyte culture-negative related donor, who was either 2 antigen (HLA A and B) mismatched (UPN 143) or phenotypically matched (UPN 188). A matched unrelated donor was used in 5 cases (UPN 147, 164, 184, 203, and 206). Recipients of TCD BM received additional immunosuppression with rabbit antithymocyte globulin at 2 mg/kg BW × 5, plus anti–lymphocyte function-associated antigen-1 (LFA1; from day −2 onwards) at 0.2 mg/kg BW × 8 (UPN 143), Campath-1G at 0.2 mg/kg BW × 5 (days −7 through −5) plus anti-LFA1 at 13 (UPN 147, 184, 188, 203, and 206), or Campath-1G plus anti-LFA1 × 8 (UPN 164).

Prophylaxis of acute GVHD consisted of cyclosporine A (CsA) in combination with a short course of metotrexate (MTx), or of CsA alone or a long course of Mttx. Acute GVHD was treated with methylprednisolone, IV, at 2 mg/kg BW during 2 to 4 weeks, followed by tapering and discontinuation.

Outcome. At the time of statistical analysis (May 1, 1993), 27 of the 53 children included in this study were alive in continuous CR. Seventeen children had died after relapse. Six children had died from causes other than leukemia relapse, ie, toxoplasma encephalitis in association with acute GVHD grade II (UPN 48), cytomegalovirus (CMV)-interstitial pneumonitis (UPN 55), hemolytic uremic syndrome in association with extensive chronic GVHD (UPN 90), adult-type respiratory distress syndrome (UPN 143), or Epstein-Barr virus (EBV)-lymphoproliferative syndrome (UPN 164 and 203). The remaining 3 children were alive after hematologic relapse: UPN 103 was in remission 4 years after reinduction chemotherapy, and UPN 197 and 204 were alive 4 and 5 months after relapse, respectively. Patient-, disease-, and transplant-related variables are summarized in Table I.

Chimerism analysis. Chimerism analysis was performed by amplification of VNTRs within flow-sorted cell populations (FACS/PCR-VNTR or FFP analysis) and PCR products were visualized by Southern blot hybridization, as previously described.² Chimerism analysis in PB was performed for all cases at regular time intervals after BMT (2, 6, and 12 months or until relapse or death). Chimerism patterns in the BM were investigated for 17 children, ie, at 2 to 3 weeks after BMT in 3 children and at 6 to 8 weeks after BMT in 14 children. PB mononuclear cells (PBMC) and BM mononuclear cells (BMMMC) were obtained by Ficoll-Isoaque density gradient centrifugation and stained with appropriate directly conjugated monoclonal antibodies (MoAbs), according to the manufacturer’s instructions. In the PB, we analyzed unfractonated PBMC, CD3+CD4+ and CD3+CD8+ T cells, CD20+CD19+ B cells, CD16+CD14+ natural killer (NK) cells, CD14+CD16+ monocytes, and, more recently, CD15+CD33+ granulocytes. The purity of the cell populations obtained by cell sorting was estimated to be more than 98%; the sensitivity for the detection of mixed chimerism was shown to be 1% to 5%.

Chimerism analysis of BM-derived cell populations was performed to evaluate the chimerism pattern at early differentiation stages and to detect the possible persistence of residual host-type cells within the affected cell lineage at the maturation stage of leukemia transformation. Therefore, after enrichment of progenitor cells (vide infra), we investigated unsorted BMMMC as well as appropriately flow-sorted cell populations according to the immunophenotype of the leukemia in individual patients. Before cell sorting, progenitor cells were enriched by a negative selection procedure to increase the probability of detecting residual disease and to assure the purity of the flow-sorted cell populations. BMMMC were incubated with a cocktail of unconjugated MoAbs (2 μg/mL each, 10 BMMMC/mL) specific for various mature cell surface markers (CD3, CD20, CD16, glycophorin A, and CD11b) at room temperature (RT) for 30 minutes. Then, BMMMC were washed extensively in phosphate-buffered saline supplemented with 1% bovine serum albumin and incubated with immunomagnetic beads coiled to sheep-antimouse IgG antibodies (Dynal, Oslo, Norway) on a rocking platform at RT for 20 minutes (bead/cell ratio = 5/1, 10 BMMMC/mL). The efficiency of the procedure was tested by enumeration of CD34+ progenitor cells by flow cytometry both before and after negative selection. This negative selection procedure resulted in a fivefold to 10-fold enrichment and 80% to 100% recovery of CD34+ progenitor cells (data not shown). Depletion of the cells expressing the mature cell surface markers was always efficient (>95%), as determined by remaining and flow cytometric analysis of the final cell suspension, and the viability of the cells exceeded always 95% (as determined by propidium-iodide staining). To avoid depletion of leukemic cells by this procedure, the anti-CD11b MoAb was omitted from the cocktail if the BM was obtained from a patient suffering from a myeloid malignancy. This, of course, reduced the overall enrichment of progenitor cells considerably. In three cases (UPN 202, 203, and 206), an insufficient number of cells (<5 × 10⁶ BMMMC) was obtained 2 to 3 weeks after BMT to perform negative selection. In all cases studied, immunophenotypic analysis of the original leukemic
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</table>

