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Results of Minimally Toxic Nonmyeloablative Transplantation in Patients with Sickle Cell Anemia and β-Thalassemia

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ABSTRACT

We describe previously transfused patients with sickle cell disease (n = 6) and thalassemia (n = 1) who received nonmyeloablative hematopoietic stem cell transplantation (HCT) to induce stable (full or partial) donor engraftment. Patients were 3 to 20 years (median, 9 years) old. All 7 received pretransplantation fludarabine and 200 cGy of total body irradiation; 2 patients also received horse antithymocyte globulin. Patients received bone marrow (n = 6) or peripheral blood stem cells (n = 1) from HLA-identical siblings, followed by a combination of mycophenolate mofetil and cyclosporine or tacrolimus for postgrafting immunosuppression. After nonmyeloablative HCT, absolute neutrophil counts were $<0.5 \times 10^9$/L and $<0.2 \times 10^9$/L for a median of 5 days (range, 0-13 days) and 0 days (range 0-13 days), respectively. A median of 0 (range, 0-9) platelet transfusions were administered. No grade IV nonhematologic toxicities were observed. One patient experienced grade II acute graft-versus-host disease. Two months after transplantation, 6 of 7 patients had evidence of donor chimerism (range, 25%-85%). Independent of red blood cell transfusions, these 6 patients initially had increased total hemoglobin and hemoglobin A concentrations and a reduction of reticulocytosis and transfusion requirements. There were no complications attributable to sickle cell disease during the interval of transient mixed chimerism. However, after posttransplantation immunosuppression was tapered, there was loss of the donor graft, and all patients experienced autologous hematopoietic recovery and disease recurrence. One patient did not engraft. The duration of transient mixed chimerism ranged from 97 to 441 days after transplantation in patients 4 and 6, respectively, and persisted until immunosuppressive drugs were discontinued after transplantation. In summary, the nonmyeloablative HCT regimens described here produced minimal toxicity and resulted in transient donor engraftment in 6 of 7 patients with hemoglobinopathies. Although complications from the underlying hemoglobinopathies did not occur during the period of mixed chimerism, these results suggest that stable (full or partial) donor engraftment after nonmyeloablative HCT is more difficult to achieve among immunocompetent pediatric patients with hemoglobinopathies than among adults with hematologic malignancies, perhaps in part because recipients may have been sensitized to minor histocompatibility antigens of their donor by preceding blood transfusions.

KEY WORDS

Sickle cell disease • Thalassemia • Nonmyeloablative bone marrow transplantation

INTRODUCTION

Despite advances in supportive treatment and preventive measures, sickle cell disease (SCD) remains a chronic disease with attendant risks of significant morbidity and early mortality. As a result, there has been considerable interest in defining the role of hematopoietic stem cell transplantation (HCT) for SCD as a curative alternative to therapies such as supportive care, regular red blood cell (RBC) transfusions, and hydroxyurea. The potential for a successful outcome after transplantation was illustrated in a recent review that described 175 patients with SCD who received

myeloablative HCT from HLA-identical sibling donors. One hundred fifty-nine (91%) patients survived, and 143 (82%) survived free of SCD [1]. However, efforts to expand the application of HCT for SCD have been tempered in part by the risk of significant toxicity that accompanies this intensive procedure. Among the 175 patients treated by HCT, graft rejection and recurrent disease was observed in 16 (9%) and 17 (9%) patients who died of transplant-related causes. In a prospective series of 59 patients, 4 patients died from transplant-related causes that included intracranial hemorrhage (n = 1) and graft-versus-host disease (GVHD; n = 3) [2]. There were other reversible acute toxicities, such as seizures and other neurologic complications, in 13 patients. Long-term complications included infertility and gonadal failure, particularly among females, and a potential for secondary malignancies. Although these initial transplantation studies show that most very high-risk patients with SCD would benefit from this therapy, concerns about mortality and long-term effects have generated attitudes among families and clinicians that justifiably curb their willingness to consider transplantation more broadly [3-5]. Thus, currently this therapy is generally reserved for those who have experienced significant complications of SCD. To consider transplantation for SCD before irreversible vital organ damage has occurred and thereby expand its availability, it is likely that improvement in the risk-benefit ratio must first be achieved.

Nonmyeloablative HCT may represent an effective strategy to reduce the toxicity of transplantation, as was shown in canine transplantation models [6] and in older debilitated patients with hematologic malignancies [7-10]. There is considerable evidence from preclinical models [11,12] and from clinical transplantation trials that mixed hematopoietic chimerism, a possible outcome of nonmyeloablative HCT, is associated with clinical benefit for SCD [13] and thalassemia [14]. These observations have supported the development of pilot clinical trials to evaluate nonmyeloablative HCT for these disorders. Here we illustrate the challenge of establishing full or partial donor chimerism that is stable in immunocompetent patients with hematological disorders by describing a series of 7 patients with SCD and β-thalassemia major who underwent HLA-identical sibling HCT after nonmyeloablative conditioning.

