

Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency

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Background: Currently, hematopoietic stem cell transplantation allows long-term survival in a high proportion of infants with congenital severe T-cell immunodeficiency. However, relatively little is known of their long-term quality of life.

Objective: We sought to assess the long-term immune reconstitution and clinical status in children treated with stem cell transplantation for severe T-cell immunodeficiency.

Methods: Immune function and clinical status have been analyzed in a cohort of 40 patients with severe T-cell immunodeficiency who are alive at a follow-up of at least 5 years after transplantation.

Results: Most patients have attained normal T- and B-cell function. Weight and height were normal at last follow-up in most patients. Endocrine and severe neurologic abnormalities have been observed in 17.5% and 10% of the patients, respectively. **Conclusions:** These data indicate that with current management strategies, stem cell transplantation can lead to long-term survival and good quality of life in the majority of patients with severe T-cell immunodeficiency.

Clinical implications: Prompt recognition of congenital severe T-cell immunodeficiency, followed by stem cell transplantation, allows excellent perspectives of long-term survival and good quality of life for these otherwise fatal disorders. (*J Allergy Clin Immunol* 2007;120:892-9.)

Key words: T-cell immunodeficiency, severe combined immune deficiency, hematopoietic stem cell transplantation, immune reconstitution, quality of life

Congenital severe T-cell immunodeficiency comprises a heterogeneous group of genetic disorders of the immune system that profoundly affect T-cell development,

Abbreviations used

ADA: Adenosine deaminase
ATG: Anti-thymocyte globulin
CR: Conditioning regimen
GvHD: Graft-versus-host disease
HSCT: Hematopoietic stem cell transplantation
IVIG: Intravenous immunoglobulins
JAK3: Janus kinase 3
MMR: Measles-mumps-rubella
MMRD: Mismatched related donor
MSD: Matched sibling donor
MUD: Matched unrelated donor
PIRD: Phenotypically identical related donor
RAG: Recombinase activating gene
SCID: Severe combined immune deficiency
TREC: T-cell receptor excision circle

function, or both.^{1,2} Unless treated with hematopoietic stem cell transplantation (HSCT) or in selected cases with enzyme replacement therapy or gene therapy, these patients usually die within the first years of life because of overwhelming infections.³⁻⁵

Use of HSCT from matched sibling donors (MSD-HSCT) in infants with severe combined immune deficiency (SCID) results in a current 3-year survival rate of approximately 85%.⁴ Results of HSCT from mismatched related donors (MMRD-HSCT) are more controversial, with overall survival ranging from 54% to 78% in selected large experiences.^{3,4} More recently, matched unrelated donors (MUDs) have become another interesting source of stem cells for transplantation in infants with SCID and other forms of severe T-cell immunodeficiency.⁵

Despite remarkable improvement in survival, decreasing or incomplete T-cell reconstitution and impaired humoral immunity have been reported long-term after HSCT for SCID^{2,6-8} and could account for significant morbidity.⁹⁻¹¹ However, few data are available on the long-term clinical status and quality of life after HSCT for SCID and other severe T-cell immunodeficiencies. In the present single-center report, we review immune reconstitution and current clinical status in 40 patients with severe T-cell immunodeficiency who are alive at least 5 years after HSCT.

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METHODS

Patients and transplantation

To assess long-term clinical outcome and quality of life in patients treated with HSCT for severe T-cell immunodeficiency, we reviewed data on 58 consecutive children with severe T-cell immunodeficiency who received HSCT at the Department of Pediatrics, University of Brescia, Italy, in the period from March 1, 1991, to June 30, 2002. Analysis of subjects who, if alive, would be at least 5 years after HSCT provides more accurate analysis of disease- and transplant-related mortality and chronic complications, as well as of the long-term immune reconstitution and clinical benefits of the procedure.⁹ Of the 58 patients, 12 received MSD-HSCT, 33 were treated with MMRD-HSCT, 3 received transplants from a phenotypically identical related donor (PIRD), and 10 received transplants from a MUD. Forty-two (72.4%) of these patients are alive. Tables E1 and E2 in the Online Repository (available at www.jacionline.org) report on the genotype and immunologic phenotype in the overall cohort of 58 children and on the causes of death in 16 patients. Among the 42 long-term survivors, 2 patients are known to be alive and in good health, both at 6 years after HSCT; however, after returning to their home countries, they could not be studied in sufficient detail and have therefore been excluded from the present study. The characteristics of the remaining 40 patients are reported in Table I. Of these, 5 patients treated with MMRD-HSCT required 2 (n = 4) or 3 (n = 1) transplantations.

