CONCISE REPORT

Mixed Blood Chimerism in T Cell-Depleted Bone Marrow Transplant Recipients: Evaluation Using DNA Polymorphisms

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We have used DNA sequence polymorphism analysis to document engraftment after T cell-depleted bone marrow transplantation (BMT), with a selected panel of four DNA probes. In contrast to nondepleted BMT recipients, the patients who received T cell-depleted marrow exhibited a mixed blood chimerism. This mosaicism was observed before graft failure or relapse in six patients. However, in five other patients, this mixed chimerism was not followed by these complications with a follow-up of 9 to 31 months after transplantation. Our results support the hypothesis that transplanted bone marrow T cells may help to maintain engraftment by eliminating host cells that can cause graft failure.

THE ABILITY to establish the origin of cells following bone marrow transplantation (BMT) and to detect change in a patient's hematopoietic and lymphoid cell compartments from host to donor origin is clinically useful in assessing both the occurrence and completeness of engraftment. In particular, the pattern of engraftment is of interest with regard to the development of in vitro T cell depletion of the BMT. This technique, which has proved useful in the prevention of graft-versus-host disease (GVHD), dramatically increases the risk of graft failure and relapse, a risk that is probably associated with the existence of residual host cells multiplying after transplantation. In order to explore this phenomenon, we have documented and characterized the pattern of engraftment in T cell-depleted and nondepleted BMT recipients using DNA sequence polymorphism analysis.

PATIENTS AND METHODS

Patients

Beginning in September 1984, certain patients transplanted in our unit received a T cell-depleted graft (Table 1). In this prospective study, recipients >30 years were selected for in vitro T cell depletion: 19 of these patients were analyzed for peripheral blood chimerism (12 in the depleted group and seven in the nondepleted group). In addition, three patients (patients no. 13, 14, and 15) having received a nondepleted graft demonstrating total body irradiation (3 Gy) before the beginning of this study were examined retrospectively and are included in the control group.

All 22 patients received an allogeneic BMT from a HLA-identical mixed lymphocyte culture negative sibling as therapy for hematologic-malignant diseases. Acute myeloblastic leukemia (AML), 7; acute lymphoblastic leukemia (ALL), 6; chronic myeloid leukemia (CML), 6; preleukemic state, 3. The BMT conditioning regimen consisted of chemotherapy (cyclophosphamide, 60 mg/kg x 2) and total body irradiation (10 Gy single dose with mean dose rate 3 cGy/min and lung shielding above 8 Gy). Six patients (no. 2, 5, 6, 10, 11, and 21) received a more ablative preparation (additive drugs or total lymphoid irradiation) (Table 1). All were given methotrexate (15 mg/m2 on day 1, 10 mg/m2 on days 3, 6, and 11, and weekly up to day 100) as GVHD prophylaxis. Twelve patients (no. 1 to 12) received T cell-depleted BMT either with monoclonal antibodies CD2-CD5-CD7 plus rabbit complement (patients no. 1, 2, 6, and 7) or with T101 immunotoxin (patients no. 3, 4, 5, 8, 9, 10, 11, and 12). These two techniques are equally efficient (mean depletion 99% and mean injected T cells 0.12 × 106/kg). All patients were advised of chemotherapeutic and bone marrow procedures and their attendant risks, and gave informed consent.

DNA Analysis

In all the patients studied, engraftment was established and monitored with the use of DNA sequence polymorphism analysis as previously described or with a Y chromosome-specific probe when donor and recipient sex were different. The genotype of the posttransplant cell fractions was compared with the pretransplant host and donor genotype.

Preparation of genomic DNA samples. Peripheral blood specimens from donors and patients before and after BMT were collected in EDTA. High molecular weight DNA was prepared by standard techniques. Ten micrograms of DNA were digested to completion with the restriction enzyme TaqI and 5 µg with HaeIII in the case of sex mismatch. DNA fragments were then fractionated by electrophoresis in 1% agarose gels and transferred to nitrocellulose filters by the Southern's technique.