METHODS

Patients

Between December 1999 and November 2001, 6 patients with SCD and 1 with β-thalassemia major received nonmyeloablative HCT from HLA-matched sibling donors at the Johns Hopkins Hospital, Stanford University Medical Center, Children's Hospital and Research Center at Oakland, and the Fred Hutchinson Cancer Research Center. This case series describes all patients who were treated according to different institutional review board–approved clinical protocols that used similar pretransplantation conditioning and posttransplantation immunosuppression. There were 3 independent clinical trials conducted at Fred Hutchinson Cancer Research Center/Oakland, Stanford, and Johns Hopkins.

Treatment Regimen

After written, informed consent was obtained, all 7 patients were enrolled in prospective clinical investigations that were reviewed and approved by each center's institutional review board. Patients were informed about conventional myeloablative HCT as an alternative therapy and either were ineligible or chose instead to participate in an institutional review board–approved clinical trial of nonmyeloablative HCT.

Pretransplantation conditioning regimens are summarized in Table 1. Patients 1, 2, and 3 were treated at Johns Hopkins Hospital, and all 3 patients received gonadal and head shielding during total body irradiation. Patients 4 and 5 were treated at Stanford University Medical Center and also received horse antithymocyte globulin 10 mg/kg/d on each of 4 consecutive days, commencing 4 days before nonmyeloablative HCT. Patients 4 and 5 received granulocyte-macrophage colony-stimulating factor after nonmyeloablative HCT. Patient 5 received an infusion of umbilical cord blood from the same donor approximately 8 months after the initial transplantation, without any preinfusion preparation, in an unsuccessful attempt to rescue donor hematopoiesis. Patient 6 was treated at the Children's Hospital and Research Center at Oakland, and patient 7 was treated at the Fred Hutchinson Cancer Research Center. Both received 3 days of fludarabine.

Six of the 7 patients received HLA-identical sibling bone marrow, and patient 4 received HLA-identical, mobilized peripheral blood stem cells (PBSC). Other donor characteristics are summarized in Table 1.

For postgrafting immunosuppression, patients 1, 2, and 3 received a combination of tacrolimus (FK-506) and mycophenolate mofetil (MMF). FK-506 dosing commenced 1 day before nonmyeloablative HCT and was administered until approximately 200 days after nonmyeloablative HCT, when it was tapered and discontinued over 30 days. MMF dosing was commenced on the day of transplantation and was administered until 2 weeks after FK-506 was discontinued, when it was tapered and discontinued over 30 days. Patients 4 and 5 received cyclosporine (CsA), which was commenced 1 day before nonmyeloablative HCT,

Table 1. *Patient and Donor Characteristics and Transplantation Procedure*

Patient No.*	Age (y)/Sex	Disease	Major Complications before Transplantation	RBC txn	Treatment Regimen†	Stem Cell Source	Donor			Graft Composition		
							Host/ Donor ABO	Hb Type	Sex	TNC per kilogram ($\times 10^8$)	$CD34^+$ per kilogram ($\times 10^6$)	$CD3^+$ per kilogram ($\times 10^7$)
1	7/M	Hb SD^{LA}	Infarct on MRI, neurocognitive changes	2.5 y	Flu × 5, 200 cGy, FK-506/ MMF	Unprocessed BM	Compatible	AS	F	5.5	11.3	3.9
2	9/M	Hb SS	Right middle and anterior cerebral artery strokes while on transfusion for TIA	5 y	Flu × 5, 200 cGy, FK-506/ MMF	Unprocessed BM	Compatible	AA	M	5.1	9.6	5.3
3	20/F	Hb SS	Frequent VOE, ataxia, and dysarthria from idiopathic cerebritis	1 y‡	Flu × 5, 200 cGy, FK-506/ MMF	Plasma-depleted BM	A^-/O^+	AS	F	1.21	1.11	4.1
4	11/F	β^0	Cholelithiasis	11 y	Flu × 5, 200 cGy, CsA/MMF, ATG	PBSC	Compatible	AA	M	17	9.7	31
5	3/M	Hb SS	ACS × 1, splenic sequestration, splenectomy	16 u	Flu × 5, 200 cGy, CsA/MMF, ATG	Unprocessed BM	Compatible	AS	F	5.8	8.3	3
6	5/F	Hb SS	Hospitalization for VOE × 2	<5 u	Flu × 3, 200 cGy, CsA/MMF	Unprocessed BM	Compatible	AA	M	4.6	N/A	N/A
7	12/M	Hb SS	Frequent VOE, ACS × 1, aplastic crisis, seizures, ischemic changes on MRI, NI MRA, NI TCD	6 u	Flu × 3, 200 cGy, CsA/MMF	RBC-depleted BM	O^+/A^+	AS	F	1.5	4.8	9.2

β° indicates β thalassemia major; MRI, magnetic resonance imaging; TIA, transient ischemic attack; VOE, painful vaso-occlusive episode; ACS, acute chest syndrome; NI, normal; MRA, magnetic resonance arteriography; TCD, transcranial Doppler; RBC, red blood cell transfusions; Flu, fludarabine; FK-506, tacrolimus; MMF, mycophenolate mofetil; CsA, cyclosporine; BM, bone marrow; TNC, total nucleated cells; N/A, not available; txn, transfusion exposures/duration; y, years; u, units.

