

Long-term immune reconstitution after anti-CD52-treated or anti-CD34-treated hematopoietic stem cell transplantation for severe T-lymphocyte immunodeficiency

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Background: Results of treatment of severe T-lymphocyte immunodeficiencies by means of hematopoietic stem cell (HSC) transplantation have improved. T cell-depleted haploidentical transplantations are successful if there is no HLA-identical donor. Methods to remove T lymphocytes include addition of anti-CD52 antibodies and CD34⁺ HSC selection.

Objective: Assessment of long-term immune function is important after these treatments. We looked at immune reconstitution in 36 survivors for more than 2 years after HSC transplantation for severe T-lymphocyte immunodeficiencies and compared engraftment quality between the 2 T-lymphocyte depletion methods.

Methods: Chimerism, T- and B-lymphocyte subsets, immunoglobulin levels, and specific antibody production at last follow-up were examined. The χ^2 (Fisher exact test) and Wilcoxon rank sum analyses were used to compare the groups.

Results: Nineteen patients received anti-CD52-treated and 19 anti-CD34-treated HSCs. More anti-CD52-treated patients had full donor myeloid chimerism ($P = .025$). All patients had full donor T-lymphocyte chimerism. There was no difference in donor B-lymphocyte chimerism, but significantly more anti-CD52-treated patients had class-switched memory B lymphocytes ($P = .024$), normal IgG levels, and normal responses to tetanus and *Haemophilus influenzae* type B vaccination. More anti-CD52-treated patients with common γ chain or Janus-associated kinase 3 severe combined immunodeficiency had donor B lymphocytes.

Conclusion: Long-term T-lymphocyte function is good with either treatment method, with a low incidence of graft-versus-host disease. The results imply more incomplete donor

chimerism in anti-CD34-treated patients with less B-lymphocyte function.

(J Allergy Clin Immunol 2007;119:1005-1011.)

Key words: Severe combined immunodeficiency, hematopoietic stem cell transplantation, T-cell depletion, CD34⁺ stem cell selection, immune reconstitution

Severe combined immunodeficiencies (SCIDs) are inherited genetic defects in T-lymphocyte differentiation with or without defects in other lymphoid (B and natural killer cells) or myeloid lineages.¹ Conventional treatment for these disorders is hematopoietic stem cell transplantation (HSCT). Improvements in tissue typing, advances in stem cell manipulation, new chemotherapy conditioning regimens, and new methods for detecting and treating viral and fungal infections have led to improving survival and cure over the past 2 decades.²

For many patients without an HLA-identical relative or unrelated donor, the use of T lymphocyte-depleted hematopoietic stem cells (HSCs) from an HLA-mismatched donor has been successful, with survival rates of 75% to 85%.^{3,4} A number of methods have been used to remove viable T lymphocytes from the graft, including the use of E-rosette lectin depletion and *in vitro* CAMPATH-1M anti-lymphocyte antibodies (anti-CD52). Since the late 1990s, European centers performing T lymphocyte-depleted HSCT for patients with primary immunodeficiency have used CD34⁺ stem cell selection rather than T-lymphocyte depletion. The most commonly used method, the Miltenyi CliniMACS system (Miltenyi Biotec, Surrey, United Kingdom), uses an organic iron bead attached to an anti-CD34 antibody to isolate purified CD34⁺ HSCs from the other cells by passing the HSC source through a magnetic column.⁵ The purified CD34⁺ HSC fraction is infused into the patient. By using this method, 4 log depletions of T-lymphocyte numbers can be achieved.

There are important differences between CD34⁺ stem cell-selected and T lymphocyte-depleted bone marrow. Although the residual T-lymphocyte count in anti-CD52-treated marrow can be relatively high, very few of the T lymphocytes remain viable because they are still coated with anti-CD52 when infused into the recipient and are then destroyed by complement-mediated lysis. In the anti-CD34-treated HSC product, although the number of T lymphocytes infused into the patient might be very low, those that are infused are viable and could cause graft-versus-host disease (GvHD). Additionally, the anti-CD52-treated product will contain component blood cell precursors and cells already in early differentiation from the stem cell, as

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Disclosure of potential conflict of interest: M. A. Slatter and A. R. Gennery have received grant support from Miltenyi Biotec. The rest of the authors have declared that they have no conflict of interest.

Presented in abstract form at the 33rd Annual Meeting of the European Group for Blood and Marrow Transplantation, Lyon, France, March 2007.

Received for publication September 10, 2007; revised October 25, 2007; accepted for publication October 26, 2007.

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doi:10.1016/j.jaci.2007.10.035

Abbreviations used

APC:	Allophycocyanin
C γ C:	Common γ chain
FITC:	Fluorescein isothiocyanate
GvHD:	Graft-versus-host disease
HSC:	Hematopoietic stem cell
HSCT:	Hematopoietic stem cell transplantation
IL-7R α :	IL-7 receptor α
JAK3:	Janus-associated kinase 3
PE:	Phycoerythrin
PerCP:	Peridinin-chlorophyll-protein complex
SCID:	Severe combined immunodeficiency
TEE:	Thymic emigrant equivalent

well as other stromal factors that might aid engraftment of HSCs into the bone marrow space,⁶ thus achieving better engraftment.