Abbreviations: FU, follow-up; ND, not done.

* Conditioning regimens: 1, BuCy4; 2, 5.0 Gy TBI + Cy; 3, 7.0 Gy TBI + Cy; 4, 7.5 Gy TBI + Cy; 5, 8.0 Gy TBI + Cy; 6, 2 × 6.0 Gy TBI + Cy; 7, 4 × 3 Gy TBI + VP16.
clone at diagnosis (kindly supplied by the Dutch Children’s Leukemia Study Group, The Hague, The Netherlands) did not show a positive staining pattern for the MoAbs used for negative selection. For each patient, we selected appropriate populations for cell sorting on the basis of the immunophenotype of the malignancy at diagnosis. For instance, progenitor cell-enriched unsorted BMMMC and CD34+CD19+, CD34+CD19+, CD10−CD19+, as well as CD34+CD33− cell populations were analyzed in a patient with common ALL (UPN 178) or pre-B ALL (UPN 182). Similarly, unsorted BMMMC, CD34+CD33−, and CD34+CD33− cells were investigated in a patient with AML M7 (UPN 152).

Leu-fluorescein isothiocyanate (FITC) (CD3), Leu9-phycocerythrin (PE) (CD7), Leu12-PE (CD19), Leu16 (CD20), Leu11c-PE (CD16), LeuM1-FITC (CD14), LeuM3-FITC (CD15), and LeuM9-PE (CD33) MoAbs were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA), OKT4-FITC (CD4), OKT8-FITC (CD8), OKB20-FITC (CD20), and OKM1 (CD11b) MoAbs were obtained from Ortho Diagnostic Systems (Raritan, NJ). The antilymphocytrophorin A (GpA) MoAb was obtained from Immunotech SA (Marcelle, France) and the J5-FITC (CD10) MoAb was obtained from Coulter Immunology (Hialeah, FL). The 8G12-FITC (CD34) MoAb was kindly supplied by Dr P.M. Lansdorp (Terry Fox Laboratory, Vancouver, Canada). CLB-T3 (CD3) was obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands).

Statistical analysis. The relation between clinical variables and the chimerism pattern was determined by the Pearson χ2 test for categorical data and by one way analysis of variance for continuous data. Survival curves of the patients in each chimerism group were estimated by the table-method with intervals of 1 month (SPSS PC). Three different endpoints were considered: the duration of the LFI, the overall survival, and the LFS. Children who died of other causes without leukemia recurrence were withdrawn (censored) from the LFI survival curve at the end of the observation period but included in the overall survival and LFS analysis. For LFS, the endpoint is death or disease recurrence. Differences between survival curves were tested by the logrank statistic. Statistical analyses were performed for the whole group of children (n = 53) as well as for those receiving HLA identical non-TCD BMT, separately (n = 46).