*Patients 1, 2, and 3 were treated at Johns Hopkins, patients 4 and 5 at Stanford, patient 6 at Oakland, and patient 7 at the Fred Hutchison Cancer Research Center.

†Fludarabine dose: 30 $mg/m^2/d$; ATG dose, 10 mg/kg/d × 4 days.

‡Patient 3 also received hydroxyurea for 1 year.

was administered until 60 days after nonmyeloablative HCT, and was tapered thereafter. MMF was administered for 30 days after nonmyeloablative HCT, when it was discontinued without a taper. Patients 6 and 7 received CsA for 120 days and MMF for 35 days after nonmyeloablative HCT, respectively. In general, CsA and FK-506 were administered intravenously and converted to oral administration after therapeutic blood concentrations were achieved. Levels were monitored on a weekly basis to maintain CsA levels between 150 and 300 ng/mL and FK-506 levels between 5 and 15 ng/mL. Once a steady-state plasma level was established, therapeutic monitoring was performed at the discretion of the attending physician. MMF was administered orally without therapeutic monitoring.

Before nonmyeloablative HCT, all SCD patients received RBC transfusions to achieve a fraction of hemoglobin (Hb) S $\leq$30% in the blood. Thereafter, RBC transfusions were administered by the treating physicians in consultation with the patient's hematologist on an individual basis. All patients who were seronegative for cytomegalovirus received cytomegalovirus antibody–screened negative or leukocyte-poor blood products. Patients also received prophylactic antibiotics after transplantation, according to institutional standards. To prevent neurologic complications after transplantation, the following guidelines were used: anticonvulsant prophylaxis was initiated with CsA/FK-506 dosing and continued for 6 months after transplantation (or until CsA was discontinued); strict control of hypertension; prompt repletion of magnesium deficiency; and maintenance of platelet counts $>50 \times 10^9$/L.

Donor Chimerism Analysis

Chimerism in the bone marrow was measured by fluorescence in situ hybridization with an X and Y chromosome–specific enumerator probe [15] in patients 1 and 7 and by restriction fragment length polymorphism [16] analysis in patients 2 and 3, according to previously published methods. Patients 1, 2, and 3 also had chimerism in the blood measured by polymerase chain reaction–based analysis of germline microsatellite short tandem repeats (STRs), exploiting DNA differences between the recipient and donor (ABI Profiler; Applied Biosystems, Foster City, CA). Patients 4 and 5 had chimerism measured by using minisatellite variable number tandem repeat genetic markers to distinguish donor and host cells. Donor chimerism was measured in patient 6 with informative STR markers or amelogenin (X and Y) markers amplified by polymerase chain reaction by using the Geneprint Fluorescent STR Quadriplex assay (Promega, Inc., Madison, WI). Patients 4 through 7 also had donor chimerism measured in specific lineages after cell sorting [16], according to previously described methods.

Assessments of Disease Status

All patients were monitored regularly with complete blood counts, absolute neutrophil counts (ANC), reticulocyte counts, and quantification of Hb types by high-performance liquid chromatography. The attending physicians also carefully monitored and treated patients for manifestations of SCD or thalassemia, as dictated by good clinical practice.

Statistical Analysis

Correlation coefficients between paired bone marrow and peripheral blood chimerism assessments were derived from calculations of Pearson product moments by using SigmaPlot statistical software (SPSS Inc., Chicago, IL).

RESULTS

Patient Characteristics

Seven patients with SCD (n = 6) or β-thalassemia major (n = 1) from 4 transplantation centers underwent nonmyeloablative HCT with bone marrow (n = 6) or PBSC (n = 1) from HLA-identical sibling donors. Patients ranged in age from 3 to 20 years (median, 9 years; mean, 9.6 years). Prior complications in patients with SCD included cerebral infarction in 3 patients, frequent painful episodes in 2 patients, and acute chest syndrome in 1. Four patients received regular RBC transfusions with a duration ranging from 1 to 10 years before nonmyeloablative HCT, 1 patient had 16 RBC transfusions, and 2 patients had 6 or fewer pre–nonmyeloablative HCT transfusion exposures. There was 1 major (patient O^+, donor A^+) and 1 minor (patient A^-, donor O^+) ABO antigen incompatibility among the 7 donor-host pairs. Four of 7 sibling donors had sickle cell trait. Table 1 summarizes patient and donor characteristics.