We have previously published the results of the long-term follow-up of 19 children who received anti-CD52–treated marrow transplanted between 1987 and 1998.⁷ It was subsequently noted that since changing to CD34⁺ stem cell selection of marrow, B-lymphocyte function failed to develop in more patients. Results of long-term follow-up of all these patients are presented, particularly comparing the quality of engraftment in patients receiving anti-CD52–treated HSCs with those given CD34⁺ selected HSCs.

METHODS

A retrospective review was performed of all patients undergoing HSCT for severe T-lymphocyte immunodeficiencies who had survived more than 2 years treated at our center with either anti-CD52–treated or anti-CD34–treated HSCT. Anti-CD52 and anti-CD34 treatment was performed on whole, freshly harvested marrow, as previously described.^{5,8}

The following parameters were analyzed: myeloid chimerism, T-lymphocyte number and chimerism, CD3⁺/CD4⁺/CD45RA⁺/CD27⁺ and CD3⁺/CD4⁺/CD45RA⁺/CD27⁺ (naïve) T-lymphocyte number, B-lymphocyte number and chimerism, memory B-lymphocyte (CD19⁺/CD27⁺/IgM⁺) and class-switched memory B-lymphocyte (CD19⁺/CD27⁺/IgM⁺) number, and serum IgG, IgA, and IgM levels, as well as specific antibody levels to tetanus toxoid, *Haemophilus influenzae* type B, and pneumococcal polysaccharide antigens after vaccination.

Lymphocyte subset analysis was measured by means of 4-color flow cytometry, as previously described.⁹ Briefly, lymphocyte surface marker studies were performed on fresh whole blood collected in EDTA by using appropriate markers (CD45 peridinin-chlorophyll-protein complex [PerCP], CD3 fluorescein isothiocyanate [FITC], CD4 allophycocyanin [APC], CD8 phycoerythrin [PE], CD19 APC, CD16/CD56 PE, CD3 PerCP/CD4 APC/CD45RA-FITC/CD27 PE, CD19 PerCP/CD27 FITC/IgM APC/IgD PE; Becton Dickinson, UK Ltd, Oxford, United Kingdom) and analyzed on a Becton Dickinson FACSCalibur flow cytometer. The T- and B-lymphocyte numbers were defined as normal or low by using age-specific reference ranges. Class-switched memory B lymphocytes were defined as less than 1% or greater than 1% of the B-cell population. The markers CD3⁺/CD4⁺/CD45RA⁺/CD27⁺ and CD3⁺/CD4⁺/CD45RA⁺/CD27⁺ were used as surrogates for recent thymic emigrant T lymphocytes (thymic emigrant equivalent [TEE]).¹⁰ Briefly, the CD3 subset was gated, and CD4⁺/CD45RA^{bright+}/CD27⁺ lymphocytes were identified. It was assumed that the majority of CD4⁺ lymphocytes in this population were CD8⁺. By identifying CD4⁺/CD45RA⁺/CD27⁺ lymphocytes, effector cells (which are CD45RA⁺/CD27⁺), were excluded. We have previously demonstrated that the CD45RA^{bright+}/CD27⁺ lymphocyte population correlates with CD45RO⁺ lymphocytes (unpublished observations). Lymphocytes were described as present or absent. Lymphocyte responses to stimulation with PHA were performed by using standard tritiated thymidine uptake methods and measured as a percentage of the response

of a healthy adult control subject.⁷ Immunoglobulin concentrations were measured by means of rate nephelometry, and specific antibody responses to tetanus toxoid, *Haemophilus influenzae* type B conjugate vaccine, and pneumococcal polysaccharide vaccine antigens were measured by means of ELISA.¹¹ Immunoglobulin levels were defined as normal or low by using age-specific reference ranges, and specific antibody levels were defined as present or absent after vaccination.

Cells were separated into separate lineages before measuring chimerism. Whole blood was stained with CD3, CD19, or CD15 microbeads, and cell lines were separated by using an autoMACS automated bench-top magnetic cell sorter (Miltenyi Biotec Ltd). Chimerism was measured in sex-mismatched cases by means of XY-fluorescent *in situ* hybridization with standard cytogenetic techniques. Briefly, interphase fluorescent *in situ* hybridization was performed with a Vysis 2-colour CEP X/CEP Y probe set (Abbott Diagnostics, Maidenhead, Berkshire, United Kingdom), according to the manufacturer's protocol. Where donor and recipient were of the same sex, chimerism was measured by means of short-tandem repeat marker analysis of genomic DNA, as previously described¹²; sensitivity was accurate to within 5%. For the purposes of analysis, chimerism was defined as donor, mixed, or recipient in specific cell lineages.

