Highly Sensitive Polymerase Chain Reaction Methods Show the Frequent Survival of Residual Recipient Multipotent Progenitors After Non–T-Cell–Depleted Bone Marrow Transplantation

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Twenty-four male patients grafted for various pathologies with the marrow of a female donor and presenting a complete donor-type hematopoiesis when analyzed by polymerase chain reaction (PCR) amplification of minisatellite sequences 33.6.3 and MS51 (0.1% to 1% sensitivity) were studied by the highly sensitive technique of PCR amplification of the Y-chromosome–specific DYZ1 sequence (0.01% sensitivity). Residual recipient male cells were detected in all peripheral blood samples collected within 1 year posttransplantation. These residual cells were present in both the lymphocyte and polymorphonuclear cell fractions when such a separation was performed by Ficoll gradient centrifugation and, for samples of 13 of 15 patients, at comparable levels in both fractions. In 3 samples collected from 3 patients 4 months or more posttransplantation, residual recipient cells were detected in the polymorphonuclear cell fraction but were present at a lower level or were undetectable in the lymphocyte fraction. These cells are of hematopoietic origin because they were detected at equivalent levels in whole blood and in B and T lymphocytes sorted with antibody-coated magnetic beads. They were not detected in samples collected more than 15 months posttransplantation for 6 of 7 patients. The persistence of residual recipient cells within 1 year posttransplantation is not restricted to male patients receiving a transplant from a female donor because they were also detected in 2 female patients using an allele-specific amplification method for the thyroid peroxydase gene that also has a high sensitivity (0.01%). Our results indicate that at least residual recipient myeloid progenitors and possibly totipotent hematopoietic stem cells may survive intensive pretransplant conditioning regimen and support a transient residual hematopoiesis of the host posttransplantation.

ALLOGENEIC BONE marrow transplantation (BMT) is now a widely used form of therapy for malignant hematologic diseases[1-3] and for some nonmalignant hematologic disorders such as Fanconi anemia[4] and severe aplastic anemia.[5] Monitoring hematopoietic chimerism early after allogeneic BMT allows us to document donor cells engraftment and to detect the eventual persistence of recipient cells. This enables early detection of graft rejection or of secondary marrow failure. After the early hematopoietic reconstitution phase, chimerism studies are also important to document the relationships between chimeric status and other parameters such as the conditioning regimen, graft-versus-host disease (GVHD), or minimal residual disease.[6] Assessment of the prognotic value of the persistence of residual recipient cells is clearly dependent on the sensitivity of the technique used to detect these cells. For some hematologic malignancies, polymerase chain reaction (PCR) amplification of translocated sequences allows the specific detection of residual cells from the malignant clone with a sensitivity of 10^{-5}.[7] PCR amplification of minisatellite sequences is a much more general method to study chimerism after BMT, but its sensitivity limit for the detection of residual recipient cells is of 0.1% to 1%.[5,8-11] In the case of male patients grafted with the marrow of a female donor, PCR amplification of the DYZ1 sequence, which is specific of the Y chromosome,[12] has been used to detect residual recipient cells.[5,13] The sensitivity of this method reaches 10^{-4}. Our own results [5,14] as well as those of others[15-19] have shown that, using PCR amplification of minisatellite sequences to assess chimeric status after BMT, the majority of the patients grafted with non–T-cell–depleted marrow from an HLA-identical sibling donor apparently presents an early complete donor cell engraftment. In contrast, we have previously found on a limited number of cases[5] that low levels (10^{-2} to 10^{-4}) of residual male cells were detected when chimerism study can be performed by amplification of the DYZ1 sequence. These cells were detected in patients whose clinical condition and hematologic parameters (including an analysis of chimerism by PCR amplification of minisatellite sequences) indicated an early complete engraftment. Similar results have been reported by others.[13,20,21] Therefore, we were interested to know whether this is a frequent situation and, in particular, whether these residual recipient cells could be detected in other situations than in male patients grafted with the marrow from a female donor, whether these cells are of hematopoietic origin, and whether they can be detected on the long term. We present evidence for positive answers to these three questions.

PATIENTS AND METHODS

Patients. Patients were aware of the objective of the study and samples were collected according to the rules established by the local ethical committee of the hospital where they were treated. They were grafted at Hôpital Saint-Louis (Paris, France) or at the Institut Gustave Roussy (Villejuif, France) with the marrow from an HLA-identical sibling donor. Patients grafted for severe aplastic anemia received a conditioning regimen consisting of the association of thoraco-abdominal irradiation (TAI; 6 Gy) and cyclophosphamide (CPM; 150 mg/kg).[22] Patients with Fanconi anemia received a modified protocol (TAI, 5 Gy; CPM, 20 mg/kg).[4] For other patients, the

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conditioning regimen consisted in total body irradiation (TBI; 10 Gy) delivered in a single dose or in 12 fractionated doses over 3 days and associated with chemotherapy (60 mg/kg CPM or 16 or 24 g/m^2 aracytine and 140 mg/m^2 melphalan). Two patients received TBI, etoposide (VP16; 60 mg/kg), and CPM (120 mg/kg) and 1 patient had aracytine in addition. Three patients were treated with CPM alone (200 mg/kg) or associated with busuflan (14 or 16 mg/kg).[23,24] None of the patient had ex vivo T-cell depletion of the donor bone marrow. GVHD prophylaxis regimen consisted in the association of cyclosporin A and methotrexate,[25] with the exception of Fanconi anemia patients, who received cyclosporin A alone.[14]