Complications after Nonmyeloablative HCT

Nonmyeloablative pretransplantation conditioning and posttransplantation immunosuppression were associated with only low-grade toxicity, and all 7 patients were alive a median of 28 months (range, 16-40 months) after nonmyeloablative HCT. Patients initially were hospitalized during the procedure for a median of 16 days (range, 0-52 days; mean, 15.7 days), and 2 patients never required hospitalization. Four of 7 patients required subsequent hospitalizations. The median number of total hospital days was also 16 (range, 0-81; mean, 24.6). There were no life-threatening infections. Two patients had transient grade III mental status changes. Patient 3 had visual and audi-

tory hallucinations while receiving opioid analgesics, phenytoin, and FK-506. FK-506 levels were in the therapeutic range, but phenytoin was increased. These symptoms resolved after the phenytoin dosing was reduced. Patient 7 developed ataxia and dysarthria when plasma phenytoin levels were supratherapeutic; these symptoms also resolved after phenytoin was discontinued. Neither patient had evidence of new abnormalities by neurologic examination or by magnetic resonance imaging.

Patient 7 developed grade II acute GVHD after nonmyeloablative HCT. He had a complete response after corticosteroid treatment. There was no evidence of chronic GVHD in any of the 7 patients.

Hematologic Recovery

Donor graft composition for all patients is detailed in Table 1. Notably, patient 3 received a decreased $CD34^+$ dose; however, there were no apparent donor clinical characteristics to account for the poor yield. The tempo of hematologic recovery after nonmyeloablative HCT is depicted in Figure 1. Among all 7 patients, the median number of days that the ANC remained $<0.5 \times 10^9$/L was 5 (range, 0-13 days; mean, 4.4 days), and for a level of $<0.2 \times 10^9$/L, the median time was 0 days (range, 0-13 days; mean, 1.9 days). As depicted in Figure 1A, patients 1, 2 and 5 experienced a nadir in ANC between 10 and 14 days after transplantation. Patient 6 had a nadir in ANC later, at approximately 3 weeks, and patients 3 and 7 had a nadir at approximately 4 to 5 weeks. Patient 3, who received a total nucleated donor cell dose of 1.21×10^8/kg, did not recover with donor cells after nonmyeloablative HCT and thus experienced a prolonged period of neutropenia that accompanied autologous recovery.

In general, thrombocytopenia after nonmyeloablative HCT was mild and had a short duration. Four of 7 patients had platelet measurements that were never $<50 \times 10^9$/L (Figure 1B). Most patients had full recovery by 4 to 7 weeks after nonmyeloablative HCT. Patient 3, who received a low donor cell dose, had prolonged neutropenia and thrombocytopenia that required platelet transfusion support between 20 and 40 days after nonmyeloablative HCT. However, among all 7 patients, the median number of platelet transfusions administered was 0 (range, 0-9; mean, 1.6). The number of platelet and red cell transfusions given after transplantation for each patient, and other observations made after transplantation, are detailed in Table 2.

Engraftment and Mixed Chimerism after Nonmyeloablative HCT

The fraction of donor cells in mononuclear cell populations in the blood and marrow for patients who had engraftment of donor cells ranged from 25% to 85% approximately 2 months after nonmyeloablative HCT (Figure 2A). Three of 4 patients who had $CD3^+$ T-lymphocyte donor chimerism measured 28 days after nonmyeloablative HCT showed levels that were <10%, whereas a higher fraction of 45% was observed in patient 4, who received PBSC in lieu of bone marrow (Figure 2B). However, by 100 days after nonmyeloablative HCT, the fraction of donor T lymphocytes in the blood decreased to <20% in all 4 patients.

Engraftment of donor cells resulting in donor-host mixed hematopoietic chimerism was detected by day 30 in 6 of 7 patients (Figure 2A). Mixed chimerism was associated with a reduction in the Hb S and Hb D fractions and the reticulocyte count and, in some, increased Hb concentrations. The effect of donor engraftment on hematologic parameters was most pronounced in patients 1, 2, and 6, one of whom had donor engraftment that persisted for up to 14 months