Patients, when administered cytoreductive chemotherapy, were treated in accordance with the European Group for Blood and Marrow Transplantation/European Society for Immunodeficiencies Inborn Errors Working Party treatment guidelines current at time of transplantation. No patients received radiotherapy. Nucleated cell doses in the stem cell products were compared between groups because CD34⁺ cell doses were not available for many of the earliest patients who received anti-CD52–treated marrow.

The χ^2 (Fisher exact test) and nonparametric Wilcoxon rank sum analyses were used to detect statistical differences between the groups (GB-Stat PPCworks 6.5.4; Dynamic Microsystems Inc, Silver Spring, Md). A 2-sided *P* value of .05 or less was considered significant.

From May 1987 until the end of August 2004, 72 patients with severe T-lymphocyte immunodeficiencies were transplanted with 57 (79%) survivors; 47 patients underwent T lymphocyte-depleted HSCT, of whom 36 (77%) survive. Before 1999, marrow from HLA-mismatched donors was depleted *in vitro* with anti-CD52 antibody (27 patients). Subsequently, marrow has been CD34⁺ stem cell selected, as described above (22 patients). All patients surviving for more than 2 years from transplantation for severe T-lymphocyte immunodeficiencies who were still alive at the time of the study were included.

RESULTS

Patient characteristics are detailed in Table I; there were 19 long-term survivors in each group. Two patients (nos. 12 and 13) in the anti-CD52 group underwent retransplantation with CD34⁺ HSCs. There were 8 deaths in the anti-CD52–treated group (3 of fungal infection, 3 of parainfluenzae pneumonitis, 1 of cytomegalovirus pneumonitis, and 1 of idiopathic pneumonitis; median time of death, 2.5 months after HSCT; range, 5 days–7 months) and 3 deaths in the anti-CD34–treated group (1 of hepatic veno-occlusive disease, 1 of EBV infection, and 1 of adenovirus infection; time of death, 3 days–1 month after HSCT). There were no deaths associated with GvHD in either group. Of the survivors, 10 had common γ chain (C γ C)–deficient SCID, 8 had T-B⁺NK⁺ SCID (3 confirmed IL-7 receptor α chain [IL-7R α] defects), 8 had recombinase activating gene 2 or Artemis deficiency, 3 had adenosine deaminase SCID, 3 had Janus-associated kinase 3 (JAK3) deficiency, 2 had zeta-associated protein 70 kinase deficiency, and 4 had other phenotypes. There was no significant difference in the number of patients in each group who received fully myeloablative cytoreductive chemotherapy with 16 mg/kg busulfan and 200 mg/kg cyclophosphamide.

There was no significant difference in age at transplantation between the groups (anti-CD52–treated group: median of 7

TABLE I. Characteristics of patients receiving anti-CD52-treated or anti-CD34-treated HSCT for severe T-lymphocyte immunodeficiency