Sample collection and cell sorting. Ten to thirty milliliters of blood was collected from the donor and from the recipient before transplantation and at least once after BMT. Lymphocytes (Ly) and polymorphonuclear (PMN) cells were separated by Ficoll gradient centrifugation.[5] One volume of blood was carefully loaded on top of a 2-vol cushion of Ficoll (d = 1.077). After centrifugation (450*g* for 15 minutes), the Ly fraction was collected by pipetting from the top and the pellet containing PMN and red blood cells was resuspended in phosphate-buffered saline (PBS). Cells from both fractions were collected by centrifugation and washed in PBS. After such a separation, the Ly fraction consisted of 88% ± 3% mononuclear cells and 12% ± 2% PMN cells and the PMN fraction of 87% ± 4% PMN cells and 13% ± 4% mononuclear cells.

In some experiments, B and T Ly were sorted from the Ly fraction using magnetic beads (Dynabeads; Biosys; Dynal, Oslo, Norway) coated with pan-B anti-CD19 or pan-T anti-CD2 antibodies, respectively.[26] Ly from 10 to 20 mL of blood were washed and resuspended in 3 mL of PBS. From the values of blood cell concentrations in healthy adults,[27] we assumed this represented 2 to 4 × 10^7 cells with a B/T cell ratio of about 1/10. According to the supplier's recommendations, a ratio of about 3 beads per cell was used. For the equivalent of 10 mL of blood, 12 μL of anti-CD19–coated beads at 4 × 10^8 beads/mL was added and the mixture was incubated for 30 minutes at 4°C under gentle agitation. The tube was then placed for 2 minutes close to a magnet. The supernatant was removed and 120 μL of anti-CD2–coated beads at 4 × 10^8 beads/mL were added to it. Incubation and bead separation were performed as described above. Both bead pellets were washed by centrifugation in PBS. In the experiment aimed at assessing the efficiency of the method, 10^6 HEL cells, established from a male patient with erythroleukemia,[28] were mixed with 20 mL of heparinized blood from a healthy female individual. We had previously checked that HEL cells do not express the CD2 or CD19 antigens by incubating them with the corresponding beads followed by microscopic examination of the suspension and that they do carry the DYZ1 sequence of the Y chromosome (data not shown).

DNA extraction. Cell nuclei were prepared from whole blood and from the PMN cell fraction by a Triton X-100–based method.[29] DNA was extracted from these nuclei and from the Ly fraction by the rapid method of Miller et al[30] and directly from the bead pellets by sodium dodecyl sulfate (SDS)-proteinase K treatment and phenol/chloroforme extractions.[31]

PCR amplification and Southern blot analysis. Chimerism analysis by PCR amplification of minisatellite sequences 33.6.3 and MS51 was performed as described.[14] PCR amplification of DYZ1 was performed as described[5] with 30 cycles of amplification, unless otherwise specified. We have previously reported that, after 30 cycles of amplification, the intensity of the DYZ1 signal reflects the proportion of male cells in the range 1% to 0.01%. An interferon (IFN) γ sequence was amplified under the same conditions as the DYZ1 sequence, using primers TCTTTTCTTTCCCGATAGGT and CTGGGATGCTCTTCGACCTC located at positions 4582 and 4731, respectively, of the IFNγ gene.[32,33] The thyroid peroxydase (TPO) gene presents an A/C polymorphism in intron 9, 212 bp upstream of exon 10.[34] One of the PCR primers was chosen with its last nucleotide on the 3′ end corresponding to the polymorphic nucleotide (Fig 1). Two forms of this primer were synthesized: TPO-T (AGCCAAGCATGGTAGCGGGA) had an adenosine at the 3′ end and matched entirely the A allele; TPO-G (AGCCAAGCATGGTAGCGGGC) had a cytosine and matched the C allele. It was important to choose a gene with such a polymorphism and not an A/G polymorphism (which is more frequent) because, in this case, the mismatched basepair at the 3′ end is a GT pair that allows initiation of DNA synthesis by the polymerase to occur efficiently (unpublished observations). The second PCR primer (TPO-C: GCACAAGGCAAGGACAGCTC) was chosen downstream to yield a PCR product of 218 bp. The polymorphic TPO sequence was amplified with primers TPO-G and TPO-C or TPO-T and TPO-C in 100 μL of reaction mixture containing 1 μg of DNA, 1 μmol/L of each primer of the appropriated pair, 200 μmol/L of each of the four deoxyribonucleoside triphosphates, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris-HCl, pH 8.2, and 2.5 U of Taq DNA polymerase (Amersham, Amersham, UK). Thirty cycles of amplification were performed consisting of 1 minute at 94°C, 1 minute at 69°C, and 1 minute at 72°C. Amplification products were analyzed by electrophoresis on 1.5% agarose gels and Southern blotting.[5,35] The TPO probe was the amplified 218-bp fragment purified on agarose gel and labeled by random oligonucleotide priming (Amersham mega-prime labeling system) in the presence of [α^{32}P]-dCTP.