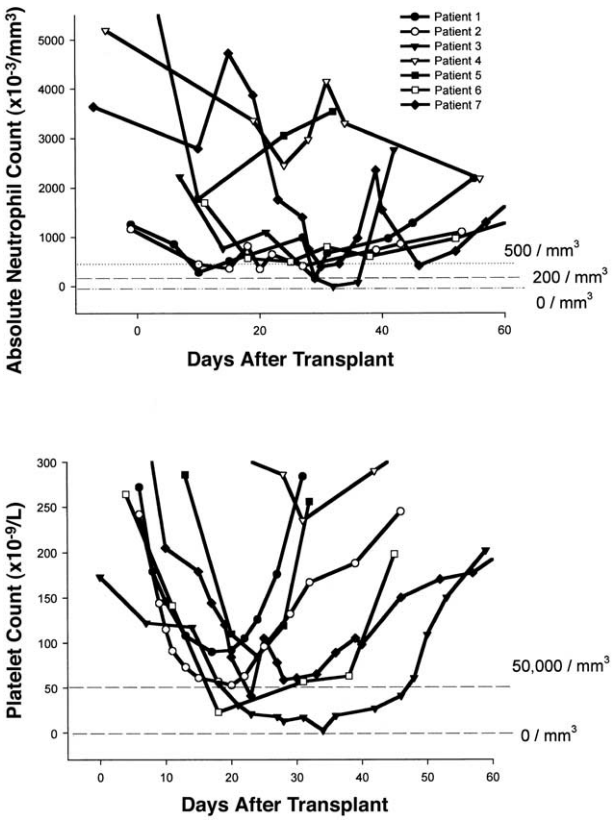

Figure 1. Hematologic recovery after nonmyeloablative hematopoietic stem cell transplantation (HCT). A, Neutrophil levels after nonmyeloablative HCT are depicted. The duration of having an ANC $<0.5 \times 10^9$/L was <5 days for most patients. In patient 3, the ANC decreased to 0 after a low dose of $CD34^+$ cells in the marrow inoculum; donor engraftment was never shown. B, Serial platelet count determinations after nonmyeloablative HCT are depicted. After nonmyeloablative HCT, the platelet count was never $<50 \times 10^9$/L in 4 of 7 patients. Patient 3 experienced profound thrombocytopenia and received multiple platelet transfusions from 20 to 40 days after nonmyeloablative HCT.

Table 2. *Posttransplantation Outcomes*

Patient No.	Initial Hospital Days	Total Hospital Days	Days with ANC $<0.5 \times 10^9$/L	Days with ANC $<0.2 \times 10^9$/L	Platelet Transfusions*	Red Blood Cell Transfusions during Study	MMF/CsA† Stop Day
1	10	10	5	0	0	None after day 18	224/209
2	16	16	8	0	0	None after day 25	217/197
3	52	81	13	13	9	6	110/110
4	0	6	0	0	0	None after day 7	31/67
5	0	1	0	0	0	Days 10 and 217	28/60
6	7	16	0	0	1	Days 10, 21, 38, and 98	34/120
7	25	42	5	0	1	6; 4 before day 150, became alloimmunized	35/120

*Platelet transfusions were given to maintain counts $\geq 50 \times 10^9$/L.

†Some patients received tacrolimus rather than CsA (see Methods).

after nonmyeloablative HCT. In these patients, a small fraction of donor cells in the marrow was sufficient for a clinically significant decrease in the patients' endogenous Hb fraction, a reduction in reticulocytosis, and a modest increase in the Hb concentration (Figure 3). During the period when patient 1 had 30% to 50% donor chimerism in marrow, the endogenous Hb fraction (Hb D^{LA}) was less than 3%, indicating <6% host RBC in the blood. Patient 2 had an endogenous Hb fraction (Hb S) that was <13%, with 25% donor chimerism, and patient 6, who had peripheral blood donor chimerism of 4% to 7% that persisted for >1 year after nonmyeloablative HCT, had an endogenous Hb fraction (Hb S) that varied from 20% to 50%. Patient 4, who had thalassemia major and received a single RBC transfusion in the first week after nonmyeloablative HCT, maintained an Hb concentration that ranged from 9 to 10.6 g/dL during the 3 months after nonmyeloablative HCT when donor chimerism was detectable. Of interest, although Hb concentrations increased and the fraction of abnormal Hb was low, most patients had mild to moderate anemia with relatively low reticulocyte counts during CsA and MMF administration.

During the period of mixed donor-host hematopoietic chimerism, none of the 5 SCD patients who had evidence of donor engraftment experienced sickle-related clinical events, such as pain, acute chest syndrome, or stroke. After posttransplantation immunosuppression was withdrawn, each of these 6 patients experienced nonfatal graft rejection. The duration of transient mixed chimerism ranged from 97 to 441 days after transplantation in patients 4 and 6, respectively. A decline in donor chimerism was temporally associated with discontinuation of immunosuppression in all patients, corresponding to approximately day 220 in patients 1 and 2, day 60 in patients 4 and 5, and day 120 in patients 6 and 7 (Figures 2A, 3A, and 3B). The level of donor chimerism in patient 6 stabilized after treatment with MMF was reinstituted on day 161 but declined after the dose of MMF was decreased again on day 182 (Figure 3C). After the loss of donor hematopoiesis, all patients reverted to their pre–nonmyeloablative HCT hemoglobinopathy condition with autologous hematopoiesis.

Patient 4 subsequently received a second HCT from the same sibling donor after myeloablative conditioning with total body irradiation (12 Gy), cyclophosphamide (120 mg/kg), and antithymocyte globulin. Twenty-two months after myeloablative transplantation, she has stable full donor chimerism.

Patient 7 had a major ABO incompatibility with significant isohemagglutinin titers before nonmyeloablative HCT (anti-A immunoglobulin M, 1:2035; immunoglobulin G, 1:512). Although the anti-A titers declined after nonmyeloablative HCT, there was no evidence of circulating donor RBC until anti-A titers became undetectable. Six months after nonmyeloablative HCT, this patient developed an acute exacerbation of anemia with circulating anti-c, anti-E, anti-FyA, and anti-JkB antibodies that coincided with loss of the donor graft. Increasing anti-A titers were noted, and the anti-c antibody was donor specific.