Patient no.	Age at transplantation	Diagnosis	Preexisting respiratory tract infection	Donor	Conditioning	Complications	Follow-up	Status
Anti-CD52								
1	8/52	C γ C	Nil	Pat	Bu8 Cy200	<i>S aureus</i> osteomyelitis 8 y after	17 y 8 mo	A & W
2	7 mo	C γ C	PJP	Pat	Bu8 Cy200	CMV plantar warts, pneumonia 5 y after	14 y 1 mo	A & W
3	12 mo	C γ C	Nil	Pat	Bu8 Cy 200	Atypical mycobacteria	12 y 11 mo	Behavioral disorder
4	11 mo	C γ C	PJP	Pat	Bu8 Cy200	Nil	9 y 11 mo	A & W
5	4 mo	ADA	Nil	Pat	Bu16 Cy200	AIHA, plantar warts	17 y 7 mo	A & W
6	3 mo	ADA	Nil	URD	Bu16 Cy200	Nil	6 y 7 mo	ADHD
7	8 mo	IL-7R α	BCG	Pat	Bu8 Cy200	VOD, splenectomy, BCGosis, plantar warts	14 y	A & W
8	8 mo	IL-7R α	?PJP	Pat	Bu8 Cy200	VOD	10 y 7 mo	Bronchiectasis, Ig
9	12 mo	T-B ⁺ NK ⁺	Nil	Pat	Bu16	VOD, pneumothoraces 14 y after	18 y 11 mo	A & W
10	5/52	T-B ⁺ NK ⁺	Nil	Pat	Bu8 Cy200	Osteomyelitis 5 y after	12 y 10 mo	A & W
11	6/52	T-B ⁺ NK ⁺	Nil	Pat	Bu8 Cy200	Nil	9 y 4 mo	A & W
12*	6/52	RAG2	Nil	Pat	Bu8 Cy 200	Graft failure	2 y	Retransplanted
13*	2 mo	Artemis	Nil	URD	Bu8 Cy200	Graft failure, cGVHD	2 y 4 mo	Retransplanted
14	7 mo	Artemis	PJP	Mat	Bu8 Cy200	Localized BCG	6 y 7 mo	A & W
15	13 mo	ZAP 70	Nil	URD	Anti-LFA1/CD2 Bu16 Cy200	Nil	13 y 3 mo	Behavioral disorder
16	3 mo	T ^{low} B ^{low}	Nil	Mat	Bu16 Cy200	Nil	18 y 11 mo	Poor growth Behavioral disorder
17	7 mo	T-B ⁺	RSV	Pat	Bu8 Cy200	Skin GvHD	15 y	A & W
18	6/52	T-B ⁺	Nil	Pat	Bu8 Cy200	Nil	13 y 6 mo	A & W
19	2 mo	T ^{low} B ^{low}	Nil	Pat	Bu8 Cy200	Familial deafness	11 y	Behavioral disorder
Anti-CD34								
20	8 mo	C γ C	?PJP	Pat	Bu8 Cy200	Nil	8 y 2 mo	A & W, Ig
21	6 mo	C γ C	PF	Mat	Nil	Nil	7 y 2 mo	A & W, Ig
22	9 mo	C γ C	PJP	Mat	Bu8 Cy200	Nil	7 y	A & W, Ig
23	5/52	C γ C	Nil	Pat	Bu8 Cy200	EBV BLPD	6 y 2 mo	A & W, Ig
24	6/52	C γ C	Nil	Pat	Bu8 Cy200	Nil	7 y 7 mo	A & W, Ig
25	6/52	C γ C	Nil	Pat	Bu8 Cy200	Nil	5 y 5 mo	A & W, Ig
26	6/52	JAK3	Nil	Pat	Bu8 Cy200	Nil	5 y 9 mo	A & W
27	8 mo	JAK3	PJP	Pat	Nil	Nil	5 y 6 mo	A & W, Ig
28	6/52	JAK3	Nil	Mat	Bu8 Cy200	Plantar warts	4 y 10 mo	A & W
29	4 mo	ADA	Unidentified	Pat	Bu16 Cy200	AI hypothyroid	4 y 3 mo	A & W
30	9 mo	IL-7R α	PJP	Mat	Bu8 Cy200	VOD, BCG fever	6 y 7 mo	A & W
31	8 mo	T-B ⁺ NK ⁺	PJP	Pat	Bu8 Cy200	Nil	6 y 4 mo	A & W, Ig
32	6/52	T-B ⁺ NK ⁺	Nil	Pat	Bu16 Cy200	Nil	5 y 9 mo	A & W
12*	2 y 1 mo	RAG 2	Nil	Mat	Bu16 Cy200	AI hypothyroid	6 y 8 mo	Mild ADHD
33	6/52	RAG 2	HHV6	Mat	Bu16 Cy200	HHV6, pharyngeal abscess 2.5 y after	4 y 10 mo	A & W
13*	2 y 6 mo	Artemis	Nil	Pat	ATG	AIHA, ITP	6 y 10 mo	Renal impairment, impaired growth
					Bu16 Cy200	GB-like 2 y after, hearing loss		
34	4/52	Artemis	Nil	Pat	Bu16 Cy200	Nil	3 y	A & W
35	6/52	Artemis	Nil	Pat	Bu16 Cy200	Nil	5 y 11 mo	A & W
36	1 y 3 mo	ZAP 70	Nil	Mat	Alemtuzumab Bu16 Cy200	VOD	4 y 2 mo	A & W

Pat, Paternal; Bu, busulfan (8 or 16 mg/kg); Cy, cyclophosphamide (200 mg/kg); A & W, alive and well; (?) PJP, (presumed) *Pneumocystis jiroveci* pneumonia; CMV, cytomegalovirus; ADA, Adenosine deaminase SCID; AIHA, autoimmune hemolytic anemia; URD, unrelated donor; ADHD, attention deficit hyperactivity disorder; VOD, veno-occlusive disease; Ig, immunoglobulin replacement; NK, natural killer; ZAP 70, zeta-associated protein kinase 70 SCID; Mat, maternal; RSV, respiratory syncytial virus; PF, parainfluenzae virus; BLPD, B-lymphoproliferative disease; AI hypothyroid, autoimmune hypothyroidism; RAG 2, recombinase activating gene 2 SCID; HHV6, human herpes virus 6 infection; ITP, immune thrombocytopenia; GB-like, Guillain-Barre like.

*Patient appears in the anti-CD52 and anti-CD34 groups.

months and range of 1.25–17 months; anti-CD34–treated group: median of 6 months and range of 1–25 months). The anti-CD52–treated group had significantly longer follow-up than the anti-CD34–treated group (median of 12.83 years and range of

1.17–18.92 years vs median of 5.92 years and range of 2.67–8.17 years, $P < .0001$). The anti-CD52–treated group received significantly higher nucleated cell doses (median of $5 \times 10^7/\text{kg}$ and range of $0.4\text{--}525 \times 10^7/\text{kg}$ vs median of $0.13 \times 10^7/\text{kg}$ and range

of $0.0114\text{--}0.57 \times 10^7/\text{kg}$, $P < .0001$) and T-lymphocyte doses (median of $225 \times 10^3/\text{kg}$ and range of $0\text{--}6690 \times 10^3/\text{kg}$ vs median of $15 \times 10^3/\text{kg}$ and range of $2.2\text{--}180 \times 10^3/\text{kg}$, $P = .0001$), respectively. Three patients in each group received a boost infusion of T lymphocyte-depleted HSCs from the original donor without further chemotherapy for incomplete chimerism.¹³ There were no significant differences in survival between the groups (70% vs 86%, $P = .19$) and no late deaths (>12 months after HSCT) in either group. Only 4 patients in the anti-CD52-treated HSC group and 1 in the anti-CD34-treated HSC group had grade I acute GvHD of the skin, and 1 patient in each group had mild localized chronic GvHD of the skin.