A conditioning regimen (CR) was used according to the guidelines of the European Bone Marrow Transplantation/European Society for Immune Deficiency and in most cases included busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). Typically, myeloablative CR was used for recipients of MMRD-HSCT. However, 3 patients received MMRD-HSCT *in utero* without chemotherapy, whereas patient 12 received 2 attempts at MMRD-HSCT with anti-thymocyte globulin (ATG) alone or with ATG and cyclophosphamide. Among recipients of MSD-HSCT, only 2 (patients 6 and 8) received CR because of residual pretransplantation T-cell immunity. Finally, patient 32 was the only one among 10 patients treated with MUD-HSCT who received a nonmyeloablative CR with ATG only.

T-cell depletion was used in all cases of MMRD-HSCT and in 1 patient treated with MUD-HSCT. Methods for T-cell depletion consisted of *in vitro* use of Campath-1M (kindly provided by G. Hale and H. Waldmann, Sir William School of Pathology, Oxford, UK) until 1995 (in 6 patients) and, since then, of positive selection of CD34⁺ cells (in 15 patients).

Each patient received a detailed auxologic evaluation on a yearly basis, with evaluation of weight and length and of thyroid function tests. Growth hormone and insulin growth factor 1 levels were assessed when indicated.

Neurologic assessment was performed every 2 years, and respiratory function tests were performed at least once in children older than 7 years of age. Assessment of vision and hearing (audiometric tests and, when necessary, auditory evoked potentials) were performed before admission to school, unless differently indicated. To evaluate quality of life, a questionnaire was administered to the children's parents by means of telephone or at the time of a yearly control visit after obtaining informed consent, as approved by the institutional review board. Data were collected for all 40 children and included analysis of their lifestyle, ability to interact with other children and adults, and attendance and proficiency at school.

This study was approved by the Institutional Review Board at Spedali Civili, Brescia, Italy, and informed consent was obtained from patients, legal guardians, or both, as appropriate.

Immunologic investigations

Laboratory analysis of immune reconstitution included assessment of humoral and cellular immunity, as previously described.⁵

CD3⁺ T-lymphocyte counts of less than 1000/ μ L, CD3⁺CD4⁺ counts of less than 500/ μ L, CD19⁺ B-lymphocyte counts of less than 50/ μ L, and natural killer cell counts of less than 50/ μ L were considered abnormally low, as previously reported.⁵

In vitro proliferative response to PHA was considered normal at 35,000 or more counts per minute, with the mean value \pm 2 SDs in 40 control subjects being 156,029 \pm 120,768. The analysis of T-cell repertoire included evaluation of the complexity of the repertoire by means of heteroduplex analysis at the various TCRBV families. In this assay amplified products of TCRBV chains migrate in polyacrylamide gels as smears, whereas homoduplex or heteroduplex bands indicate an oligoclonal T-cell repertoire.¹² Thymic output was measured by evaluating signal-joint T-cell receptor excision circles (TRECs).¹³ TRECs were enumerated per 10⁶ PBMCs, taking into account that 1 μ g of DNA can be obtained from 150,000 PBMCs.⁹ The mean number of TRECs (\pm 2 SDs) in 26 healthy age-matched control subjects (age range, 6-17 years) was 81,790 \pm 68,058/10⁶ PBMCs. Accordingly, levels of TRECs were considered abnormally low at less than 13,700/10⁶ PBMCs.

Humoral immunity was assessed by measuring serum immunoglobulins and antibody responses to immunization with tetanus toxoid, hepatitis B vaccine, and measles-mumps-rubella (MMR) vaccines. Patients were immunized with toxoids, inactivated vaccines, or recombinant products not earlier than 1 year after HSCT, provided that they were no longer receiving intravenous immunoglobulins (IVIGs). Live attenuated vaccines were administered when there was evidence of normal cellular and humoral immunity.

Chimerism analysis was performed by means of DNA analysis at highly polymorphic loci (D1S80, HLA-DQ α , ApoB).

Antinuclear antibodies and Coombs' tests were performed on a yearly basis. Other autoantibodies were investigated depending on the clinical status of the patients.