RESULTS

Ubiquitous detection of residual recipient cells in male patients receiving a graft from a female donor. Residual recipient cells were searched by PCR amplification of the Y-chromosome–specific DYZ1 sequence in 24 male patients receiving a graft of the marrow of a female donor. The results are presented in Table 1. All the patients were in continuous remission of their disease at the time of analysis. For all samples collected within 1 year post-BMT, male residual cells were detected (Fig 2A). All these patients were considered complete chimeras when analyzed by PCR amplification of minisatellite sequences (Fig 2B; see also Socié et al[5,14]), with the exception of patients SLA 228 (d41 sample) and SLA 241 (d28 and d100 samples), who were partial chimeras.[14] Chimerism of patients SLA 112 and SLA 210 could not be analyzed. This indicated that residual male cells were in the range of 1% to 0.01%, given the respective sensitivity limits of the two methods, thus confirming previous results.[5,13,20,21] These residual cells were detected in both the Ly and PMN cell fractions when such a separation was performed and, for samples of 13 of 15 patients, at comparable levels in both fractions according to the signal intensity (Fig 2A; see also Fig 5 for additional examples). For the d35 sample from patient AL 8905 but not for the subsequent d45 sample (shown in Fig 5B) and for the d35 (not shown) and d90 (shown in Fig 4) samples from patients AL 9112 and AL 9212, respectively, the signal of the Ly fraction was at least five time higher than that of the PMN cell fraction.

Hematopoietic origin of residual recipient cells. The low level of recipient cells detected in blood samples of male patients grafted with marrows from female donors raised the possibility that these cells might not be of hematopoietic origin but, for example, fibroblasts, adipocytes, epithelial, or endothelial cells contaminating the samples. The fact that these cells were detected in the Ly fraction after separation by Ficoll gradient centrifugation was an indication that these cells might indeed be of hematopoietic origin. To answer

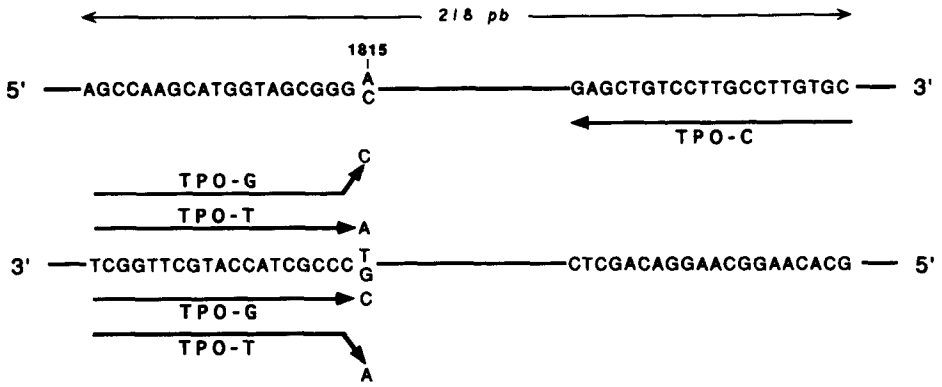

Fig 1. Allele-specific amplification of the human TPO gene. The two strands of the DNA around the polymorphic nucleotide at position 1815 of the gene[34] are schematized and the positions of the allele-specific primers (TPO-G and TPO-T) and of the common primer (TPO-C) are indicated.

this question, we used magnetic beads coated with anti-CD19 or anti-CD2 monoclonal antibodies to sort out B or T Ly, respectively. First, we showed that this method is able to efficiently leave out nonlymphocytic cells. HEL cells, established from a male patient with erythroleukemia,[28] were mixed with blood from a healthy female donor in the proportion of about 1 male HEL cell for 100 female leukocytes. This mixture was subjected to Ficoll gradient centrifugation to separate the Ly and PMN cell fractions. The Ly fraction was incubated, first with anti-CD19 and then with anti-CD2–coated beads to collect the B-cell and T-cell fractions, respectively. PCR amplification of the DYZ1 sequence was performed on the DNAs extracted from the five fractions (whole blood, Ly, PMN, and B and T cells) to determine the proportion of male HEL cells. PCR reactions were run for 15 cycles because we have previously shown[5] that, under these conditions, the intensity of the signal reflects the proportion of male cells in the sample in the range of 1% to 50%. The results are presented in Fig 3A. Most of the HEL cells are found in the Ly fraction. The signal is lower with the B- and T-cell fractions than with the unsorted Ly fraction, indicating that the sorting process has reduced the proportion of male HEL cells in these two Ly fractions. Serial dilution of the PCR product obtained with the unsorted Ly fraction indicates that the signal corresponding to this fraction is 30-fold more intense than that corresponding to the B- or T-cell fractions (Fig 3B). These fractions therefore contain only about 3% of the male HEL cells that were present in the Ly fraction, indicating that the majority (97%) of nonlymphoid male cells can be substracted from the Ly fraction by this method.

The method was then applied to a blood sample obtained 3 months after BMT from a male patient (AL 9212) grafted for a granulocytic sarcoma with the marrow of a female donor. Results are shown in Fig 4. Residual male cells were detected, as for other analyzed patients, mainly in the Ly fraction in this particular case. The intensity of the DYZ1 signal is the same for this fraction and for the sorted B- and T-cell fractions, showing that the proportion of male cells was not affected by sorting. This indicates that a large proportion of residual recipient cells present in this patient was of hematopoietic origin.