RESULTS

Identity of persistent host-type cells in PB and BM. We have previously reported the results of FPV chimerism analysis of PB T, NK, and B-lymphoid cell populations as well as monocytes and granulocytes in 25 children after allogeneic BMT for leukemia. Here, these results are extended to a larger group of children (n = 53) and summarized in Table 1. Thirty children showed complete chimerism (CC) in all cell lineages analyzed throughout the period of investigation. FPV analysis was performed at multiple occasions in 4 of 10 children with CC who relapsed or died within 6 months after BMT. Nine other children showed mixed T-lymphoid chimerism (MTLC), characterized by the presence of host-type cells in the T-cell lineage only. In 4 of these 9 cases, host T cells were detected at 2 months, but not 6 or 12 months after BMT, thereby demonstrating the transient nature of MTLC. The transient nature of MTLC could not be evaluated in the other 5 cases because of disease recurrence (n = 2) or short follow-up (n = 1) or because PBMC were not available for analysis (n = 2). Fourteen children showed stable mixed chimerism (SMC), characterized by the presence of mixed chimerism in all cell lineages (Fig 1) throughout the period of investigation. The stable nature of this chimerism pattern was confirmed by multiple investigations in 3 of 4 cases who died or relapsed within 6 months after BMT.

FPV chimerism analysis was also performed on BM samples obtained from 17 children (Table 1). Ten of 17 cases showed only donor-type cells in progenitor cell-enriched unsorted BMMMC, including only children with CC (n = 8) or MTLC (n = 2) in the PB. The latter finding confirms the concept that children with MTLC show complete donor-type hematopoiesis and indicates that the persistence of host-type T cells is a peripheral phenomenon. The BM of the remaining 7 children contained a mixture of recipient- and donor-type cells, all of whom showed SMC in the PB, thereby confirming the persistence of host-type hematopoiesis.

We previously suggested that chimerism analysis should be performed within the affected cell lineage at the maturational stage of leukemic transformation to predict leukemia relapse in individual patients. Therefore, we analyzed appropriate cell populations of the affected cell lineage in the BM on the basis of the immunophenotype of the malignancy at diagnosis. However, the persistence of host-type hematopoiesis in children with SMC (n = 7) precluded identification of residual malignant host-type cells. We failed to obtain evidence for residual disease in appropriately flow-sorted cell populations of the affected cell lineage in children with CC (n = 6) or MTLC (n = 2), who have remained in continuous CR for 6 to 34 months after BMT. However, we also failed to detect residual disease in flow-sorted CD19−CD10+ (UPN 160) or CD34+CD33− (UPN 161) cells of the remaining 2 children who subsequently relapsed, even though...
The incidence of persistent host-type hematopoiesis was significantly higher in younger transplant recipients \((P < .0001)\). Furthermore, we found a significant relation between the incidence of SMC and the type of conditioning regimen \((P = .004)\), a recipient age-dependent variable (see Materials and Methods). SMC was frequently observed after low-dose TBI \((\leq 7.5\) Gy), but not after \(8.0\) Gy single-dose TBI \((n = 12)\), \(2 \times 6.0\) Gy fractionated TBI \((n = 7)\), or \(4 \times 3.0\) Gy fractionated TBI \(+ VP16 (n = 2)\). In addition, SMC was found in 3 infants less than 2 years of age \((UPN 188, 194,\) and 196) after BuCy4 conditioning with a total dose of 20 to 24 mg/kg BW busulfan, whereas CC was observed in a 16-year-old child \((UPN 161)\) after a total dose of 16 mg/kg BW busulfan.

To investigate the influence of the TBI dose on the persistence of host-type hematopoiesis, we excluded children who received BuCy4 \((n = 4)\) or TBI \(+ VP16 (n = 2)\) conditioning regimens from analysis. The remaining children \((n = 47)\) received equal doses of Cy plus varying doses of TBI \((conditioning regimens 2 through 6 in Table 1)\). We found a significantly higher incidence of SMC in patients receiving a lower TBI dose \((P = .006)\).

The incidence of SMC was significantly higher after TCD HLA nonidentical \((4 of 7)\) versus non-TCD HLA-identical \((10 of 46) BMT (P = .047)\). However, we cannot exclude the confounding influence of other variables. For instance, the children who showed SMC after HLA nonidentical TCD BMT received a relatively mild conditioning regimen, ie, BuCy4 \((n = 1)\) or \(\leq 7.5\) Gy single-dose TBI \((n = 3)\), whereas the other children showed CC after 7.5 Gy TBI \((n = 2)\) or \(2 \times 6.0\) Gy TBI \((n = 1)\). Furthermore, additional immunosuppression was administered to recipients of HLA nonidentical TCD BM (see Materials and Methods).

