Brief report

Cotransplantation of ex vivo–expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation

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Haploidentical hematopoietic stem-cell transplantation (HSCT) is associated with an increased risk of graft failure. Adult bone marrow–derived mesenchymal stromal cells (MSCs) have been shown to support in vivo normal hematopoiesis and to display potent immune suppressive effects. We cotransplanted donor MSCs in 14 children undergoing transplantation of HLA-disparate CD34$^+$ cells from a relative. While we observed a graft failure rate of 15% in 47 historic controls, all patients given MSCs showed sustained hematopoietic engraftment without any adverse reaction. In particular, children given MSCs did not experience more infections compared with controls. These data suggest that MSCs, possibly thanks to their potent immunosuppressive effect on alloreactive host T lymphocytes escaping the preparative regimen, reduce the risk of graft failure in haploidentical HSC transplant recipients. (Blood. 2007;110:2764-2767)

Introduction

T-cell–depleted hematopoietic stem-cell transplantation (HSCT) from an HLA-haploidentical relative is a feasible option for children needing an allograft and lacking an HLA-compatible donor.[1] However, both primary (defined as lack of hematologic recovery or absence of donor chimerism) and secondary (defined as loss of donor chimerism after initial engraftment)[2] graft failure, mainly mediated by host alloreactive T cells escaping the preparative regimen, have been reported in up to 15% to 18% of children given mismatched HSC transplants,[3] despite the infusion of large numbers of hematopoietic stem cells.[4] Recipients of T-cell–depleted HSC transplants from an HLA-disparate relative are also exposed to an increased risk of life-threatening infections, especially of viral origin, due to the delay in reconstitution of adaptive immunity.[1,3]

Bone marrow (BM) contains pluripotent mesenchymal stromal cells (MSCs), which form cartilage, fat, bone, and muscle.[5] MSCs have been shown to modulate the function of T lymphocytes,[6] including that of alloreactive T cells involved in graft-versus-host disease (GvHD) pathophysiology.[7] In adult patients undergoing transplantation from an HLA-identical sibling, MSC infusion was shown to be safe and possibly to accelerate hematopoietic recovery, as well as to reduce the incidence of both acute and chronic GvHD.[8] However, it is still unknown whether cotransplantation of MSCs in haploidentical HSC transplant recipients can reduce graft failure.

We carried out a phase 1/2 pilot study of cotransplantation of BM-derived, ex vivo–expanded MSCs of donor origin in children undergoing transplantation of granulocyte colony stimulating factor (G-CSF)–mobilized, CD34-selected progenitor cells from an HLA-disparate relative. The procedure was intended to reduce graft failure rate compared with historic controls.

Patients, materials, and methods

Patients

Children with hematologic malignancies or nonmalignant disorders, including primary immune deficiencies, lacking an HLA-matched donor were enrolled in the study by the 2 participating centers (Leiden University Medical Center and Fondazione IRCCS Policlinico San Matteo). Institutional Review Board approval was provided by the 2 participating centers. Parents or legal guardians of patients provided written informed consent for inclusion in the study. Written informed consent in accordance with the Declaration of Helsinki was also obtained from donors by an independent physician trained to explain risks associated with mesenchymal and hematopoietic stem cell donation.

Preparation of MSCs

Approximately 5 weeks before HSCT, mononuclear cells were isolated from 50 to 70 mL donor BM by density gradient centrifugation on Ficoll. These were plated in noncoated 75- to 175-cm^2 polystyrene culture flasks at a density of 160 000/cm^2 in complete culture medium (LG-DMEM [Invitrogen, Paisley, United Kingdom] supplemented with penicillin and streptomycin [Lonza, Logan, UT] and 10% fetal bovine serum [FBS; HyClone, Verviers, Belgium]). We used characterized and defined FBS batches preselected for their potential to support MSC expansion.

All procedures were carried out under strict Good Manufacturing Practic (GMP) conditions. Flasks were incubated at 37°C in a CO_2 incubator and culture medium was replaced twice weekly. After reaching at least 70% confluence, MSCs were replated at 4000 cells/cm^2 using trypsine/EDTA (Lonza). MSCs were infused, fresh or after cryopreservation, at passage 3 or less to reduce the risk of genetic instability. MSC release criteria for clinical use were as follows: spindle-shape morphology,

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absence of contamination by pathogens, viability, and an immune phenotype proving the expression of CD73, CD90, and CD105 surface molecules and the absence of CD34, CD45, and CD31. The target dose for infusion was 1×10^6/kg to 5×10^6/kg body weight. MSCs were infused at a final concentration of 1×10^6 to 2×10^6 cells/mL.

Cotransplantation of MSCs and haploidentical peripheral blood stem cells

At day 0, under monitoring of vital signs, patients were given MSCs intravenously via a central venous catheter and 4 hours later received T-cell–depleted, G-CSF–mobilized $CD34^+$ cells, positively selected using the CliniMacs 1-step procedure (Miltenyi Biotech, Bergisch Gladbach, Germany). The target number of $CD34^+$ cells to be infused was 20×10^6 $CD34^+$ cells/kg recipient weight.