DISCUSSION

This report describes our initial experience of allogeneic transplantation for SCD and thalassemia major after preparation with minimally toxic regimens, administered with the aim of reducing the toxicity of allogeneic transplantation while retaining its efficacy by achieving full or partial donor engraftment. The rationale for pursuing this approach in light of the very good results of conventional allogeneic transplantation for sickle cell anemia has to do with (1) concerns among families and their physicians about the risks of transplantation and how these significantly limit the application of this curative therapy and (2) the observation that patients who develop full or partial donor chimerism are protected from the clinical complications of SCD [11-14]. Were a less-toxic transplantation regimen successful, HCT could more readily be used before the onset of irreversible complications rather than to prevent additional complica-

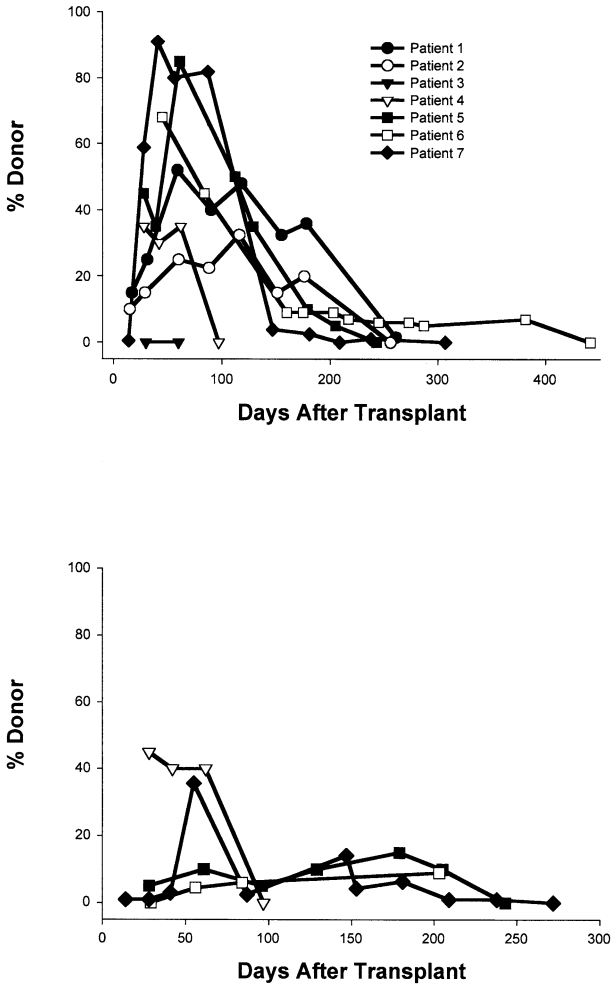

Figure 2. Engraftment of donor cells after transplantation. A, The percentages of donor engraftment measured in unfractionated blood and bone marrow mononuclear cell preparations are plotted as a function of time in days after transplantation. Chimerism fraction results from blood and bone marrow samples were closely correlated ($r = 0.85$). In cases in which blood and marrow measurements were performed on the same day, results from marrow are shown. There was a decrease in donor chimerism 200 days after nonmyeloablative HCT in patients 1 and 2, 60 days after nonmyeloablative HCT in patients 4 and 5, and 120 days after nonmyeloablative HCT in patients 6 and 7. B, The level of donor T-cell chimerism ($CD3^+$ cells) in peripheral blood after nonmyeloablative HCT for patients 4 through 7 is depicted. These low levels contrast with a higher level of donor chimerism among unfractionated mononuclear cells (patients 5 through 7; panel A).

tions in already severely affected patients. The preliminary experience presented here confirmed that, transiently, even partial donor engraftment was sufficient in most patients to suppress clinical expression of the underlying hemoglobinopathy. In addition, the degree of neutropenia and thrombocytopenia was modest and tended to confirm a reduction in toxicity and other life-threatening complications after nonmyeloablative HCT compared with conventional myeloablative allografting. Unfortunately, donor engraftment was not sustained in any of the 7 patients, and all had full autologous hematopoietic recovery after the tapering of postgrafting immunosuppression.

These observations seem to underscore the problem of graft rejection and disease recurrence that occurred in approximately 10% of patients after conventional myeloablative transplantation for SCD and thalassemia, a 5- to 10-fold increased incidence compared with that in patients who undergo myeloablative transplantation for hematologic malignancies. The increase may be due in part to sensitization to minor histocompatibility antigens from prior blood transfusions. Our results also contrast sharply with similarly minimally toxic regimens in adults with hematologic malignancies, 80% of whom had stable donor engraftment [10]. Thus, there are important differences between pediatric patients with hemoglobinopathies and adults with hematologic malignancies which hinder current efforts to induce donor-specific tolerance. Like those with chronic myelogenous leukemia and myelodysplastic syndrome, patients with hemoglobinopathies are unlikely to have been immunosuppressed by exposure to intensive chemotherapy before nonmyeloablative HCT, possibly predisposing them to a higher rate of graft rejection [10]. Unfortunately, whereas augmenting the pretransplantation regimen by the addition of fludarabine produced stable engraftment among 28 consecutive chronic myelogenous leukemia patients [17], it did not produce stable engraftment in our patients with SCD and β-thalassemia major.