Statistical analysis

Depending on the nature of the variables examined, the Student *t* test, the Wilcoxon rank sum test for nonparametric analysis, the χ^2 test, and percentile distribution were used for statistical analysis.

RESULTS

Long-term survival and immunologic reconstitution

Overall, of the 58 patients with severe T-cell immunodeficiency treated with HSCT at our center in the period from March 1, 1991, to June 30, 2002, all 42 subjects who reached at least 5 years of follow-up are currently alive (72.4% survival rate), with a median follow-up of 132 months (range, 68-196 months). Survival was 90% (9/10) for recipients of MSD-HSCT, 60.6% (20/33) for recipients of MMRD-HSCT, 83.3% (10/12) for recipients of MUD-HSCT, and 3/3 for recipients of PIRD-HSCT.

Immunologic abnormalities were observed in a minority of the 40 long-term survivors for whom detailed information is available (Tables I and II).

Matching between recipient and donor did not significantly affect T-cell reconstitution because the absolute number of CD3⁺, CD4⁺, CD8⁺, and CD4⁺CD45RA⁺ cells was not significantly different in recipients of MSD-HSCT versus recipients of MMRD-HSCT or MUD-HSCT. Moreover, analysis of the kinetics of immune reconstitution in these 3 groups of patients was

TABLE I. Characteristics of patients and long-term immune reconstitution after HSCT

Patient no.	Diagnosis/defective protein	Age at HSCT (mo)	CR	Donor	Years after HSCT	ALC (μ L)	CD3 (μ L)	CD4 (μ L)
1	γ c	10	None	MSD	16.3	2874	2385	775
2	IL7R	8	None	MSD	16.2	2320	1669	846
3	JAK3	11	None	MSD	16.1	1280	853	517
4	FOXN1	4	ATG	MSD	13.2	990	641	354
5	γ c	6	None	MSD	11.1	2723	2083	955
6	T-cell activ def	12	Bu/Cy	MSD	11.0	3955	3167	1125
7	ADA	7	None	MSD	9.8	1616	1290	483
8	Omenn (RMRP)	9	Bu/Cy	MSD	9.0	1210	668	365
9	T-cell activ def	34	Bu/Cy/TT	PIRD	11.2	3670	2521	1460
10	IL7R	5	Bu/Cy	PIRD	7.1	3170	2133	1191
11	JAK3	3	Bu/Cy	MMRD	15.1	1510	1270	504
12	JAK3	4	ATG	MMRD				
		7	ATG/Cy	MMRD	14.6	7440	7402	915
13	JAK3	8	ATG	MMRD				
		10	Bu/Cy	MMRD	13.5	1360	979	338
14	Artemis	4	ATG/Cy/TT	MMRD	13.3	3870	2580	1460
15	JAK3	4	Bu/Cy	MMRD	11.9	1920	1271	802
16	γ c	IUT	None	MMRD	11.2	2620	2033	691
17	Omenn (RAG)	7	Bu/Cy/TT/ATG	MMRD	11.0	1600	1326	515
18	RAG	18	Bu/TT/Cy	MMRD	10.9	2439	1968	1024
19	JAK3	7	None	MMRD				
		9	Bu/Cy	MMRD	10.7	2938	2303	1128
20	Omenn (RAG)	12	Bu/Cy/TT/ATG	MMRD	10.3	2290	1798	804
21	IL7R	IUT	None	MMRD	9.7	2980	1722	754
22	T-B-NK ⁺	6	Bu/Cy/TT	MMRD	9.2	1650	1311	597
23	Omenn	4	Bu/Cy	MMRD				
		5	Cy/ α -CD3	MMRD				
		10	Flu/Mel/TT/ATG	MMRD	8.8	2720	1618	574
24	γ c	6	Bu/Cy/TT	MMRD	8.1	3750	2955	1676
25	T-B ⁺	2	Bu/Cy	MMRD	7.7	1200	918	448
26	T ^{low} B ⁺	26	Bu/Cy/TT/ATG	MMRD	6.8	3650	2576	1899
27	JAK3	7	Bu/Cy	MMRD				
		10	Flu/Mel/ATG	MMRD	6.6	5280	3643	2040
28	γ c	IUT	None	MMRD	6.3	3540	2330	580
29	γ c	8	Bu/Cy	MMRD	6.2	2400	2040	1008
30	T-B-	3	Flu/Mel/ATG	MMRD	5.7	4490	3187	1625
31	Omenn (RAG)	8	Bu/Cy/VP16	MUD	14.6	3100	2182	1370
32	γ c	1	ATG	MUD	13.5	1770	1584	330
33	JAK3	12	Bu/Cy	MUD†	13.1	2020	1705	844
34	Omenn (RAG)	8	Bu/Cy/ATG	MUD	13.0	2767	2042	1184
35	T-B ⁺	7	Bu/Cy	MUD	12.3	2810	1964	1360
36	Artemis	7	Bu/Cy	MUD	11.3	3790	3236	1500
37	RAG	11	Bu/Cy/ATG	MUD	9.6	1470	1317	408
38	JAK3	10	Bu/Cy	MUD	9.1	1470	1230	595
39	γ c	10	Bu/Cy/ATG	MUD	7.1	4520	3200	1640
40	JAK3	27	Bu/Cy/ATG	MUD	6.9	3700	2120	1213