Long-term evolution of residual recipient cells. To address the question of whether residual recipient cells could be detected on the long term, we analyzed blood samples collected 8 months to 8 years after BMT from 11 of the male patients previously analyzed (Table 1). Residual male cells were detected in 4 patients whose samples were collected within 15 months after BMT (SLA 243, SLL 424, AL 8905, and AL 9003) and in 1 patient (AL 8908) analyzed 4.5 years after BMT. For the 6 other patients (SLA 112, SLA 210, SLA 235, SLA 241, SLL 219, and AL 9114), residual male cells could not be detected within the sensitivity of the method (Fig 5A). For 2 patients (SLA 241 and AL 9114), blood samples were obtained within months after BMT and on the long term. In the case of patient SLA 241, residual recipient cells were detected both on days 28 and 100 posttransplantation but could not be detected on days 511 and 910. For patient AL 9114, such cells were detected on day 39 but not on day 681 after BMT. Failure to detect residual male cells in most long-term samples was not caused by inefficient PCR with these DNA preparations because amplification of a control IFNγ sequence could be performed on the same samples (Fig 5A). We also noticed that, for 3 samples (d140, d365, and d450 from patients AL 9209, AL 8905, and AL 9003, respectively), residual male cells could be detected in the PMN cell fraction but were present at a lower (AL 8905 and AL 9209) or undetectable (AL 9003) level in the Ly fraction (Fig 5B).

A highly sensitive method to detect residual recipient cells irrespective of the donor and recipient sexes. For methodologic reasons, the results reported above were obtained with male patients grafted with marrows from female donors. To extend the validity of these findings to other situations (donor and recipient of the same sex; male donor and female recipient), we first tried, but failed, to improve the sensitivity of our method based on PCR amplification of minisatellite sequences (data not shown). We then decided to use the allele-specific amplification method described by others[36,37] applied to the TPO gene. The expected results were that, with DNAs of AA- or CC-type homozygote individuals, amplification will occur only with the TPO-T/TPO-C or TPO-G/TPO-C primers, respectively. In the case of an heterozygote individual, amplification should occur with both primer pairs. Screening of healthy individuals by this method yielded the three expected situations. The genotypes of putative AA-type and CC-type homozygote individuals were confirmed by digestion with *Acy* I restriction enzyme, of which one of the sites contains the polymorphic nucleotide.[34]

To assess the sensitivity of the method, DNA from an

Table 1. Residual Recipient Cells in Male Patients After BMT

UPN	Age (yr)	Pathology	Conditioning Regimen	aGVHD (grade)	cGVHD	Detection of Residual Cells		
						WB	Ly	PMN
AL 8905	4	ALL	STBI-CPM	3	−	d17(+)		
						d35(+)	d35(+)	d35(+)
							d45(+)	d45(+)
							d365(±)	d365(+)
AL 9003	6	ALL	FTBI-ARAC-MPH	1	−	d28(+)		
							d90(+)	d90(+)
							d450(−)	d450(±)
AL 9114	29	ALL	STBI-CPM	0	−		d39(+)	d39(+)
						d681(+)		
AL 9204	24	ALL	STBI-CPM-VP16	0	−		d44(+)	d44(+)
AL 9206	9	ALL	FTBI-ARAC-MPH	3	+		d28(+)	d28(+)
AL 9209	11	ALL	FTBI-CPM	3	+	d30(+)		
							d140(±)	d140(+)
SLL 219	7	ALL	STBI-ARAC-CPM-VP16	0	−		d2281(−)	d2281(−)
AL 9112	43	AML	FTBI-CPM	2	NE		d35(+)	d35(+)
AL 9205	41	AML	FTBI-CPM	3	NE		d29(+)	d29(+)
AL 8908	29	CMLa	FTBI-ARAC-MPH	0	+	d30(+)		
							d39(+)	d39(+)
							d60(+)	d60(+)
						d255(+)		
						d1684(+)		
SLL 412	56	CML	STBI-CPM	0	−	d22(+)		
							d60(+)	d60(+)
SLL 424	17	CML	BSF-CPM	1	−	d283(±)		
SLA 112	27	SAA	TAI-CPM	3	+		d3231(−)	d3231(−)
SLA 196*	14	SAA	TAI-CPM	0	+		d30(+)	d30(+)
SLA 210	35	SAA	TAI-CPM	1	+		d1562(−)	d1562(−)
SLA 213*	14	SAA	TAI-CPM	2	+	d18(+)		
						d31(+)		
							d38(+)	d38(+)
						d60(+)		
SLA 216*	31	SAA	TAI-CPM	1	+	d21(+)		
						d40(+)		
						d100(+)		
SLA 228*	7	FA	TAI-CPM	2	+	d41(+)		
						d64(+)		
						d111(+)		
								d111(+)
								d130(+)
SLA 241*	7	FA	TAI-CPM	2	−		d28(+)	d28(+)
							d100(+)	d100(+)
							d511(−)	d511(−)
						d910(−)		
SLA 243*	6	FA	TAI-CPM	2	+		d60(+)	d60(+)
						d402(+)		
SLA 268*	4	FA	TAI-CPM	2	−	d75(+)		
SLA 235	7	DC	CPM	2	+	d579(−)		
SLC 10	11	TAR	BSF-CPM	3	+		d116(+)	d116(+)
AL 9212	20	GS	STBI-CPM-VP16	1	+		d90(+)	d90(+)