It is possible that donor T-cell engraftment and the accompanying allogeneic effect was not sufficient in these patients to establish stable donor chimerism. Of interest, none of the 4 patients evaluated in our series had donor T-cell chimerism that exceeded 50% after transplantation, and only 1 patient developed GVHD, which was grade II. In addition, the median donor marrow chimerism sampled at 56 days after transplantation was 45% (range, 0%-85%), compared with 95% (range, 2%-100%) among adults with hematologic malignancies [10]. This suggests that the level of host myelosuppression and immunosuppression was generally lower in our series compared with patients who received intensive chemotherapy treatment for hematologic malignancies before nonmyeloablative HCT. It is not known to what extent inherent immunologic differences between children and adults could explain the greater barrier to achieving stable donor engraftment in our series. Patient 6 illustrates that prolonging posttransplantation immune suppression is not likely to result in stable donor chimerism. In this patient, reinstitution of MMF reversed the trend toward waning donor chimerism, but only while MMF therapy was continued. The use of higher doses of hematopoietic stem cells by the mobilization of allogeneic peripheral blood mononuclear

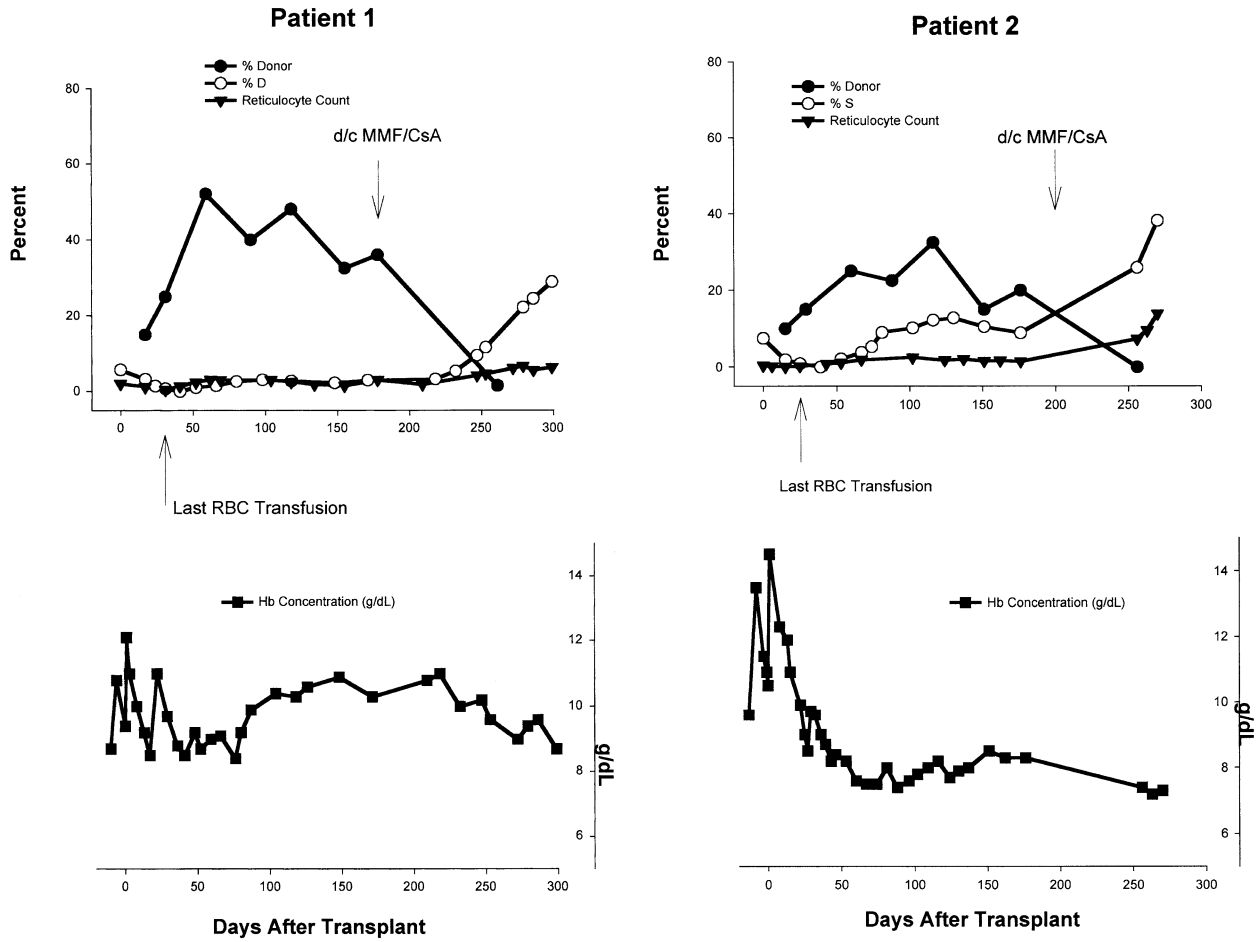

Figure 3. Improvement in hematologic parameters after donor engraftment. The fraction of sickle Hb (S and D) is depicted in relation to serial measurements of Hb concentration, reticulocyte count, and donor chimerism after transplantation. Patients 2 and 6 had Hb SS and donors with Hb AA. Patient 1 has the genotype Hb SD^{LA} and a donor with Hb AS; thus, the percentage of Hb DLA is plotted. Because the fraction of Hb D was equivalent to the fraction of Hb S in sickle erythrocytes before transplantation in this patient, the total fraction of abnormal Hb in recipient cells should be twice what is plotted. A suppression of reticulocytosis was observed among patients who had mixed chimerism, even when the Hb concentration was less than normal. MMF was restarted in patient 6 on day 161 when donor chimerism was noted to have decreased. After several stable donor chimerism measurements, the dose of MMF was decreased on day 182 because of myelosuppression. It was increased on day 224 when donor chimerism was again noted to have decreased, but donor engraftment was lost. d/c indicates discontinued.