The proliferative response to PHA was considered normal at 35,000 cpm or greater (for all age groups). The normal value for TRECs is 13,700/10⁶ PBMCs or greater.

ALC, Absolute lymphocyte count; H, host; D, donor; IL7R, IL-7 receptor; M, mixed; FOXN1, forkhead box N1; T-cell activ def, T-cell activation deficiency; Bu, busulfan; Cy, cyclophosphamide; TT, thiotepa; IUT, intrauterine transplantation; Flu, fludarabine; Mel, melphalan; Und., undetectable.

*After treatment with rituximab for autoimmune hemolytic anemia.

†This patient received a T cell-depleted MUD HSCT.

also similar (see Fig E1 in the Online Repository available at www.jacionline.org).

Fourteen (35%) of the 40 patients had low TREC levels (<13,700/10⁶ PBMCs) at their last follow-up. When patients were grouped according to thymic output at long-term follow-up, those with low TREC levels had

significantly fewer CD4⁺ and CD4⁺CD45RA⁺ cells than patients with normal TREC levels (Fig 1, A and B).

Oligoclonality of the T-cell repertoire was demonstrated at the last follow-up in 11 (27.5%) of 40 patients (Table II). Five (45.4%) of 11 patients who received no CR or a nonmyeloablative CR had an oligoclonal T-cell

TABLE I. (Continued)

CD8/ μ L	CD4		CD16/ μ L	TREGs ($\times 10^6$ PBMCs)	PHA (cpm $\times 10^3$)	IVIG	Chimerism		
	CD45RA/ μ L	CD19/ μ L					Myeloid cell	T cell	B cell
1549	413	364	97	1,611	149	No	H	D	H
563	147	314	282	10,881	137	No	H	D	H
256	11	170	184	16,447	66	No	H	M	H
241	8	205	103	152	59	No	H	D	H
876	585	606	71	44,062	136	No	H	M	H
1615	545	410	217	100,817	172	No	D	D	D
345	140	220	68	37,822	101	No	M	D	M
202	125	164	290	3,960	118	No	H	M	M
928	891	844	256	10,684	231	No	D	D	D
706	773	431	513	329,340	208	No	H	D	H
627	234	172	31	4,157	76	No	H	D	M
6316	15	29	22	3,060	17	Yes	H	D	H
503	137	293	70	11,748	178	No	M	M	M
909	1450	885	325	33,551	141	No	D	D	D
403	539	326	286	67,751	245	No	D	D	D
1190	57	390	99	617	217	Yes	H	D	H
662	60	Und.*	262	7,544	146	No	H	D	M
690	529	260	129	79,099	282	No	D	D	D
951	393	428	123	43,390	167	No	H	D	H
845	506	293	172	328,664	220	No	D	D	D
717	354	472	708	237,427	194	No	H	D	H
506	251	163	186	11,594	219	No	M	D	M
508	87	533	456	2,622	57	No	D	D	D
956	1264	731	41	105,643	118	Yes	M	D	D
347	340	212	24	133,636	262	No	D	D	D
469	1447	435	244	110,010	232	No	D	D	D
1362	897	696	728	141,499	283	No	D	D	D
1165	160	1040	32	7,098	90	Yes	H	D	H
900	648	230	93	226,187	165	No	D	D	D
1508	695	305	664	173,194	75	No	D	D	D
660	852	598	340	73,735	205	No	D	D	D
844	127	152	19	9,536	171	Yes	H	D	H
741	480	244	77	127,851	170	No	M	D	H
442	734	439	254	51,892	150	No	D	D	D
483	880	548	228	125,947	135	No	D	D	D
1580	826	356	170	117,724	289	No	D	D	D
873	41	5*	145	1,630	81	No	H	D	M
527	223	163	61	234,429	138	No	M	D	D
1120	1150	866	366	229,124	203	No	D	D	D
614	828	1135	236	70,250	126	No	M	D	M