Abbreviations: UPN, unique patient number; CML, chronic myelogenous leukemia; CMLa, CML in acute phase; ALL, acute lymphoblastic leukemia; AML, acute myelogeneous leukemia; FA, Fanconi anemia; DC, dyskeratosis congenita; SAA, severe acquired aplastic anemia; GS, granulocytic sarcoma; TAR, thrombocytopenia with absent radius; STBI, single-dose TBI; FTBI, fractionated TBI; ARAC, aracytine; MPH, melphalan; BSF, busulfan; aGVHD, acute GVHD; cGVHD, chronic GVHD; NE, not evaluable; d, day posttransplantation; WB, whole blood; ±, weak or barely detectable signal.

* Chimerism analysis by PCR amplification of minisatellite sequences has been reported for these patients.[5,14]

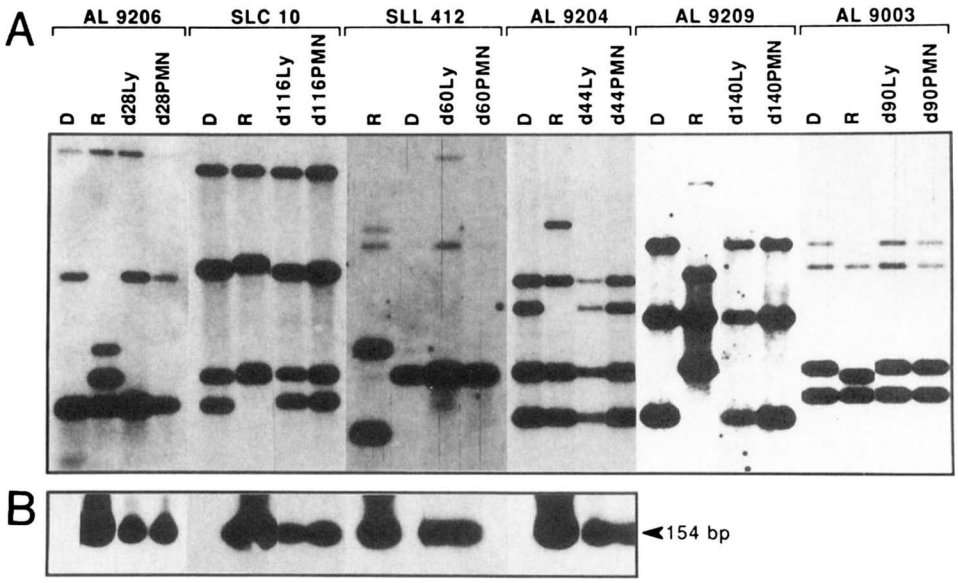

Fig 2. Detection of male residual recipient cells in grafted patients with an apparently complete donor-type hematopoiesis. (A) Chimerism was analyzed on the indicated samples obtained from the indicated patients by PCR amplification of minisatellite sequences 33.6.3 and MS51. Ly and PMN cell fractions were separated by Ficoll gradient centrifugation. R, recipient; D, donor; d, day postgraft. (B) The same samples as in (A) were analyzed by amplification of the DYZ1 sequence. Arrowhead points to the 154-bp amplified fragment. DYZ1 analysis of samples from patients AL 9209 and AL 9003 is shown in Fig 5B.

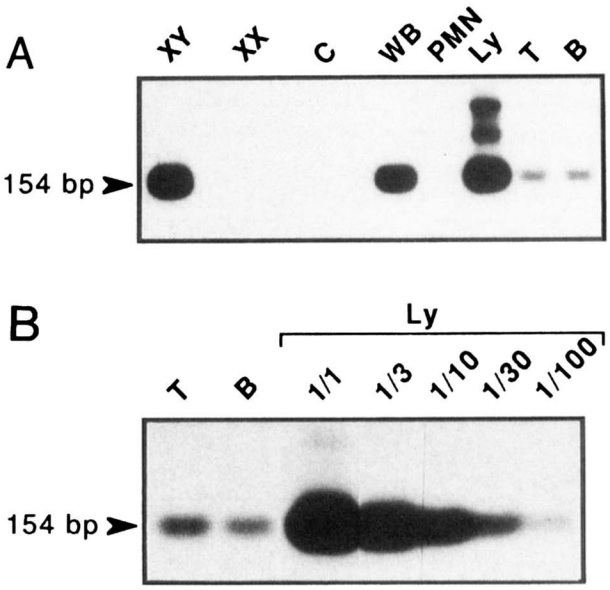

Fig 3. Efficiency of nonlymphocytic male cell removal by sorting with antibody-coated magnetic beads. (A) Erythroleukemia (HEL) male cells were mixed with female blood (1 HEL cell for 100 female leukocytes) and the mixture (WB) was subjected to Ficoll gradient centrifugation to separate the Ly and PMN cell fractions. B and T lymphocytes were sorted from the Ly fraction using magnetic beads coated with anti-CD19 or anti-CD2 monoclonal antibodies, respectively. DNA extracted from these various fractions was analyzed by PCR amplification (15 cycles) of the Y-chromosome–specific DYZ1 sequence. Arrowhead points to the 154-bp amplified fragment. XY and XX, DNAs from male and female individuals; C, control amplification without added DNA. (B) The PCR product obtained with the Ly fraction in (A) was serially diluted, as indicated, for quantitative comparison with the products of the B- and T-cell fractions.