cells in lieu of bone marrow is a strategy that might be used to promote donor engraftment; however, this was not sufficient in patient 4.

Driven by a desire to mitigate the risk of rejection, several recent reports of stable engraftment after transplantation for SCD used intermediate-intensity regimens that relied on immunosuppression and myelosuppression to prevent a host-versus-graft reaction and promote engraftment [18-20]. However, also in contrast to the minimally toxic regimens described here, the intermediate-intensity regimens were associated with prolonged hospitalization and were accompanied by an increased risk of regimen-related toxicity. Patients who received intermediate-intensity regimens benefited from augmented pregrafting immunosuppression that facilitated engraftment of donor cells, but severe acute and chronic GVHD occurred that was fatal in some, particularly among those who received PBSC allografts and developed full donor chimerism [20]. Thus, the problem of transplant-related mortality was not eliminated by the intermediate-intensity regimens, especially among older recipients.

In summary, the barrier to stable full or partial donor engraftment after nonmyeloablative HCT in pediatric patients with hemoglobinopathies seems more difficult to overcome than in adults with hematologic malignancies. The minimal toxicity observed in this case series and the lack of symptoms attributable to SCD that accompanied transient low-level donor chimerism provide a rationale to pursue this approach of reducing the toxicity of transplantation and thus make it more widely available to patients with hemoglobinopathies. Follow-up studies are under way to determine whether it is possible to modulate the intensity of this regimen without significantly

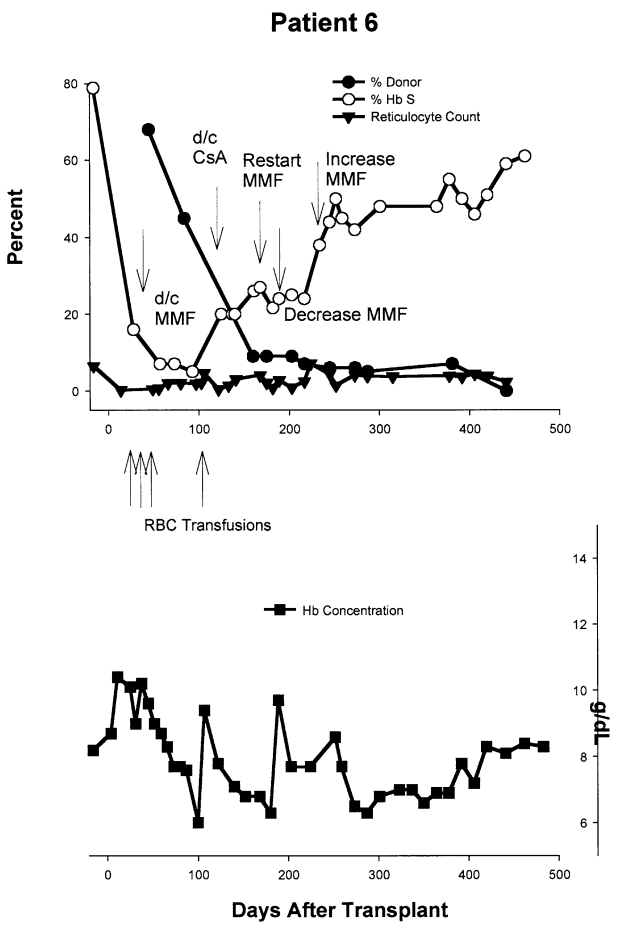

Figure 3 Continued.

increasing its toxicity and establish stable donor engraftment in most recipients. By incrementally increasing the intensity of transplant conditioning, it may be possible to identify an immunosuppressive regimen that is sufficient for engraftment yet minimizes the risk of toxicity. Strategies that might also reduce the risk of GVHD include the use of T cell–depleting antibodies and posttransplantation cyclophosphamide, and these additions to the regimens tested here might represent suitable modifications. Finding the minimally toxic regimen for achieving the goal of stable donor engraftment may transform the application of allogeneic transplantation for SCD.

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