Chimerism and immune reconstitution

There was no significant difference in the number of patients who achieved some donor myeloid chimerism between the 2 groups (Table II). However, significantly more patients who received anti-CD52-treated HSCs had full donor myeloid chimerism (7 vs 1, $P = .025$; Fig 1). More patients treated with anti-CD34 HSCs with some donor myeloid chimerism received a fully myeloablative dose of busulfan (16 mg/kg) than patients treated with anti-CD52 HSCs (8 vs 2, $P = .03$). All patients in both groups had full donor chimerism in the T-lymphocyte lineage. After immune reconstitution, all patients treated with anti-CD34 HSCs had TEE cells compared with 15 of 19 in the anti-CD52-treated HSC group. Neither the B-lymphocyte number nor the number of patients with at least some donor B-lymphocyte chimerism at last follow-up was significantly different between the 2 groups. However, more patients who had received anti-CD52-treated HSCs had class-switched memory B lymphocytes (15 vs 9, $P = .024$). There was no difference between the groups in the number of patients who had normal levels of IgM or IgA at the latest follow-up. However, significantly more patients who had received anti-CD52-treated HSCs had normal IgG levels (16 vs 10, $P = .036$). Correlating with this, at the time of the last follow-up, more patients in the anti-CD52-treated HSC group had achieved a sustained response to tetanus toxoid vaccination (16 vs 10, $P = .036$) and to *Haemophilus influenzae* type B conjugate vaccine (16 vs 9, $P = .019$). There was no difference between groups in patients mounting a response to pneumococcal polysaccharide antigen. More patients in the anti-CD34-treated HSC group had $\text{C}\gamma\text{C}/\text{JAK3}$ -deficient SCID (4 vs 9, $P = .09$). None of these patients with $\text{C}\gamma\text{C}/\text{JAK3}$ -deficient SCID in either group received 16 mg/kg busulfan, and there was no difference in the number of these patients receiving no conditioning compared with 8 mg/kg busulfan. The numbers of patients with $\text{C}\gamma\text{C}/\text{JAK3}$ SCID were too small to make confident comparisons, but significantly more patients with $\text{C}\gamma\text{C}/\text{JAK3}$ SCID had donor B lymphocytes in the anti-CD52-treated HSC group (3/4 vs 2/9, $P = .014$).

Complications

Four patients had a single significant invasive infection more than 2 years after transplantation. Four had plantar warts, only 2 of whom had $\text{C}\gamma\text{C}$ - or JAK3 -deficient SCID; in no patient were they severe or persistent. Four had autoimmunity, 2 of whom had autoimmune thyroid disease (Table I). There was no late GvHD, except for patient 13, who had chronic skin GvHD after the first transplantation. Six patients had mild-to-moderate behavior-related problems, only one of whom had adenosine deaminase SCID.

DISCUSSION

This study adds to those previously reporting long-term follow-up of patients with SCID receiving T lymphocyte-depleted HSCT^{14,15} but is the first to compare the quality of immune reconstitution after anti-CD52- or anti-CD34-treated HSCT in severe T-lymphocyte disorders. Our overall survival of 77% compares favorably with that of other reported series.^{4,16} Overall survival was better in the anti-CD34-treated HSC group, but there was no significant difference between the 2 groups. This improvement over time is likely to reflect improvement in transplantation care, including more accurate diagnoses and earlier detection of viral and fungal infections by means of PCR, newer and more effective treatments, and better supportive care, as has been demonstrated in other studies,² rather than differences in the method of marrow manipulation. Although follow-up was longer in patients receiving anti-CD52-treated HSCs, no deaths occurred beyond 12 months in either group.