AA-type homozygote individual was serially diluted in the DNA of a CC-type homozygote individual and PCR amplification was performed with the TPO-T/TPO-C primer pair. Southern blot analysis of the products (Fig 6A) showed that, under these conditions, the AA-type DNA can be detected down to the 10^{-4} dilution. This indicated that the method has a sensitivity comparable to that based on the amplification of the DYZ1 sequence.

The method was then applied to blood samples collected from patients after BMT. Donor/recipient pairs were first screened (data not shown) to find those whose genotypes fulfilled the needed criteria (see above). Among 43 pairs analyzed, 3 met the criteria: patient AL 9101 was a female heterozygote and the donor was a female AA-type homozygote; patient SLL 455 was a female CC-type homozygote and the donor was a male AA-type homozygote; and patient SLA 228 was male heterozygote and the donor was a female AA-type homozygote. This last patient has been previously analyzed by amplification of the DYZ1 sequence. Patients AL 9101 and SLL 455 received BMT for acute lymphocytic leukemia or chronic myelogenous leukemia, respectively. Patient SLL 455 suffered from acute (grade 1) and chronic GVHD. They could not be evaluated in patient AL 9101 because of early death (d51). Analyzed samples were collected on days 25, 65, and 64 and 130 post-BMT for patients AL 9101, SLL 455, and SLA 228, respectively. In none of these patients could residual recipient cells be detected when posttransplantation circulating blood cells were analyzed by PCR amplification of minisatellite sequences 33.6.3 and MS51 (Fig 7). However, when the same samples were analyzed by the TPO allele-specific amplification method (Fig 6B), residual recipient cells were detected in all 3 cases and in the PMN cell fraction in the cases of patients AL 9101 and SLA 228.

DISCUSSION

We have found that residual host cells can be detected in peripheral blood samples of all analyzed male recipients within 1 year post-BMT when they are search for by a highly

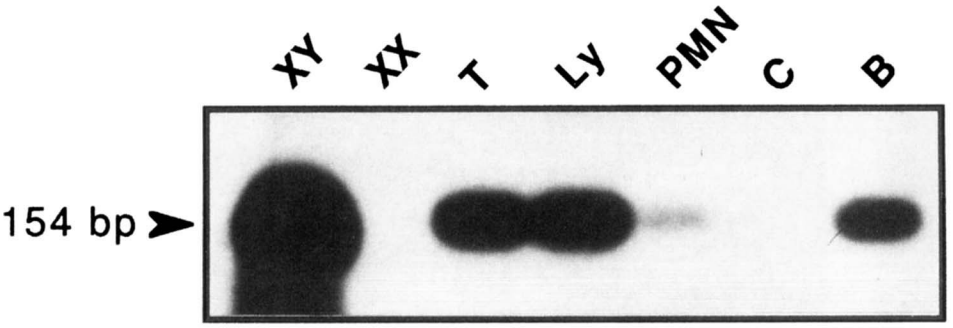

Fig 4. Hematopoietic origin of male residual recipient cells. A blood sample obtained from patient AL 9212 on day 90 post-transplantation was treated and DNAs were analyzed as described in Fig 3A. Abbreviations are the same.

sensitive technique, ie, PCR amplification of a Y-chomosome–specific sequence (0.01% sensitivity). This result is in agreement with those reported by others.[16,20,21,38] The same patients appeared to have a complete donor-type hematopoiesis when analyzed with the less-sensitive technique (0.1% to 1%) of PCR amplification of minisatellite sequences, indicating that these residual recipient cells are in the range of 0.01% to 1% of peripheral white blood cells. The persistence of residual recipient cells within 1 year post-BMT is not restricted to male patients grafted with the marrow from a female donor because we also detected them in 2 female patients (with a male or female donor) using an allele-specific amplification method that also has a high sensitivity. Lo et al[39] have recently reported similar results.

Residual recipient cells are of hematopoietic origin and not contaminating cells of other origin because (1) they were detected in the PMN cell and Ly fractions after Ficoll gradient centrifugation and, in 13 of 15 of the cases analyzed, at comparable levels in both fractions; (2) they were detected at equivalent levels in whole blood and sorted B and T Ly of a patient; and (3) they were no longer detected in most long-term samples.

Residual recipient cells were not detected in samples collected more than 15 months post-BMT in 6 of 7 cases. For 2 patients, both short- and long-term samples could be analyzed. Residual male cells were detected in the former but not in the later. Altogether, our results strongly suggest that low level of hematopoietic residual recipient cells are present in the vast majority, if not all, of non–T-cell–depleted transplant recipients within 1 year post-BMT and that their level decreased below detection threshold after about 15 months in the majority of the patients. This phenomenon is apparently unrelated to the pathology treated by BMT. In one case, residual recipient cells were detected more than 4 years post-BMT. Viard et al[20] and Stuppia et al[21] have also reported few cases of long-term persistence of residual recipient cells in male patients grafted with a female donor. An analysis of serially collected samples in several patients is now needed to clearly establish the kinetic of this population. No correlation could be found between GVHD and the detection of residual recipient cells. However, this point should be re-evaluated when data on the kinetic and the level of residual recipient cells are available for a cohort of patients. In addition, many more patients will have to be studied serially to determine whether the inability to detect host cells late post-transplant predicts for long-term remission or cure.