repertoire; in contrast, a restricted T-cell repertoire was observed only in 6 (29.7%) of 29 patients who received a more aggressive CR. Finally, only 1 patient showed a reduced *in vitro* proliferative response to PHA at the last follow-up, indicating that even in subjects with low thymic output, T-cell function was grossly normal.

Engraftment of donor-derived myeloid and B cells was associated with use of myeloablative CR. In particular, of 11 patients who received no CR or who were treated with ATG (as a single agent in 1 patient and with cyclophosphamide in another patient), 10 showed autologous B-cell reconstitution. In contrast, among 29 patients who

TABLE II. Clinical and immunologic abnormalities at more than 5 years after HSCT in 40 children with severe T-cell immunodeficiency

Abnormalities	Proportion (%) of patients with defects
Clinical problems	
Growth insufficiency (weight \leq 3rd percentile)	7/40 (17.5)
Low stature (height \leq 3rd percentile)	5/40 (12.5)
Endocrine abnormalities	7/40 (17.5)
Severe neurologic problems	4/40 (10.0)
Hearing abnormalities	2/40 (5.0)
Significant infections at >1 y after HSCT	5/40 (12.5)
Hospitalizations after first year after HSCT	8/40 (20.0)
Current need for IVIGs	5/40 (12.5)
Laboratory abnormalities	
ALC $<1500/\mu\text{L}$	6/40 (15.0)
CD3 ⁺ T cells $<1000/\mu\text{L}$	5/40 (12.5)
CD3 ⁺ CD4 ⁺ T cells $<500/\mu\text{L}$	7/40 (17.5)
CD4 ⁺ CD45RA ⁺ cells $<200/\mu\text{L}$	13/40 (32.5)
CD3 ⁺ CD8 ⁺ cells $<300/\mu\text{L}$	3/40 (7.5)
CD19 ⁺ B cells $<50/\mu\text{L}$	3/40 (7.5)
CD16/56 ⁺ NK cells $<50/\mu\text{L}$	6/40 (15.0)
PHA response $<35,000$ cpm	1/40 (2.5)
TRECs $<13,700/10^6$ PBMCs	14/40 (35.0)
Oligoclonal T-cell repertoire	11/40 (27.5)
IgG <500 mg/dL	0/35* (0)
IgA <35 mg/dL	3/40 (7.5)
IgM <50 mg/dL	5/40 (12.5)
Anti-TT antibody titer <0.1 IU/mL	7/40 (17.5)

ALC, Absolute lymphocyte count; TT, tetanus toxoid.

*Not including 5 patients undergoing substitution therapy with IVIGs.

received myeloablative CR, autologous B-cell and myeloid cell reconstitution was observed in only 3 and 6 patients, respectively (Table I). The difference between myeloablative versus nonmyeloablative CR in determining engraftment in lineages other than T cells was statistically significant ($P < .001$).

Sustained myeloid engraftment (defined as a proportion of donor-derived granulocytes $\geq 10\%$) was associated with higher values of TRECs (Fig 1, C). However, even among the 16 patients with autologous myeloid reconstitution, 5 had normal levels of TRECs. All of these patients had defects in the IL-7-mediated signaling pathway.

Only 5 (12.5%) of 40 patients require IVIGs. Interestingly, all of these have defects of the γc -JAK3 signaling pathway, and 4 of them have received no CR or a nonmyeloablative preparative regimen. The fifth patient (patient 24) originally had a normal ability to produce antibodies and maintains normal IgA and IgM levels, indicating that his B-cell immunity is not absent.

Of 40 patients, 34 (85%) have produced protective titers of antibodies to tetanus toxoid and hepatitis B surface antigen on immunization, but 1 of them (patient 24) subsequently has shown a marked decrease in antibody titers and failed to respond to boosting immunizations. Overall, a sustained ability to produce antibodies to tetanus

toxoid and hepatitis B surface antigen has been observed in 7 of 8 recipients of MSD-HSCT, 15 of 20 patients treated with MMRD-HSCT, 9 of 10 recipients of MUD-HSCT, and in the 2 patients treated with PIRD-HSCT.