The use of CsA in GVHD prophylaxis was associated with a significantly increased incidence of SMC \((P = .018)\). Again, the confounding influence of other variables cannot be excluded. For instance, only 12 of 37 children receiving CsA for GVHD prophylaxis received \(\geq 8.0\) Gy total-dose TBI versus 9 of 12 children not receiving CsA. In most children, CsA was administered in combination with Mtx. The fact that we did not observe a similar relation between the use of Mtx and the incidence of SMC may be because of the small number of children not receiving Mtx (Table 2).

We found no significant differences between the two chimerism groups with respect to the incidence of acute GVHD grade I-IV or chronic GVHD. This is probably because of the low frequency of GVHD in our pediatric patient population: only 1 of 53 children had acute GVHD grade \(\geq II (UPN 48)\) and only 1 of 53 children suffered from extensive chronic GVHD \((UPN 90)\).

The incidence of SMC was significantly higher among patients with acute leukemia transplanted more than 6 months versus those transplanted less than 6 months after achievement of the second CR \((n = 18; P = .043)\). All these children received HLA identical non-TCD BMT.

Statistical analyses were performed also separately for those children receiving HLA-identical non-TCD BM \((n = 46)\). Variables that were significantly related to the persistence of host-type hematopoiesis included a lower age of the recipient, recipient age \((<.0001)\), and recipient age-dependent variables (age \(\leq 24\) mo \(2.29\), age \(>24\) mo \(2.18\), age \(\leq 6\) mo \(0.043\), age \(>6\) mo \(0.40\)).

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recipient \((P = .0001)\), a lower age of the donor \((P = .011)\),
the type of conditioning \((P = .031)\), and the use of CsA for
prophylaxis of GVHD \((P = .034)\). The increased frequency
of SMC observed in patients grafted with BM obtained from
a younger donor probably reflects the significant correlation
between the donor and recipient age in cases of HLA-identical
BMT \((r = .67)\). The latter correlation was not significant
in the whole group of patients, probably because HLA non-
identical TCD BM graft recipients received BM from adults.
Persistence of host-type hematopoiesis was also significantly
related to a lower TBI dose in those children conditioned
with cyclophosphamide plus TBI \((n = 41; P = .034)\).

Prognostic significance of mixed chimerism with respect
to survival and leukemia relapse. The prognostic influence
of persistent host-type hematopoiesis on the duration of the
LFI, the overall survival, and the LFS was determined by
life-table analysis. The life-table estimates of the survival
distributions for the whole group of children \((n = 53)\) are
plotted in Fig 2. No significant differences were found be-
tween children with complete donor-type hematopoiesis
(CC/MTLC, \(n = 39\)) and those with persistent host-type
hematopoiesis \((SMC, n = 14)\) with respect to the duration
of the LFI \((P = .84)\), the overall survival \((P = .78)\), or the
LFS \((P = .67)\). The 3-year probabilities of LFI, survival,
and LFS for children with complete donor-type hematopoie-
sis were 53%, 44%, and 44%, respectively. The 3-year prob-
abilities of LFI, survival, and LFS for children with SMC
were 70%, 71%, and 63%, respectively. Similar results were
obtained for the group of children receiving HLA-identical
non-TCD BM \((n = 46)\); results not shown.

To investigate the influence of (transient) MTLC on the
relapse incidence and survival, we also performed life-table
analysis for the children grouped according to the CC \((n =
30)\), MTLC \((n = 9)\), or SMC \((n = 14)\) chimerism patterns.
We found no significant differences between the three chimer-
ism groups with respect to the duration of the LFI \((P = .67)\),
the overall survival \((P = .56)\), or the LFS \((P = .31)\).
Similar results were obtained if the analysis was restricted
to recipients of HLA-identical non-TCD BM \((n = 46)\); results
not shown.

DISCUSSION

We have used FPV chimerism analysis in 53 children
after allogeneic BMT for hematologic malignancies to define
the identity of persistent host-type cells, to identify pro-
nostic factors for the persistence of host-type hematopoiesis,
and to determine the prognostic significance of mixed chime-
rism on the relapse incidence and survival. In agreement
with our previous report, we found either CC, SMC, or
(transient) MTLC in the PB. Children with CC or MTLC in
the PB showed only donor-type cells in the BM, whereas
those with SMC in the PB showed a mixture of donor- and
recipient-type cells in the BM. These data support the con-
cept that SMC results from the survival of host-type pluripo-
tent hematopoietic stem cells, whereas MTLC results from
the persistence of lineage-committed host-type T cells that
have survived the conditioning regimen.