The T-lymphocyte depletion method used to manipulate the graft does not appear to affect T-lymphocyte chimerism. Interestingly, there were more patients in the anti-CD34-treated HSC group who had TEE T lymphocytes. A recent study has demonstrated that TEE lymphocytes might be found in the absence of myeloid engraftment, particularly in patients with $\text{C}\gamma\text{C}/\text{JAK3}$ SCID,¹⁴ of which there were more in our anti-CD34-treated HSC group; our study appears to support this observation. We did not have data comparing the speed of T-lymphocyte immune reconstitution between the 2 marrow manipulation methods, particularly with the appearance of TEE T lymphocytes. However, previous studies have indicated that the appearance of recent thymic emigrants is similar for patients who received replete or T cell-depleted HSCT.¹⁷

Although overall there was no statistical difference in B-lymphocyte donor chimerism between the 2 groups, there was a trend toward more complete B-lymphocyte donor chimerism in the anti-CD52-treated HSC group. Over a longer period of time, humoral function might improve so that those transplanted longest have better antibody responses. It was not possible to investigate this further because the early data in the patients treated with anti-CD52 HSCs were incomplete. However, all but 3 patients (nos. 8, 12, and 13) in the anti-CD52-treated group had discontinued immunoglobulin replacement by 4 years after HSCT.⁷ It therefore seems unlikely that many of our patients who received anti-CD34-treated HSCs but continued to receive immunoglobulin treatment will have humoral immunity in the future. More likely, antibody function after transplantation might depend on the type of SCID and the chimeric state.¹⁸ Recipient B lymphocytes in $\text{IL-7R}\alpha$ -deficient SCID are functionally normal, whereas those from $\text{C}\gamma\text{C}$ - or JAK3 -deficient SCID are intrinsically defective.¹⁹ Therefore unless donor B-lymphocyte chimerism is achieved in patients with $\text{C}\gamma\text{C}$ - or JAK3 -deficient SCID, normal humoral function is unlikely because interaction with donor T lymphocytes does not result in immunoglobulin class switching and antigen-specific IgG production in recipient B lymphocytes.²⁰ Our results are difficult to interpret because more patients in the anti-CD34-treated HSC group had $\text{C}\gamma\text{C}/\text{JAK3}$ SCID with recipient B lymphocytes. There were more patients with $\text{C}\gamma\text{C}/\text{JAK3}$ SCID who achieved donor B-lymphocyte chimerism in the anti-CD52-treated HSC group, despite the fact that there was no difference in the conditioning regimens used for the $\text{C}\gamma\text{C}/\text{JAK3}$ -deficient patients, and none received

TABLE II. Immune reconstitution and function of patients receiving anti-CD52–treated and anti-CD34–treated HSCT for SCID

Patient no.	Myeloid chimerism	CD3	CD4	CD8	TEE	PHA (%)	CD16/CD56	CD19	CSM B (%)	B-cell chimerism	IgG (g/L)	IgA (g/L)	IgM (g/L)	Hib/Tet	PPS (mg/L)
Anti-CD52															
1	R	1300	488	744	507	172	83	330	5%	R	7.18	1.17	0.83	9.0/1.57	No
2	D	1441	838	635	663	99	99	219	17%	D	10.4	1.46	1.81	9.0/0.34	132
3	Mixed	2617	1128	1550	1439	146	54	615	2%	Mixed	5.55	0.84	1.1	1.0/0.26	No
4	D	1421	1105	400	998	111	171	407	4%	D	6.04	0.87	1.04	1.3/0.44	No
5	R	582	345	251	53	13	28	40	17%	R	8.97	1.96	0.8	1.2/2.33	No
6	R	1947	261	1424	136	22	31	31	NA	R	4.81	0.57	0.51	0.31/1.18	53
7	Trace D	2237	966	1162	671	72	249	418	10%	Trace D	22.5	<0.1	1.46	2.4/1.79	No
8	R	246	125	98	13	57	98	121	0	R	Ig	<0.1	0.11	Ig	Ig
9	D	804	394	396	136	89	272	137	16%	D	11.0	1.83	1.68	1.3/0.71	52
10	Mixed	1006	509	462	307	100	133	128	14%	R	12.7	2.40	1.04	1.1/1.82	39
11	R	1195	512	610	886	92	92	288	15%	R	11.1	1.22	1.33	1.9/0.53	31
12*	R	157	114	14	0	0	187	0	0	Absent	Ig	<0.1	0.01	Ig	Ig
13*	R	84	13	3	0	0	794	0	0	Absent	Ig	<0.1	0.02	Ig	Ig
14	R	2074	1044	837	456	144	255	413	2%	D	17.8	<0.1	3.02	0.6/3.84	135
15	D	648	342	228	227	163	96	218	2%	D	9.4	1.08	0.83	0.9/1.65	80
16	D	978	675	302	200	126	96	297	9%	D	10.4	2.55	1.01	1.2/0.43	No
17	D	1672	1156	440	1088	98	330	574	5%	D	7.95	1.77	1.15	1.0/0.21	59
18	D	1566	871	603	773	107	232	609	5%	D	12.7	1.86	1.07	1.7/0.4	45
19	Mixed	2349	1087	1147	940	73	400	351	4%	Mixed	7.28	0.83	1.72	2.1/0.89	57
Anti-CD34															
20	R	3744	1289	2315	1498	91	36	173 L	0	Trace D	Ig	<0.1	0.88	Ig	Ig
21	R	1797	617	864	378	125	36	353	0	R	Ig	<0.1	0.25	Ig	Ig
22	R	2651	884	1629	1152	255	29	355	<1%	R	Ig	<0.1	1.99	Ig	Ig
23	Trace D	1621	906	656	632	209	35	261	2%	Trace D	Ig	<0.1	1.86	Ig	Ig
24	R	2585	1007	972	258	39	<1%	978	<1%	R	Ig	<0.1	0.91	Ig	Ig
25	R	2208	1371	580	1148	41	0	1313	<1%	R	Ig	<0.1	0.35	Ig	Ig
26	R	3834	1999	1637	1800	16	0	1313	<1%	R	5.05	0.49	1.60	<0.1/0.86	No
27	R	4160	1217	2547	1290	88	46	1843	0	R	Ig	<0.1	0.87	Ig	Ig
28	Mixed	2866	1228	1450	1175	105	74	1014	<1%	Mixed	6.3	2.52	2.41	1.2/2.49	61
29	Mixed	1882	662	1056	657	—	219	776	<1%	Mixed	12.7	1.94	1.12	No imms	No imms
30	Mixed	1971	1087	768	1084	46	226	726	15%	Mixed	10.3	2.55	1.29	9.0/0.71	26
31	Trace D	2113	1120	701	1100	107	0	600	<1%	Trace D	Ig	1.68	1.24	Ig	Ig
32	Mixed	3060	1232	1656	1285	119	189	667	9%	Mixed	15.1	1.87	0.93	4.3/0.86	93
12*	Mixed	1814	772	1077	272	58	341	264	16%	D	9.01	1.56	1.78	9.0/4.01	71
33	Mixed	1403	726	607	717	NA	122	496	39%	Mixed	9.75	0.87	1.13	1.02/1.2	122
13*	D	2059	835	1176	844	4 (8/12 post)	167	425	7%	D	6.34	0.99	0.65	3.2/3.1	No imms
34	Mixed	3820	2520	1530	1642	42	658	322	19%	D	8.78	1.76	1.8	2.1/1.08	No imms
35	Trace D	2365	1279	1024	804	11	255	180 L	19%	D	8.26	1.25	2.02	3.6/1.87	109
36	Trace D	1827	744	951	475	NA	442	776	3%	Trace D	6.15	0.72	1.3	9.0/7.0	No