Twenty-eight subjects have been also immunized to MMR, and for all 26 in which information is available, there is evidence of protective anti-measles antibodies.

Growth and development

Most patients have attained satisfactory growth and development after HSCT (for detailed information, see Table E3 in the Online Repository available at www.jacionline.org). In particular, weight and height that are greater than the third and less than the 97th percentiles have been reached in 31 (77.5%) and 33 (82.5%) of the patients, respectively. As shown in Table II, 7 (17.5%) patients have a weight in the third percentile or less, and none of them requires enteral or parenteral nutrition. Poor statural growth (height in the third percentile or less) was observed in 5 (12.5%) patients, 4 of whom also have low weight. Among the patients with growth failure/delay, one (patient 14) has severe encephalopathy. Two patients (patients 20 and 37) with short stature have received high-dose steroids for a protracted period because of severe autoimmune manifestations.

Endocrine abnormalities have been observed in 7 patients. Among these, post-HSCT autoimmune thyroid disease has been observed in 4 patients (3 with hypothyroidism and 1 with hyperthyroidism). Congenital hypogonadotropic hypogonadism (leading to poor growth and requiring substitution therapy with growth hormone), congenital hypoparathyroidism (requiring vitamin D supplementation), and primary pretransplantation hypothyroidism have been observed in 1 patient each. Fourteen subjects have completed pubertal development and have acquired mature secondary sexual traits. Dental abnormalities have been observed in 3 patients, 2 of whom have evidence of molar agenesis, whereas 1 patient has enamel hypoplasia.

Sensorineural development

Most patients have achieved normal sensorineural development (Table II and Table E3 in the Online Repository at www.jacionline.org). Neurologic abnormalities, behavioral abnormalities, or both have been observed in 6 patients and are severe in 4 patients. Patient 7, with adenosine deaminase (ADA) deficiency, has severe hypotonia and cognitive impairment. Patient 14 has ataxia, hypotonia, and epilepsy and requires continuous treatment with antiepileptic drugs as the consequence of encephalitis that occurred early after HSCT. Patient 19 had epilepsy, tetraparesis, and severe encephalopathy and significant cognitive delay. These irreversible complications reflect a severe and protracted condition of central nervous system asphyxia that was associated with significant hypoxia and diffuse pneumonia before HSCT. Finally, patient 17, who received HSCT because of Omenn syndrome caused by recombinant activating gene (RAG) deficiency, has paraplegia. Interestingly, the

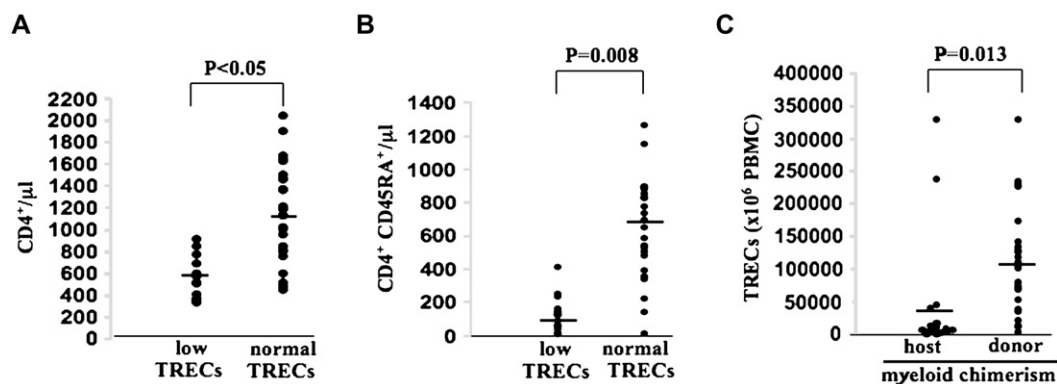


FIG 1. Long-term effect of thymic output and myeloid engraftment after stem cell transplantation for severe T-cell immunodeficiency. The numbers of CD4⁺ (A) and CD4⁺CD45RA⁺ (B) T cells at the last follow-up in patients with normal or low levels of TRECs are shown. C, TREC values in patients with autologous or donor-derived myeloid cells.

same condition is present in his younger brother, who has no evidence of immune deficiency and is heterozygous for RAG deficiency, suggesting that this patient had 2 genetically distinct rare conditions at the same time: SCID and familial paraplegia.

