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CHIMERISM ANALYSIS IN LONG-TERM SURVIVOR PATIENTS AFTER BONE MARROW TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

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ABSTRACT

Background and Objective. Allogeneic bone marrow transplantation (BMT) is the most common treatment for young patients with severe aplastic anemia (SAA). Late graft failure represents one of the possible unfavorable outcomes in this setting. Mixed chimerism might represent a risk factor for late graft failure. We examined this relationship by studying chimerism in long-term survivor SAA patients after allogeneic BMT.

Methods. We analyzed long-term hematopoietic chimerism in 15 patients who received BMTs for SAA: 9 with an irradiation-based conditioning regimen and 6 with ATG. We used a PCR method targeting VNTR loci. Sensitivity of the technique ranged between 0.5 and 1.5%.

Results. All patients conditioned with radiation-based schemes showed complete donor chimerism. Conversely, out of six patients who received cyclophosphamide and ATG as a conditioning regimen, only one of them had late graft failure (day +168). In this patient, durable mixed chimera status was first detected two months after BMT.

Interpretation and Conclusions. Our results suggest that in long-term survivors of SAA after BMT there is almost always complete donor chimerism in both irradiated and ATG-conditioned recipients. Mixed chimerism might predict graft failure in these patients.

Key words: chimerism, severe aplastic anemia, bone marrow transplantation, PCR

Allogeneic bone marrow transplantation (BMT) is now the most common type of therapy for young patients with severe aplastic anemia (SAA) who have a compatible sibling donor. Although long-term survival rates are between 60% and 80%,[1] a number of problems still have to be overcome. Late graft failure, that is the recurrence of pancytopenia and marrow hypoplasia after a period of satisfactory engraftment, is a serious complication in this setting.[2] This condition seems to be associated with the emergence of recipient cells, a kind of mixed chimerism (MC). However, the influence of MC on graft rejection is still unclear as MC *per se* in not always an indicator of impending graft rejection; in fact, long-term stable MC following bone marrow transplantation (BMT) for SAA has been documented.[3,4]

Although chimerism has been studied extensively in SAA patients receiving BMTs,[5-10] long-term studies of chimerism in this setting using the sensitive polymerase chain reaction technique (PCR) and targeting VNTR loci are scanty.[11] Moreover, studies published on the relationship between MC and graft failure have involved a patient population conditioned with cyclophosphamide (CY) which was or was not associated with irradiation, but included a small number of long-term survivors conditioned with antithymocyte globulin (ATG). For these reasons, we analyzed the long-term engraftment of 15 patients transplanted for SAA (9 with an irradiation-based conditioning regimen and 6 with ATG) using a previously reported minisatellite PCR method to determine hematological chimerism. Our objective was to assess if long-term stable MC without graft failure is possible in both groups of patients.

Materials and Methods

Patients

The study consisted of 15 patients who had received transplants for acquired SAA who had survived for more than one year after BMT. The median age at the time of BMT was 21 years (range 10-36) and median time from diagnosis to BMT was 34 days (range 21-180). All of the patients received marrow from a genotypically HLA-identical sibling donor. The characteristics of the recipients are summarized in Table 1. Nine patients were conditioned with CY at 200 mg/kg and total lymphoid

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Table 1. Characteristics of the patients.