Cell values are presented as cells per microliter, except where percentages are given. Normal values are as follows: CD3, 700 to 3500 cells/ μ L; CD4, 300 to 2000 cells/ μ L; CD8, 300 to 1800 cells/ μ L; CD16/CD56, 90 to 900 cells/ μ L; CD19, 200 to 1600 cells/ μ L; IgG, 4.8 to 15.2 g/L; IgA, 0.30 to 2.97 g/L; IgM, 0.43 to 1.9 g/L; Hib, greater than 1 mg/L; Tet, greater than 0.1 IU/L; PPS, greater than 20 mg/L.

TEE, Thymic emigrant equivalent ($CD4^{+/-}CD45RA^{bright+}CD27^{+}$); PHA, phytohemagglutinin response as a percentage of the healthy adult control value; CSM B, immunoglobulin class-switched memory B lymphocyte; Hib, *Haemophilus influenzae* conjugate vaccine antigen response; PPS, pneumococcal polysaccharide vaccine antigen response; R, recipient; D, donor; Ig, immunoglobulin replacement; Tet, tetanus toxoid protein vaccine antigen response; imms, immunizations.

*Patient appears in the anti-CD52 and anti-CD34 groups.

full myeloablative chemotherapy. Patient 1, with C γ C deficiency, has normal humoral immunity, apparently with recipient B lymphocytes: it might be that the methods of detecting donor chimerism were not sensitive enough to detect very low levels of chimerism (<5%) or that there is a significant population of donor B lymphocytes in lymphoid tissue. More sensitive methods of detecting microchimerism might detect donor cells in this patient.¹⁹ Patient 8, with IL-7R α deficiency, does not make immunoglobulin, although donor T lymphocytes would be expected to interact with recipient B lymphocytes. In this patient T-lymphocyte engraftment is poor, with few TEE cells and a restricted T-lymphocyte repertoire (data not shown); there might be too few T- and B-lymphocyte interactions for effective humoral immunity. Patient 14, with Artemis deficiency, has complete donor

B-lymphocyte engraftment with recipient myeloid engraftment. Donor early B-lymphocyte precursors might have a competitive advantage over recipient B-lymphocyte precursors, which are blocked at an early stage in B-lymphocyte development, enabling chimerism of donor mature B lymphocytes.