One patient (patient 39) has partial visual impairment in one eye caused by CMV-induced retinitis that occurred before HSCT, and 2 patients (patients 20 and 37) have initial cataracts.

Hearing abnormalities have been documented in 2 patients. In particular, patient 25 (who had an undefined form of T⁻B⁺ SCID) has congenital sensorineural deafness, whereas patient 22 has mild transmission hypoacusia.

All patients attend school, with regular progress in their careers. Three patients (patients 14, 19, and 20) require individualized support; in 2 of them (patients 14 and 19), achievement of cognitive abilities is limited by severe and irreversible neurologic problems.

Autoimmune manifestations, infections, and other significant clinical problems

Posttransplantation autoimmune manifestations, organ damage, graft-versus-host disease (GvHD), respiratory problems, hospitalization, other significant problems, and current need for drugs in the cohort of 40 patients are reported in Table E4 in the Online Repository (available at www.jacionline.org).

Clinically significant autoimmune complications after HSCT have been observed in 5 patients, 4 of whom had received MMRD-HSCT, whereas 1 patient had been treated with MUD-HSCT. Autoimmune hypothyroidism and autoimmune hemolytic anemia were the most frequently observed autoimmune manifestations, being recorded in 3 patients each. One patient had myasthenia gravis associated with autoantibodies to the acetylcholine receptor. Many of the autoimmune manifestations have responded well to immune suppressive treatment with steroids, but 2 patients have required rituximab. None of the 5 patients with autoimmunity had evidence of oligoclonal T-cell repertoire.

Significant organ damage was observed in a minority of patients. In many of these, the organ damage reflected congenital problems or complications that occurred before HSCT. Five patients had organ damage because of posttransplantation complications. Patient 14 had encephalitis early after HSCT; patient 23 has right eye keratopathy caused by acute GvHD, whereas 3 patients (patients 12, 16, and 32) had bronchiectasis, obstructive lung disease, or both after respiratory tract infections. These 3 patients show evidence of lack of antibody production and require regular IVIG substitution therapy.

Four patients (patients 14, 23, 26, and 35) had significant (grade ≥ 3) acute GvHD. None of the patients had chronic GvHD.

Apart from regular clinical and laboratory controls, 7 patients have required inpatient hospital care in the period after the first year after HSCT. Four patients have required hospitalization because of bacterial (patients 12, 16, and 24) or *Pneumocystis jiroveci* (patient 37) pneumonia. In the latter patient, the opportunistic pneumonia followed immune suppressive treatment with high-dose steroids and rituximab because of autoimmunity.

Seven patients had warts after HSCT. These include not only patients with γc (patient 16) or with Janus kinase 3 (JAK3; patients 11, 13, and 19) deficiency but also patients with RAG defects (patients 18 and 33) and with IL-7 receptor deficiency (patient 10). However, severe warts (defined as >30 lesions persisting for at least 2 years during follow-up)¹⁰ were present only in patient 19, who had JAK3 deficiency.

Currently, 24 (60%) of 40 patients do not require any treatment. Six (15%) patients require prophylaxis of infections with IVIGs, antibiotics, or both. Thyroid hormone replacement therapy is used in 4 patients with subclinical hypothyroidism, whereas 3 other subjects require treatment with antiepileptic drugs. In 1 patient (patient 25) use of ursodiol and omeprazole is needed because of portal hypertension associated with portal cavernoma, a complication of umbilical vein catheterization at birth.

DISCUSSION

We have reported on the long-term immune reconstitution and clinical outcome in 40 patients with severe T-cell immunodeficiency who survived for 5 years or longer after HSCT. Overall survival after HSCT for the patients with severe T-cell immunodeficiency during the period considered was 72.4%, which is similar to that reported in other studies. Importantly, an 80% survival rate was observed among 15 patients with T-cell defects other than SCID, even if only 3 of these patients had an MSD. Our results confirm that HSCT is a very effective form of treatment for severe T-cell immunodeficiency, even in the absence of MSD, and demonstrate that it offers perspectives of good quality of life.