Hybridization with DNA probes. A panel of four probes comprising two X chromosome-specific probes (S14 and L1.28), a Y chromosome-specific sequence (HSS), and an autosomal probe (MetH) were used. The S14 probe for locus DXS52 (Xq28) is a 3.0 kb EcoR1 fragment that detects a multiallelic TaqI polymorphism.8 Probe L1.28 detects two alleles TaqI polymorphism at the locus DXS7 on the short arm of chromosome X.9 The HSS probe is a cloned DNA fragment that represents a major component of the human Y chromosome-specific repeated DNA family (DY21). Male DNA digested by HaeIII contains 800 to 5000 copies of a 3.4 kb DNA fragment,10 a fragment that we never observed, in our experimental conditions, in DNA of female origin. The MetH probe detects a two allele TaqI polymorphism at a locus localized on the long arm of chromosome 7 (7q22-31).11 The probes were labeled with 32P deoxynucleotide triphosphates (Amersham International plc, Buckinghamshire, UK). Nitrocellulose filters were hybridized, washed, examined by autoradiography and the films were scanned with a spectrophotometer (Cellosystem, SEBIA, Issy-les-Moulineaux, France).

Fractionation of posttransplantation cells. In five patients (no. 3, 4, 8, 11, and 13) blood cells were fractionated into granulocytes, T...
and B lymphocytes by Ficoll centrifugation, E-rosetting, and plastic adherence.

RESULTS

We first estimated the sensitivity of the assay by dilution experiments in which patient and donor DNAs were mixed in varying proportions. The minor cell population is still visible at levels in the order of 1% to 5% of total DNA with the use of the probes St14, LI.28, and MetH. This level of sensitivity is dependent on the growth rate of the cell population and varies proportionally. The minor cell population is still visible (90% of the cells) at levels as high as 0.1%, ie, a hybridization fragment was detectable in DNA from the female donor or cells of a male BMT in a female recipient.

The chimerism was analyzed with the HS5 probe in eight male recipients of female donor (patients no. 1 through 8), and with the probes St14, LI.28, and MetH in the remaining male/male pairs (patients no. 12 and 22). These probes and MetH failed to distinguish between recipient and donor DNA. In the two remaining male/male pairs (patients no. 12 and 22) these probes failed to distinguish between recipient and donor DNA.

In four recipients, the use of both St14 and HS5 probes was necessary to quantify the chimerism. In patient no. 9, male host cells exceeded 10% of total cells as demonstrated by the intensity of the hybridization signal with the Y-specific sequence, and probe St14 was used to evaluate the proportion of female donor DNA. In patients no. 10 and 11, probe St14 did not detect any DNA of male origin but the use of probe HS5 showed that engraftment of male cells persisted although clinical diagnosis of graft rejection had been made (up to 5 months after clinical diagnosis of graft rejection for patient no. 11). In patient no. 5, probe St14, in contrast to probe HS5, failed to detect residual male host cells at day 30 but could detect a male-specific allele at day 90 (Fig 1). It thus appears that the precocity with which the diagnosis of partial chimerism can be made depends on the sensitivity of the test used and on the growth rate of the residual cell population. Of the nine nondepleted BMT recipients studied (patients no. 13 to 21), eight exhibited total blood chimerism (from 2 months to 3 years post-BMT) and one (patient no. 13) (ALL) displayed a partial chimerism.
ism at 6 years, detectable only with the HS5 probe (ie, <1%).

In contrast, all T-depleted BMT recipients (patients no. 1 to 11) exhibited a mixed chimerism. This chimerism was detected in seven cases with probe St14 and in four with probe HS5. This mosaicism was observed before graft failure in three patients (no. 9, 10, and 11) at 4, 2, and 3 months, respectively, or before relapse in the three others (no. 6, 7, and 8) at 5, 5, and 4 months post-BMT, respectively. In every instance the chimerism was above 10%. In five other recipients (no. 1, 2, 3, 4, and 5) (two ALL and three CML) this partial chimerism did not exceed 5% and was not followed by these complications with a follow up of 9 to 31 months after transplantation.