Statistics

A Student *t* test, Fisher exact test, and chi-square test with Yates correction were used to assess differences between study and historic control groups. A *P* value of less than .05 was considered to be significant.

Table 1. Characteristics of patients and controls

	Patients	Controls	*P*
n	14	47	—
Mo/y of transplant, range	10/2004 to 2/2007	3/1998 to 10/2004	—
Mean age, y (range)	8 (1-16)	7.1 (1-17)	NS
Patient sex, no. (%)			
Male	9 (61)	28 (60)	NS
Female	5 (39)	19 (40)	NS
Original disease, no. (%)			
Hematologic malignancies	11 (79)	35 (75)	(Distribution) .2
ALL	4 (36)	21 (57)	—
CR1	0	2	—
CR2	3	11	—
Greater than CR2	1	8	—
AML	7 (64)	12 (34)	—
CR1	0	2	—
CR2	4	5	—
Greater than CR2	0	2	—
Secondary	1	1	—
Refractory	2	2	—
CML, chronic phase	0 (0)	2 (6)	—
Immune deficiencies	2 (14)	2 (4)	—
Other nonmalignant disorders	1 (7)	10 (21)	—
Fanconi anemia	1	4	—
Hemoglobinopathies	—	1	—
HLH	—	4	—
Other	—	1	—
Donor sex, Male-female ratio	8:6	27:20	NS
Conditioning regimen, TBI-based/chemotherapy-based, no. (%)	9/5 (64/36)	30/17 (63/37)	NS
Graft characteristics			
No. of $CD34^+$ cells infused $\times 10^6$/kg, median (range)	21.5 (11.6-38.6)	24.3 (12.1-47.5)	NS
No. of $CD3^+$ cells infused $\times 10^5$/kg, mean (SD)	0.3 (0.3)	0.5 (0.7)	NS
Hematopoietic recovery, median (range)			
No. of d to PMN recovery*	12 (10-17)	13 (9-28)	.15
No. of d to PLT recovery†	10 (9-18)	13 (9-100)	.13
No. of d to reticulocyte recovery‡	12 (10-31)	23 (9-41)	.03
No. of d to leukocyte recovery§	11.5 (9-15)	14.9 (10-26)	.009
Post-HSCT complications, no. (%)			
Graft failure	0 (0)	7 (15)	.14
Primary	—	4	—
Secondary	—	3	—
Acute GvHD	—	—	.15
Grade I-II	2 (14)	12 (26)	—
Grade III-IV	0 (0)	2 (4)	—
Chronic GvHD	1 (7)	6 (13)	.34
Limited	1	4	—
Extensive	0	2	—

ALL indicates acute lymphoblastic leukemia; CR, complete remission; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; HLH, hemophagocytic lymphohistiocytosis; TBI, total body irradiation; PMN, polymorphonuclear neutrophil; PLT, platelet; HSCT, hematopoietic stem cell transplantation; GvHD, graft-versus-host-disease; SD, standard deviation; NS, nonsignificant; and —, not applicable.

*Time needed to reach an absolute neutrophil count equal to or greater than 0.5×10^9/L.

†Time needed to reach an unsupported platelet count equal to or greater than 20×10^9/L.

‡Time needed to reach a reticulocyte count equal to or greater than 20×10^9/L.

§Time needed to reach a leukocyte count equal to or greater than 1×10^9/L.

Results and discussion

Table 1 shows the characteristics of the 14 study patients compared with 47 historic controls that received transplants in either one of the 2 centers and were selected for an equivalent number of $CD34^+$ cells infused and matched for transplant indication. There was no significant difference between patients and controls in terms of age, sex, malignant versus nonmalignant disease, method of $CD34^+$ cell selection, and number of $CD3^+$ cells infused.

In all donors, both expansion of MSCs and mobilization of $CD34^+$ cells were successful. Patients received a mean of 1.6×10^6 MSCs/kg (range, 1×10^6 MSCs/kg to 3.3×10^6 MSCs/kg). No MSC infusion–related toxicity was observed.

Either primary or secondary graft failure occurred in 7 of the 47 children of the control group, whereas no rejection occurred in children who received cotransplants of haploidentical MSCs ($P = .14$). The number of $CD34^+$ cells infused was superimposable in the study patients (mean, 21.5×10^6/kg; range, 11.6×10^6/kg to 38.6×10^6/kg), in controls with sustained engraftment (mean, 21.2×10^6/kg; range, 12.1×10^6/kg to 47.5×10^6/kg), and in those who experienced either primary (mean, 21.7×10^6/kg; range, 14.7×10^6/kg to 39.4×10^6/kg) or secondary (mean, 21.1×10^6/kg; range, 12.4×10^6/kg to 26.6×10^6/kg) graft failure.