The vast majority of our patients had normal T-cell counts and function at late follow-up, which is in keeping with previous observations.^{3,9,14} Infants with SCID should theoretically be impaired in their ability to reject donor stem cells, and this argument has been used to avoid use of CR before MMRD-HSCT for SCID.³ Myers et al¹⁵ have clearly demonstrated that unconditioned MMRD-HSCT offers excellent perspectives of survival (95%) when performed in the neonatal period. This result might have significant clinical implications, especially if neonatal screening for SCID becomes available.^{16,17}

On the other hand, it has been shown that thymic output (as measured by levels of TRECs) decreases at greater than 10 years after unconditioned HSCT for SCID,^{6,7} raising some concerns about the long-term ability of this type of transplant to prevent infections. Such a decrease in thymic output was not observed by Borghans et al.⁹ However, in the latter study HSCT was preceded by CR in most patients. Cavazzana-Calvo et al¹⁴ have recently shown that use of CR before MMRD-HSCT for SCID allows multilineage engraftment and is associated with improved thymic output at greater than 10 years after transplantation. Our data support these latter observations.

Several studies have shown that reconstitution of humoral immunity after HSCT for SCID is often problematic. In the absence of CR, recipients of MMRD-HSCT for B⁺ SCID most often retain autologous B cells, and this is usually associated with the need for lifelong immunoglobulin replacement therapy.^{3,18-20} In our series B-cell engraftment was strongly associated with use of CR. Previous data from the European experience with HSCT for B⁺ SCID had failed to disclose a significant effect of use of CR in promoting B-cell engraftment.²¹ However, in that study most patients received low-dose busulfan (8 mg/kg) according to the protocol in use at that time, and this might have been insufficient to achieve myeloablation and B-cell engraftment.

In spite of the fact that 13 patients from our series retained autologous B cells, 35 of 40 patients no longer require substitution therapy with IVIGs, and 33 patients have produced protective titers of specific antibodies to immunization antigens that persist at last follow-up. Interestingly, all of the 5 who remain dependent on IVIGs share γ c or

JAK3 deficiency and have maintained autologous B cells. Buckley et al³ have reported that 27 of 34 recipient of unconditioned HSCT for X-linked SCID required immunoglobulin replacement therapy long-term after HSCT, and similar observations have been recently reported for patients with SCID caused by JAK3 deficiency.²⁰ The inability of autologous γ c- or JAK3-deficient B cells to sustain immunoglobulin production most likely reflects disturbed signaling through IL-4 and IL-21 receptors.^{22,23} Overall, it is likely that the ability to attain solid and sustained T- and B-cell function has resulted in the low incidence of severe infections observed in our cohort of patients.

The vast majority of patients in our series have gained normal weight and height, indicating lack of long-term transplant-related toxicity and absence of significant chronic disease. Abnormalities of endocrine function and growth are common after HSCT in the pediatric population.²⁴ In our series 7 (17.5%) patients have endocrine abnormalities, and 3 of them have had posttransplantation hypothyroidism. These data are in keeping with previous observations that primary hypothyroidism is the most common abnormality of the thyroid after HSCT²⁴⁻²⁶ and emphasize the importance of vigilance for thyroid disease after HSCT.

Neurologic abnormalities are present in 6 patients, including one with ADA deficiency. A high incidence of neurologic problems has been observed in patients with ADA-SCID during long-term follow-up after HSCT.^{11,27} Because of the generalized metabolic disturbance that is associated with ADA deficiency, nonimmunologic (and especially neurologic) complications are common in this disorder and require careful monitoring. On the other hand, viral infections of the central nervous system before transplantation have been shown to be relatively common in infants with SCID and might jeopardize the outcome of the procedure.²⁸ It is noteworthy that disseminated or isolated central nervous system infections were the cause of death in 5 patients undergoing transplantations at our center during the period from 1991 through 2002 and led to persistent sensorineural problems in 2 of the survivors.

All patients from our series have entered school and receive appropriate education, although 2 of them have significant neurologic problems that severely affect their cognitive development.

The majority of patients in our series live at home and do not require any form of treatment, indicating that HSCT for severe T-cell immunodeficiency might lead to a permanent cure. This is an important argument to take into account when considering the costs of the procedure. On the other hand, to expand on the cost/benefit analysis, additional studies are needed, in particular to compare long-term outcome in patients who receive CR versus those treated without chemotherapy.

Overall, this experience demonstrates that HSCT allows long-term survival and good quality of life in a remarkable proportion of children with severe T-cell immunodeficiency. These data are particularly encouraging in view of the possible development of newborn screening strategies for SCID.¹⁷

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