In five cases the leukocytes were fractionated. Mixed chimerism was observed in three recipients (no. 3, 4, and 11) (ALL, CML, preleukemic state) in the three subpopulations. The T lymphocytes of patient no. 8 (AML) were of recipient origin and the granulocytes and B lymphocytes were found to be of donor origin just before her death. In patient no. 13 (ALL), residual host cells were mainly detected in the B lymphocyte fraction at 6 years post-BMT.

**DISCUSSION**

Analysis of DNA sequence polymorphism has proved useful in studying the origin of cells after BMT and allows marrow engraftment to be documented, to detect and analyze mixed lympho-hematopoietic chimerism, and to evaluate posttransplant leukemic relapse.\(^5\)\(^-\)\(^7\) Cytogenetic analysis can be used to assess post-BMT chimerism but it requires dividing cells to be obtained by bone marrow puncture. DNA analysis, when performed on bone marrow cells, gave similar results to that obtained with peripheral blood cells.\(^7\)\(^-\)\(^9\) We thus studied blood cells only and DNA analysis was conclusive in 20 of the 22 patients we examined. The sensitivity of the test depends on the probe used: 1% to 5% for St14, L1.28, and MetH, and 0.1% for HS5. The clinical significance of this small amount of host residual cells is not known. Furthermore, the quantification of mixed chimerism by study of the whole blood cell population may be irrelevant since the mosaicism is often different in each hematopoietic subpopulation as observed in patients no. 8 and 13 and as previously described.\(^5\)

The most striking result in our study is the detection of a discernable amount of host DNA in peripheral blood of all T cell-depleted BMT recipients, even in those who had received a more ablative regimen (see patients no. 2, 5, 6, 10, and 11). Such partial chimerism has previously been observed after BMT either by DNA analysis\(^5\)\(^-\)\(^7\) or by cytogenetic techniques.\(^12\)\(^-\)\(^14\) Some were SCID\(^5\)\(^,\)\(^6\) who did not receive TBI but others received TBI-cyclophosphamide for hematological malignancies as did our patients. Among the nondepleted BMT, mixed chimerism remains uncommon.\(^13\) In contrast mixed chimerism appears very common after T cell depletion of bone marrow as shown by cytogenetic analysis\(^14\) and by DNA analysis in this report.

Although the present work is not a controlled comparative study, the observed differences between T cell-depleted and nondepleted BMT cannot be explained by mean age, diagnosis, and status at BMT or conditioning regimen. The high rate of mixed chimerism appears to be a consequence of T cell depletion of the graft. This result may be associated with the existence of radio-resistant residual host cells after TBI. BMT preparation cannot eliminate all hematological host cells as it has been described in humans\(^5\)\(^-\)\(^9\) and in dogs.\(^16\) Circulating T lymphocytes can survive intensive pretransplant conditioning regimens, proliferate after incubation with interleukin 2 and phytohemagglutinin, and function as effector cells in an in vitro model assay.\(^13\) These cells are probably destroyed by grafted T lymphocytes, explaining the rareness of mixed chimerism in nondepleted transplantation. In contrast, after T cell-depleted BMT, residual host cells survive and their growth results in partial chimerisms as observed in our study. These cells are probably involved both in the rejection episodes and in the hematological relapses. Nevertheless, the mixed chimerism does not systematically deteriorate the prognosis. Long-term cure of the disease remains a possibility when leukemic patients are grafted in complete remission (patients no. 1, 3, and 13). Recently, using a murine model of T cell-depleted BMT, Ferrara et al\(^17\) observed that mixed chimerism was frequent and could remain stable even at 1 year posttransplant. However, the persistence of the malignancy seems to be the rule in CML (patients no. 2, 4, and 5) grafted in the active phase of the disease.

In conclusion, our results demonstrate further the interest of DNA analysis techniques in the study of BMT recipients. In the future these techniques should be used to assess the quality of new protocols of T cell depletion or of appropriate conditioning regimens, designed to prevent graft rejection or relapse.

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