Previously, we suggested that FPV chimerism analysis
may be used to detect residual disease, provided that analysis
is performed within the affected cell lineage at the matura-
tional stage of leukemic transformation. Such an approach
may be particularly valuable to determine the relapse risk in
patients transplanted for AML, because alternatives for the
detection of minimal residual disease are not available for
the majority of these patients. As expected, the persistence of host-type hematopoiesis interferes with the identification of residual disease by FPV chimerism analysis. However, we also failed to detect residual disease in flow-sorted cell populations specifically enriched for the affected cell lineage and stage of leukemic transformation in 2 children with CC, who subsequently relapsed. Methods other than FPV chimerism analysis using leukemia-specific markers may be more useful to detect minimal residual disease after allogeneic BMT. Such methods include the amplification of translocation breakpoints by PCR using either DNA or mRNA, or the use of antigen receptor rearrangements as clonal markers for leukemic cells.

The incidence of SMC was significantly higher after HLA nonidentical TCD versus HLA-identical non-TCD BMT ($P = .047$). However, we cannot exclude other factors besides TCD, such as the increased HLA disparity, the age-related conditioning regimens, or the administration of additional immunosuppression, as the underlying cause for this finding. An increased frequency of MTLC after HLA-identical TCD BMT has been observed by numerous groups.$^{6,11}$ However, the effect of TCD on the persistence of host-type hematopoiesis is less well documented. A high frequency of transient MTLC$^9$ or CC in granulocytes$^9$ after HLA-identical TCD BMT has been reported in single studies. In other studies, no clear distinction was made between transient or stable mixed chimerism.$^{10,11}$ Taken together, these results suggest that the degree of mixed chimerism after TCD BMT may depend on additional variables, such as the intensity of the conditioning.

In support of this hypothesis, we found a significant relation between the persistence of host-type hematopoiesis and the recipient age ($P < .0001$) or the type of conditioning regimen ($P = .004$), a recipient age-dependent variable. More specifically, we observed a significant relation between the TBI dose and the persistence of host-type hematopoiesis in children conditioned with equal doses of Cy plus varying doses of TBI ($P = .006$), even if analysis was restricted to HLA-identical non-TCD transplant recipients ($P = .034$). Formally, we cannot discriminate between the effect of the recipient age and the type of conditioning on the persistence of host-type hematopoiesis. Recently, several groups have investigated the possible relation between the incidence of mixed chimerism and the type of conditioning regimen. Petz et al$^7$ suggested a relation between the TBI dose and the incidence of mixed chimerism, although this was not significant and no distinction was made between transient and stable mixed chimerism. Also, Frassoni et al$^7$ described a relation between the TBI dose and the incidence of mixed chimerism, although their definition of mixed chimerism does not exclude the presence of malignant host-type cells. Mackinnon et al$^7$ reported a relationship between the type of conditioning and the incidence of MTLC. However, in that study, CC was found in the granulocytes of all patients irrespective of the conditioning regimen, suggesting complete donor-type hematopoiesis. Bär et al$^7$ reported a relation between the type of conditioning and the incidence of mixed chimerism, although this relation may have been confounded by the increased incidence of acute GVHD in patients with CC. Finally, Chalmers et al$^7$ found a significant increase in the incidence of SMC in 13 children receiving 13 Gy TBI as compared with 35 adults receiving 14.3 Gy TBI.

Recently, it was shown that the systemic exposure after administration of equal doses (ie, 4 mg/kg BW × 4) of Bu is less in children as compared with adults because of differences in pharmacokinetics.$^{25-35}$ This finding may explain the occurrence of SMC in 3 infants less than 2 years of age after BuCy4 treatment as opposed to the complete donor-type hematopoiesis observed in a youngster, 16 years of age, despite the fact that the infants received a higher total dose of Bu. In agreement with this hypothesis, Fishleder et al$^7$ found no evidence of SMC in 26 adults after BuCy2 conditioning. In contrast, Katz et al$^7$ reported CC, as determined by Southern analysis, in 3 infants less than 2 years of age after the same BuCy4 conditioning regimen. Also, Ugozzoli et al$^7$ observed CC in 16 patients (age, 1.5 to 14 years) after BuCy4 treatment.