Neutrophil and platelet recovery was comparable in study patients and controls (see Table 1 for definitions and details). However, patients given MSCs had faster recovery of a total leukocyte count above 1.0×10^9/L in comparison to historic controls (mean, 11.5 days [95% confidence interval [CI] 9.0-14.8] versus 14.9 days [95% CI 10.1-26.0], respectively, $P = .009$). Lymphocyte recovery accounted for this finding: the absolute numbers of natural killer (NK) cells 1 month after HSCT being 497/μL (95% CI 347-646) in the study group and 252/μL (95% CI 173-330) in controls ($P = .02$). However, at 3 months, NK-andT-cell recovery was quantitatively no different between study patients and controls.

Chimerism analysis of ex vivo–expanded MSCs derived from recipient BM at 3-month intervals up to 1 year after HSCT using polymerase chain reaction (PCR) for informative donor recipient polymorphisms[9] did not show any evidence of donor cells in the majority of patients. In 3 patients, minimal (1%-2%) transient engraftment of donor MSCs was found at 3 months. Hematopoietic chimerism is detailed in Table 2.

Four study patients died (Table 2), 2 due to relapse and 2 due to infection, compared with 11 controls (7 relapse, 2 infections, 2 GvHD). Episodes of viral reactivation were common in both patients and controls, occurring in 50% of patients belonging to the study group and in 35% of historic controls. However, only 1 study patient died, as a result of disseminated adenovirus infection complicated by grade 2 acute GvHD requiring steroid treatment, compared with 2 historic controls. Since the follow-up of patients in the study group is shorter (range, 3-28 months) than that of historic controls (range, 32-110 months), both relapse rate and probability of overall survival in the study cohort (18% and 72%, respectively) and in controls (26% and 63%, respectively) are not comparable.

Our results indicate that in patients given a T-cell–depleted, HLA-disparate–related allograft from a relative, expansion of donor MSCs is feasible and their clinical use is safe. Moreover, our

Table 2. Patient follow-up data

Patient	Sex	Age at HSCT, y	Donor	Diagnosis	Follow-up, mo	Hematopoietic chimerism analysis	Time of last BM chimerism, mo	Time of last PB chimerism, mo	Outcome
1	M	15.5	Mother	Refractory AML	+7†	100% donor	+6	NE	Died in CR due to Candida sepsis
2*	M	2	Father	X-LPD	+28	100% donor	+24	+26	Alive and well
3*	M	2.3	Father	X-LPD	+24	100% donor on PMNs, 88% donor on $CD3^+$ cells	+20	+24	Alive and well
4	M	13.1	Mother	Refractory AML	+4†	100% donor	+3	+3	Died due to relapse
5	F	8.8	Father	Fanconi anemia	+16	100% donor	+14	+11	Alive and well, transfusion independent
6	F	3.7	Father	ALL CR2	+7†	100% donor	+3	+3	Died due to relapse
7	F	13.3	Father	Refractory AML with monosomy 7	+4†	100% donor	+2	+2	Died in CR due to adenovirus hepatitis
8	F	7.1	Mother	AML CR2	+12	100% donor	+7	+10	Alive and well, CR
9	M	5	Father	ALL-T CR4	+10	100% donor	+8	+7	Alive and well, CR
10	M	5.3	Mother	ALL CR2	+8	100% donor	+7	+8	Alive and well, CR
11	F	8.4	Sister	ALL CR2	+7	100% donor	+5	+6	Alive and well, CR
12	F	8.1	Mother	AML CR2	+6	95% donor on BM 80% donor on PB 30% donor on PB $CD4^+/CD8^+$ cells	+5	+6	Alive and well, CR
13	M	16	Father	AML CR2	+3	100% donor	+3	+4	Alive and well, CR
14	M	12	Father	AML 2CR	+2	100% donor	+3	+3	Alive and well, CR

Full donor chimerism was defined as greater than 98% donor cells in peripheral blood and bone marrow. Chimeric analysis was routinely performed at 1 to 2 weekly intervals in the first months after transplantation on peripheral blood mononuclear cells. Other serial controls of chimerism were subsequently performed at variable intervals. The last known analysis is shown for all study patients (+ months after HSCT). Two patients (number 3 and 12) show stable mixed chimerism at 2 years and 6 months, respectively.

mo indicates months; BM, bone marrow; PB, peripheral blood; M, male; NE, not evaluated; X-LPD,X-linked lymphoproliferative disorder; F, female.

*Patients number 2 and 3 are identical twins that received transplants from their haploidentical father.

†Dead.

data suggest that MSC cotransplantation may modulate host alloreactivity and/or promote better engraftment of donor hematopoiesis, reducing the risk of early graft failure.

A case-controlled study, with longer follow-up to exclude the risk of late rejections, can more precisely define the role played by cotransplantation of haploidentical donor MSCs on the outcome of patients given haploidentical, T-cell–depleted HSCT.

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Authorship

Contribution: L.M.B. and M.E.B. designed the study, performed research, analyzed data, and wrote the paper; H.R. designed the laboratory expansion protocol and, together with A.C., was responsible for mesenchymal stem cell expansions and quality controls; A.L. contributed to the design of the study, performed research, and analyzed data; R.M.E contributed to the design of the study, analyzed data, and wrote the paper; and F.L. and W.E.F. advised on the design of the study, analyzed data, and contributed to the final writing of the paper.

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