Partial hematopoietic chimerism, stable on the long term,

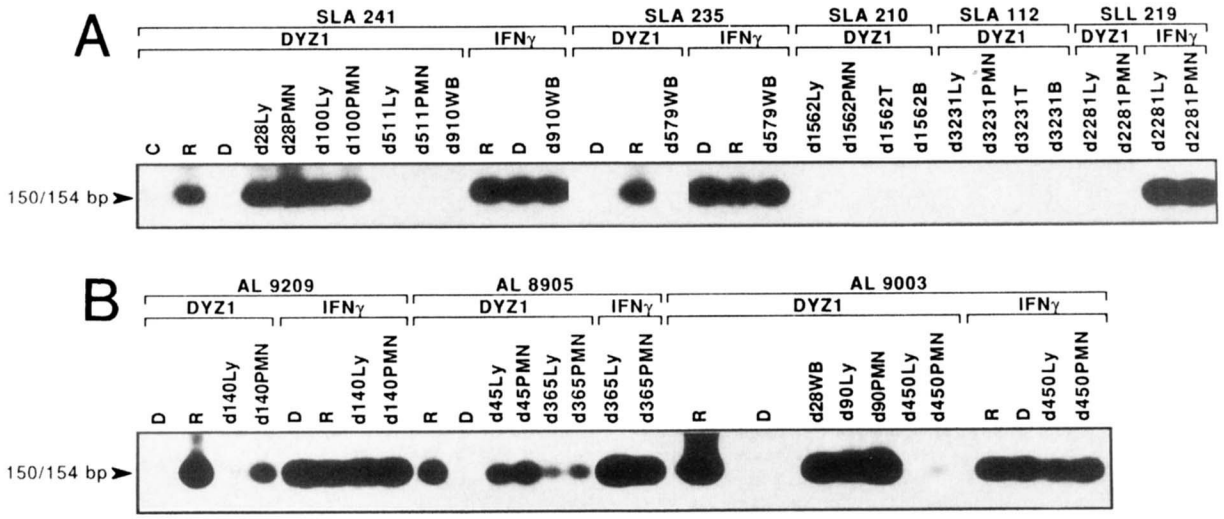

Fig 5. Long-term evolution of male residual recipient cells. The indicated samples obtained from the indicated patients were analyzed by PCR amplification of the DYZ1 (154-bp amplified fragment) or IFNγ (150-bp amplified fragment) sequences. For patients SLA 210 and SLA 112, B- and T-cell fractions were obtained by antibody-coated magnetic bead sorting. Abbreviations are as in Fig 2.

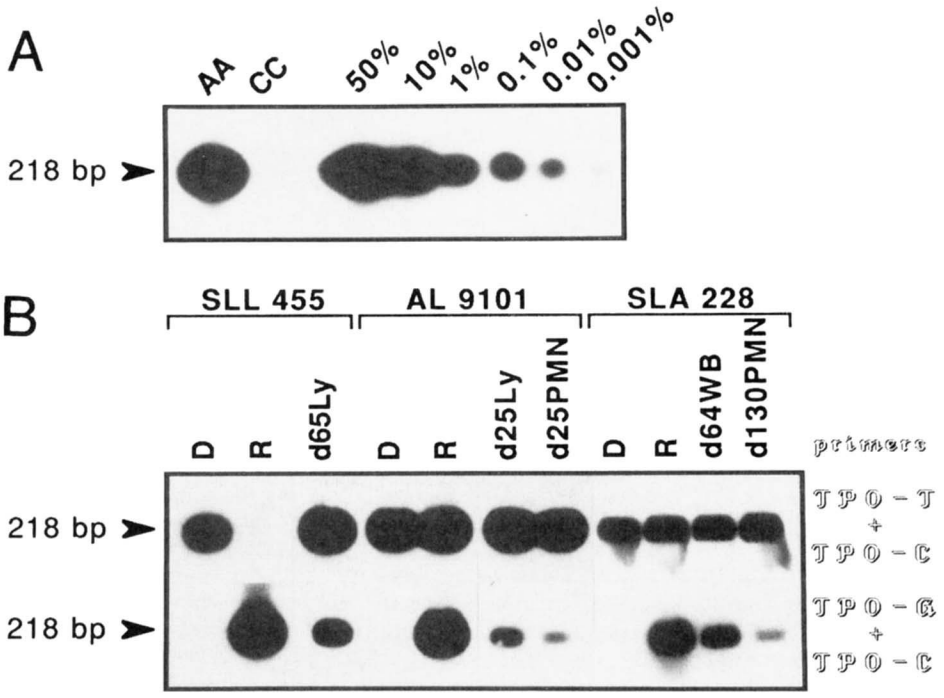

Fig 6. Detection of residual recipient cells by allele-specific amplification. (A) DNA from an AA-type homozygote individual was serially diluted as indicated in the DNA of CC-type homozygote individual and PCR amplification of the TPO sequence was performed with the TPO-T and TPO-C primers. Arrowhead points to 218-bp amplified fragment. (B) The indicated samples obtained from the indicated patients were analyzed by PCR amplification of the TPO sequence with the TPO-T and TPO-C or TPO-G and TPO-C primers, as indicated. Abbreviations are as in Fig 2.