UPN	Age (yrs)	Sex R/D	Time DX-BMT (days)	Conditioning regimen	GVHD prophylaxis	MNC 10^8/kg	acute GVHD	chronic GVHD	day Gran >500	day Plt >50,000	Graft failure	Outcome (months)
60	10	M/M	34	CY+TLI	CsA	3.06	I	no	14	18	no	live+120
104	12	F/M	33	CY+TLI	CsA	4.2	0	no	11	21	no	live+115
138	21	M/M	25	CY+TLI	CsA	2.7	I	no	16	18	no	live+93
142	14	M/M	65	CY+TLI	CsA	1.9	I	no	11	17	no	live+91
146	18	M/M	21	CY+TLI	CsA	2.81	I	limited	22	26	no	live+88
186	31	F/M	47	CY+TLI	CsA	2.73	0	no	10	24	no	live+59
206	15	F/F	118	CY+TLI	CsA+MTX	3.2	0	no	12	13	no	live+45
218	23	F/M	25	CY+TLI	CsA+MTX	2.84	0	no	19	24	no	live+37
227	36	M/F	87	CY+ATG	CsA	2.4	0	no	14	90	no	live+30
239	19	M/F	30	CY+ATG	CsA+MTX	2.39	I	no	17	30	no	live+28
241	35	M/M	150	CY+ATG	CsA+MTX	2.38	0	no	18	31	no	live+28
242	28	M/M	180	CY+ATG	CsA+MTX	3.05	I	no	13	20	no	live+26
256	19	M/M	38	CY+ATG	CsA+MTX	2.6	IV	no	12	232	no	live+25
260	27	F/F	23	CY+ATG	CsA+MTX	2.9	0	no	11	20	yes	live+22
277	27	F/F	33	CY+TLI	CsA+MTX	3.45	II	no	16	28	no	live+13

irradiation (TLI) (7.5 Gy). Six patients received CY (200 mg/kg) associated with ATG for three doses of 30 mg/kg each. Graft-versus-host disease (GVHD) prophylaxis consisted of one drug in 7 patients, specifically cyclosporine A (CsA) at the standard dose. Eight recipients received CsA and a short treatment with methotrexate (MTX). A mean dose of 2.8 10^8 nucleated cells per kilogram was infused.

Isolation of genomic DNA

High molecular weight DNA was extracted from donor and recipient peripheral blood mononuclear cells before BMT, using a *salting out* procedure.[12]

After BMT, DNA was extracted from patient peripheral blood in order to determine chimerism status. When a recipient sample was not available prior to BMT, constitutional DNA isolated from hair roots was used according to Gill *et al*.[13]

PCR analysis of chimerism

For PCR amplification, we used specific primers designed to flank the repeated units of the following human minisatellite regions: D1S80, 33.6, 33.1, YNZ-22, APO-B, lg3 and DXS52. The sequence of the primers and conditions for each reaction have been described elsewhere in earlier reports.[14-17] VNTR loci are defined as being useful if analysis of recipient and donor samples prior to BMT showed a unique band for the recipient and another unique band for the donor, or if they showed a unique band for the recipient only. Patients who exhibited complete donor hematopoiesis with all markers tested at all times were defined as donor or complete chimeras (CC). Patients who exhibited mixed populations of donor and host cells on more than one occasion with at least two different markers after day +45 or when the recipient was transfusion-independent were considered MC.

To assess the sensitivity of PCR in the evaluation of chimerism, DNA from several donors and recipients were mixed in the following proportions of host DNA: 25%, 10%, 5%, 3.5%, 2%, 1.5%, 1%, 0.5% and 0.1%. Each DNA mixture was subjected to VNTR analysis. Sensitivity was considered at the lowest proportion of host DNA in the mixtures in which host DNA could be detected. The sensitivity of the different VNTRs ranged between 0.5 and 1.5% (data not shown).

Results

Clinical results of BMT

All patients were long-term survivors with a median follow-up period of 37 months (range 13-120 months). No differences between irradiation and ATG groups in the number of cells infused, day of engraftment, age and gender were observed. The incidence of acute GVHD (grade II-IV) was 13.3% (2/5). Only one of the 15 patients developed mild, localized and transient chronic GVHD.

One patient in the ATG group (UPN 260) relapsed at day +168 post-BMT. She refused a second trasplant and was treated with ATG and CsA with total response.

Chimerism results

A total of 46 samples were examined 1 to 120 months after BMT (median 13 months) with a median of 3 samples per patient (range 1 to 6). The results obtained for each sample are despicted in Figure 1. Fourteen patients showed complete