In our group of pediatric allograft recipients, the incidence and severity of acute and chronic GVHD were too low to provide valid information on their relation with the persistence of host-type cells. We did find a higher incidence of persistent host-type hematopoiesis in patients receiving CsA for GVHD prophylaxis. It seems likely that the immunoreactivity of the graft towards the recipient is decreased by CsA-mediated immunosuppression, thereby leading to survival of host-type hematopoiesis. Hill et al$^7$ provided evidence that the incidence of acute GVHD grade II-IV is significantly lower in patients with transient mixed chimerism. Thus, a relation between the persistence of host-type hematopoiesis and the incidence of acute GVHD remains to be demonstrated.

The incidence of persistent host-type hematopoiesis was also significantly related to the time interval between achievement of the second CR and BMT. It may be that children grafted within 6 months after achieving the second CR still had an incomplete recovery of hematopoiesis and thus were more vulnerable for the myelosuppressive conditioning. Alternatively, this finding may be related to the small number of patients included in this analysis ($n = 18$) or the confounding influence of other variables.

We also performed multivariate analysis with respect to the persistence of host-type hematopoiesis using a logistic regression model including the whole study population. When either the recipient age or the type of conditioning was included in the model, no other significant covariates were identified (results not shown). However, the number of patients included in this study (ie, the statistical power of the analysis) is too small to identify significant covariates with reasonable certainty.

We previously reported the results of FPV chimerism analysis after allogeneic BMT in 25 children and suggested that persistence of host T cells is not associated with an increased risk of leukemia relapse.$^2$ However, children with SMC or MTLC were not evaluated separately. Here, we found no significant differences in the duration of the LFI, the overall survival, and the LFS between children with either CC, MTLC, or SMC; nor did we find significant differences with respect to the three endpoints between children with complete donor-type hematopoiesis (CC/MTLC) and those with persistent host-type hematopoiesis (SMC). These
results suggest that neither the persistence of mature host-type T cells, nor the persistence of host-type BM-derived antigen presenting cells, which mediate clonal deletion of host-reactive donor T cells in the thymus, are associated with a significant decrease in the graft-versus-leukemia effect of allogeneic BMT. Other investigators also failed to show a significant relation between the persistence of host-type cells and leukemia relapse after a variety of high-dose radiochemotherapy regimens.6-8,11-13,39-41.

Our finding that persistence of host-type hematopoiesis is not associated with an increased risk of leukemia relapse may be explained in view of the non-stem cell origin of most hematologic malignancies (ie, AML, ALL, MDS, and NHL). It remains to be determined whether SMC is associated with an increased relapse incidence after BMT for hematologic malignancies arising from pluripotent hematopoietic stem cells (ie, Ph+ CML). Our results do not support the latter hypothesis because the presence of SMC was compatible with long-term LFS in a patient transplanted for Ph+ CML. Furthermore, Offit et al.59 provided evidence that persistence of nonmalignant host-type cells after allogeneic TCD BMT for Ph+ CML is not associated with relapse.

Although the persistence of nonmalignant host-type cells did not affect the prognosis of the allograft recipients, other variables did. A younger recipient age was associated with improved overall survival and LFS, and the type of conditioning regimen was significantly related to the duration of the LFI (results not shown). When these parameters were included in a multivariate Cox proportional hazards model, no other covariates that significantly contributed to the progression of the disease at BMT, the conditioning regimen, and the type of transplant, these results should be interpreted with caution.

In conclusion, our results and those of others indicate that a number of clinical variables influence the posttransplant chimerism pattern. The impact of each of these variables on the persistence of host-type hematopoiesis needs further study by multivariate analysis in large cohorts of patients. In addition, our results suggest that neither the persistence of lineage-committed host-type T cells nor the persistence of host-type hematopoiesis is associated with a significantly increased risk of leukemia relapse. Thus, the persistence of nonmalignant host-type cells after allogeneic BMT for leukemia is not a useful prognostic indicator for the occurrence of relapse. However, we cannot exclude the possibility that the relation between mixed chimerism and leukemia relapse depends on variables such as the conditioning regimen or the type of leukemia.

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CHIMERISM AND RELAPSE AFTER BMT FOR LEUKEMIA


Persistence of host-type hematopoiesis after allogeneic bone marrow transplantation for leukemia is significantly related to the recipient’s age and/or the conditioning regimen, but it is not associated with an increased risk of relapse

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