has been reported for patients grafted with T-cell–depleted marrows.[10,16] It is known that the proportion of partial chimerism is much higher in such patients than in patients grafted with nondepleted marrows.[10,16,40-44] None of our patients received T-cell–depleted marrow. Some isolated cases of stable partial chimerism have been reported for patients grafted with non–T-cell–depleted marrows.[45,46] These results seem at variance with ours because, in these cases, recipient cells were abundant enough to be detected by moderately sensitive techniques. This could be due to differences in the transplantation procedure, the conditioning regimen, or the underlying disease.

The persistence of residual recipient cells within 1 year post-BMT and their disappearance thereafter raises the question of the level of commitment of their progenitors, if any. In a normal individual, a fraction of the Ly does survive for several years[27] and it has been shown that viable and potentially functional T Ly may survive intensive pretransplant conditioning regimens and are able to proliferate after incubation with interleukin-2.[47] In contrast, PMN cells are known

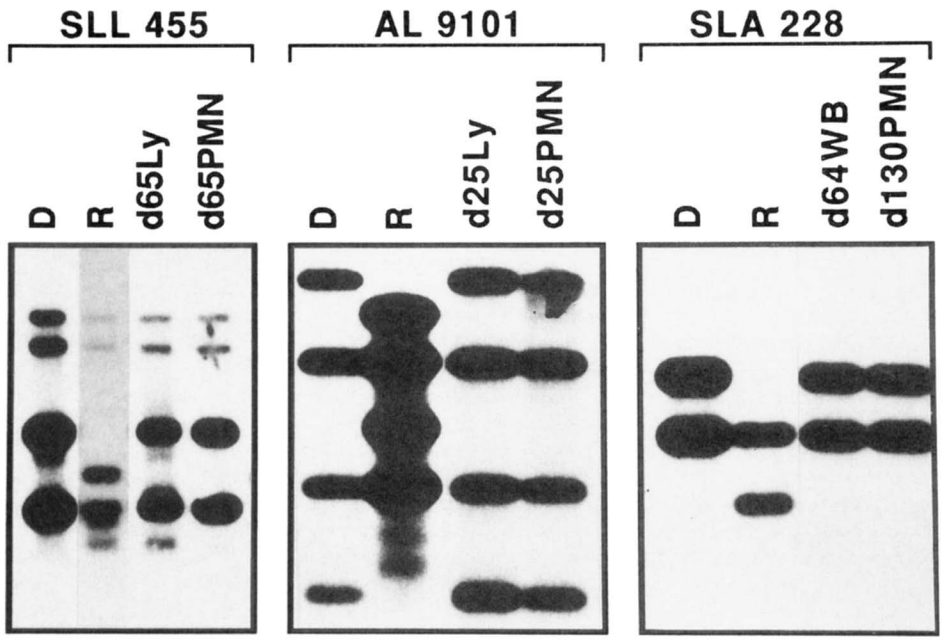

Fig 7. Chimerism status of the 3 patients studied by the allele-specific amplification method. Chimerism was analyzed on the indicated samples obtained from the indicated patients (the same as in Fig 6) by PCR amplification of minisatellite sequences 33.6.3 and MS51. Abbreviations are as in Fig 2.

to have a very short life in peripheral blood (2 to 3 days).[27] In our study, residual recipient cells were detected at comparable levels in PMN cell and Ly fractions at least up to 3 months post-BMT. We cannot distinguish with certainty between long-life recipient lymphocytes and progeny of lymphoid progenitors. In addition, it is possible that some of the residual cells detected in the PMN cell fraction are contaminating mononuclear cells (and vice versa) because, under our conditions, the Ly and PMN cell fractions were 85% to 90% pure and residual cells were detected by highly sensitive PCR techniques. However, it seems unlikely to us that these cells are only long-lived blood cells that are finally eliminated, because, in that case, they should be systematically overrepresented in the Ly fraction. In fact, this has been observed only in a few patients. Therefore, our results suggest that recipient hematopoietic stem cells may persist at the totipotent level and can give rise to both lymphoid and myeloid lineages. In three patients analyzed on days 140, 365, and 450 post-BMT, residual recipient cells were detected in the PMN cell fraction but were present at a lower or undetectable level in the Ly fraction. Because these fractions were 85% to 90% pure, this indicates the persistence of, at least, residual recipient myeloid progenitors post-BMT in these patients. In any case, these progenitors have probably a limited self-renewal capacity because this residual hematopoiesis of the host is apparently not maintained on the long term in most patients. In other respects, we cannot rule out that residual recipient hematopoietic stem cells with self-renewal capacity do persist in some cases and are eliminated through immune mechanisms because some GVHD reaction against recipient hematopoiesis may exist.[48] Some investigators have studied chimerism on sorted blood cell populations by PCR amplification of minisatellite sequences and have identified partial chimerism in various hematopoietic cell lineages.[49-51] Chimerism studies on sorted populations with highly sensitive PCR techniques are now required to further document this phenomenon and to better understand its importance.

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