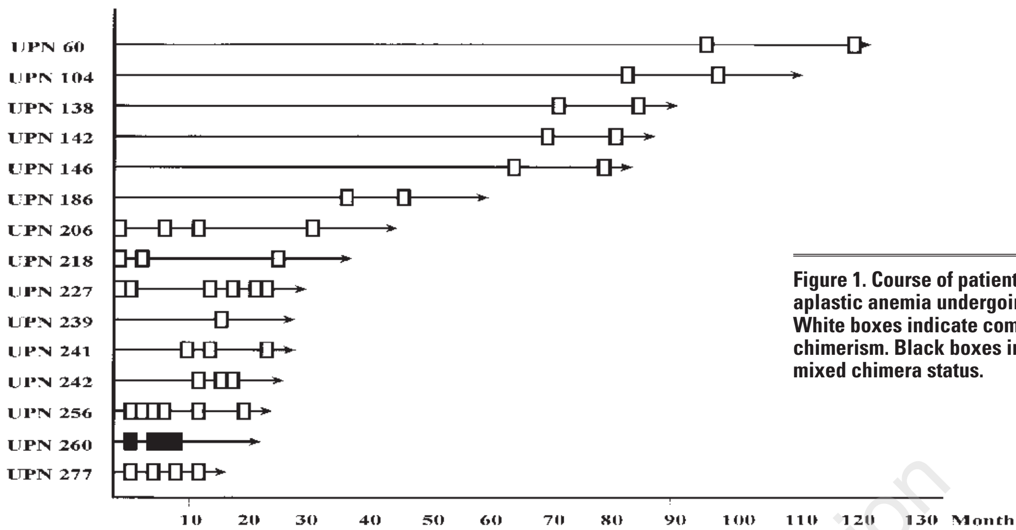

Figure 1. Course of patient with severe aplastic anemia undergoing BMT. White boxes indicate complete donor chimerism. Black boxes indicate mixed chimera status.

donor chimera status throughout their post-BMT period. In UPN, 260 residual host cells were persistently detected from day +58, and hematological relapse was evident 5.5 months after BMT.

Discussion

Allogeneic BMT remains an important method of treatment for SAA, but long-term survival is still limited because of graft failure and GVHD. Various factors such as infections, drugs and GVHD have been implicated in the development of late graft failure in marrow transplant recipients. Of these factors, persistence of residual host cells is the most serious because it is associated with a high risk of graft rejection.[5]

Determination of cell origin after allogeneic BMT has been performed by using karyotyping, red cell antigen analysis, restriction fragment length polymorphism analysis and fluorescent *in situ* hybridization.[18-20] However, only a recent report used PCR for chimerism studies in long-term survivors of non-Fanconi aplastic anemia after BMT.[11] Through the latter method, a complete chimera status was observed in all our radiotherapy-conditioned patients. These results support those of Keable *et al.*,[7] that used minisatellite probes to analyze the long-term engraftment of 21 radiotherapy-conditioned patients: they found complete donor reconstitution in all patients using DNA fingerprints.

The presence of host-type cells is more frequent in patients conditioned with CY alone,[10] and the inclusion of radiation in the preparative regimen is the main variable associated with engraftment stability in SAA.[7] A previous study from Seattle[5] showed that patients with SAA conditioned with CY had a lower incidence of acute GVHD and an increased incidence of graft rejection if they developed transient MC. Host cells persisted for a maximum of 395 days at which time the graft had either been rejected or hematopoiesis had converted them to 100% donor cells. Occasionally, patients have been presented that show evidence of stable MC following BMT for SAA up to one year post-BMT.[3,4] However, the definitive outcome of these patients is currently unknown because MC can also be seen during prolonged periods prior to graft failure in some patients conditioned with CY only.[11]

Conditioning regimens containing irradiation reduce the risk of graft rejection. However, the use of irradiation may adversely affect other transplant outcomes, causing patients to run an increased risk of development of secondary malignancy.[21,22] It is possible that a more aggressive form of immunosuppression prior to graft, such as ATG, might result in a higher number of successful sustained engraftment and overcome long-term problems.[23] However, analysis of chimerism using PCR has not been previously reported in this group of patients. Our results with the CY/ATG conditioning regimen match those of limited-field irradiation-based programs regarding the type of chimerism at a molecular level. Five patients showed complete donor chimerism, and most importantly, MC predicted impending graft failure in the only patient who had a relapse.

Our results in long-term survivors of SAA after BMT show that complete chimerism is the rule in both irradiated and ATG-conditioned patients. Studies on large groups of cases conditioned with ATC by analyzing VNTR loci using PCR could provide important therapeutic developments.

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