There is an impression of better donor stem cell engraftment with overall more complete donor myeloid and B-lymphocyte engraftment in patients who received anti-CD52–treated HSCs. This implies superior stem cell engraftment and perhaps better long-term immune function; a continuing longitudinal study is needed to see whether there is a greater waning of immune function in the anti-CD34–treated HSC group compared with the anti-CD52–treated HSC group. This observation is perhaps surprising in that fewer patients in the anti-CD52–treated HSC

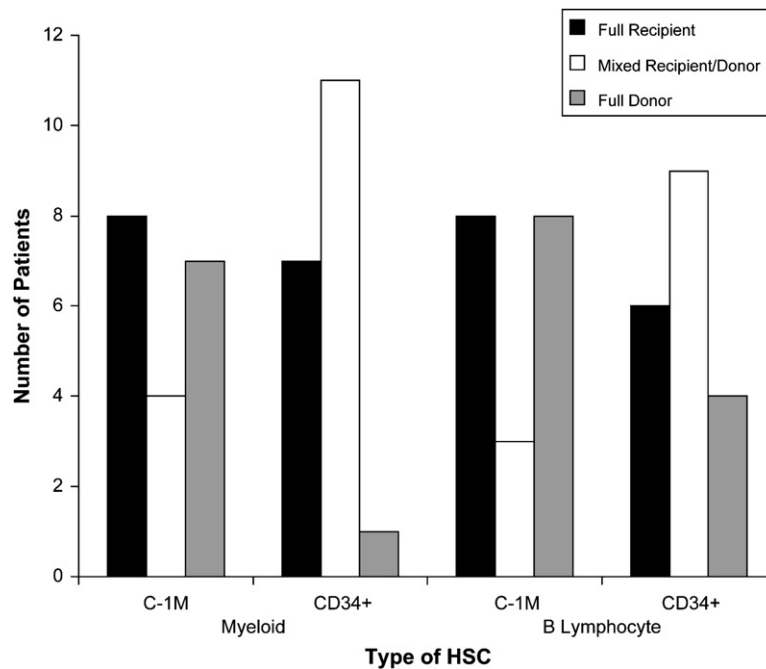


FIG 1. Number of patients with recipient, mixed, or donor chimerism in myeloid and B-lymphocyte lineages who received anti-CD52-treated or anti-CD34-treated HSCs.

group received full myeloablative conditioning with 16 mg/kg busulfan, which is more likely to result in myeloid engraftment. The depletion of most additional marrow cells other than CD34⁺ HSCs might have unintended drawbacks for engraftment because some of the other cellular elements can aid marrow engraftment of stem cells. Other methods of marrow manipulation, such as CD3/CD19 depletion, can result in decreased GvHD risk without compromising engraftment.²¹

As new information appears, the determinants of successful outcome of transplantation for SCID are becoming clearer. Long-term T-lymphocyte immunity appears to require early, sustained thymic output of donor T lymphocytes.^{14-16,22,23} There is growing evidence of a correlation between myeloid chimerism, lymphocytes containing T-lymphocyte receptor excision circles, and naive T lymphocytes, although that might be less important for patients with CγC/JAK3 SCID.^{14,16,17} Further very long-term studies are required to confirm these observations, but if there is no good evidence of newly emerging thymic T lymphocytes early after HSCT, then long-term T-lymphocyte engraftment and function is likely to be poor.¹⁶ In these instances early intervention with further therapeutic measures is more likely to be successful, before thymic atrophy is established.^{22,23} As for B-lymphocyte function, our study shows the correlation between class-switched memory B lymphocytes and the antigen-specific IgG production, and these markers should be considered an indication of long-term B-lymphocyte function. The advantages and disadvantages of chemotherapy continue to be debated.^{1,4,19} The role of cytoreductive chemotherapy in achieving long-term immune reconstitution is becoming more clear²⁴ and is confirmed in our study. It is of note that all patients who require continued immunoglobulin replacement received a less myeloablative dose of busulfan, 8 mg/kg. Although the short-term risks of gene therapy, particularly for CγC SCID, have been recognized,²⁵ it will be important to compare long-term outcomes for HSCT and gene therapy. In particular, comparison between conditioned and nonconditioned

HSCT with gene therapy will be required, and the molecular defect for which treatment has been given, as well as the method of HSC manipulation, should be considered because our data suggest this might affect the outcome.

Autoimmunity was seen in 4 of our patients and has been previously reported, as has late-occurring infection.^{15,24,26} The mechanism of autoimmunity in this setting is unclear and presumably multifactorial, but there might be a role for subsets of T lymphocytes.²⁷ Other than a requirement for continuing immunoglobulin replacement, no other significant long-term sequelae were observed.

Although in the first years after T cell-depleted HSCT as treatment for severe T-lymphocyte deficiency, long-term survival was the aim of successful treatment, quality of engraftment, long-term immunoreconstitution, and quality of life are now as important.^{1,16,22,23} Our results confirm that mismatched T lymphocyte-depleted HSCT for severe T-lymphocyte immunodeficiencies remains a successful technique, with 77% overall survival and good T-lymphocyte function and 71% good humoral immunity. Both techniques of marrow manipulation seem effective and vastly reduce the risk of GvHD. There is an impression that the B-lymphocyte function might be better after anti-CD52-treated HSC, but further studies with more patients are needed to ascertain whether T-lymphocyte depletion rather than HSC selection results in more complete immune function and in particular in better B-lymphocyte function.

Clinical implications: Retention of cells other than HSCs in the manipulated marrow can result in more complete engraftment and immune function.

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