

Long-term results of bone marrow transplantation in complete DiGeorge syndrome

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Background: Therapeutic options for DiGeorge syndrome (DGS) with profound T-cell deficiency are very limited. Thymic transplantation has shown promising results but is not easily available. Hematopoietic cell transplantation (HCT) has been successful in restoring immune competence in the short term. **Objective:** Present the long-term follow-up of 2 patients with complete DGS who received bone marrow transplants in the neonatal period from HLA-matched siblings, and perform a multicenter survey to document the status of other patients with DGS who have undergone HCT.

Methods: Immune function assessment by immunophenotyping, lymphocyte proliferation, T-cell receptor excision circles, single nucleotide polymorphism mapping arrays, spectratyping, cytogenetics, and fluorescence *in situ* hybridization were used.

Results: Among reported patients with DGS receiving HCT, survival is greater than 75%. Our patients are in their 20s and in good health. Their hematopoietic compartment shows continuous engraftment with mixed chimerism, normal T-cell function, and humoral immunity. Circulating T cells exhibit a memory phenotype with a restricted repertoire and are devoid of T-cell receptor excision circles.

Conclusion: These features suggest that T-cell reconstitution has occurred predominantly through expansion of the donors' mature T-cell pool. Although restricted, their immune systems are capable of providing substantial protection to infection and respond to vaccines. We conclude that bone marrow transplant achieves long-lived reconstitution of immune function in complete DGS and is a good alternative to thymic transplantation in patients with a suitable donor.

Clinical implications: Bone marrow transplant in complete DGS using an HLA-matched sibling donor provides long-lasting immunity and is a suitable and more available alternative to thymic transplantation. (*J Allergy Clin Immunol* 2007;120:908-15.)

Key words: DiGeorge syndrome, hematopoietic cell transplantation, bone marrow transplantation, immune deficiency, long-term outcome, treatment

DiGeorge syndrome (DGS) is a genetic disease whose phenotypic expression affects the heart, thymus, and parathyroid glands to varying degrees and is associated with a deletion of chromosome 22q11.2 in approximately 80% of patients.¹ *Complete DGS* refers to the subset of patients with severely impaired thymic function with profound T-cell deficiency.² In these infants, prompt reconstitution of the immune function is required to prevent fatal infectious complications. The optimal treatment for patients with complete DGS has not been established. Previous reports have demonstrated that reconstitution of the immune function can be achieved via thymic³⁻⁵ or hematopoietic cell transplantation.⁶⁻¹⁴ From a physiological standpoint, thymic transplantation could be considered the best choice, but expertise in this procedure is limited. In contrast, hematopoietic cell transplantation (HCT) is readily available in most tertiary care centers, making it an attractive alternative.

In 1987 and 1989, we reported 2 unrelated patients with complete DGS who had profound immune deficiency and were treated with unconditioned bone marrow transplantation (BMT) from their HLA-similar brothers (1 with an HLA-DR mismatch⁶; the other was HLA-identical⁷). Since then, others have reported similar experiences (Table I).⁸⁻¹⁴ Most of these patients have shown recovery of T-cell function that was maintained for at least 1 year after transplantation. In this report, we describe the clinical course and the current immune function of 2 patients with DGS who were transplanted more than 2 decades ago. They lead normal lives free of serious infections and have preserved immune function. This is the first report of extended survival in complete DGS treated with BMT with long-lasting immune reconstitution.

METHODS

Immunophenotyping and lymphocyte proliferation

Flow cytometry was performed on whole blood by standard methods. Absolute numbers of cells were based on absolute lymphocyte counts, and percentages of positive cells were calculated as well. Lymphoproliferative responses to phytohemagglutinin (PHA),

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Abbreviations used

BMT:	Bone marrow transplantation
CPM:	Counts per minute
DGS:	DiGeorge syndrome
FISH:	Fluorescence <i>in situ</i> hybridization
HCT:	Hematopoietic cell transplantation
PHA:	Phytohemagglutinin
ROMA:	Representational oligonucleotide microarray analysis
SI:	Stimulation index
SNP:	Single nucleotide polymorphism
TCR:	T-cell receptor
TREC:	T-cell receptor excision circle
UCB:	Umbilical cord blood

concanavalin A, pokeweed mitogen, tetanus, and *Candida* antigens were assessed by tritiated thymidine uptake and reported as total stimulation or the stimulation index (SI; counts per minute [CPM] with mitogen divided by CPM with media).

Analysis of T-cell receptor excision circles

T-cell receptor excision circle (TREC) analysis was performed on isolated DNA from whole blood and signal joint TREC copy number quantitated by real-time PCR.¹⁵ The lower limit of detection for the assay is <10 TRECs/ μ L.

Single nucleotide polymorphism mapping arrays

Representational oligonucleotide microarray analysis (ROMA) is a variant of array comparative genomic hybridization that uses oligonucleotides rather than bacterial artificial chromosomes.^{16,17} Briefly, DNA was isolated from whole blood, digested with *NspI*, and amplified in a single primer PCR amplification. The resulting PCR products were fragmented, labeled, and hybridized to Affymetrix *NspI* single nucleotide polymorphism mapping arrays (Affymetrix, Santa Clara, Calif). The *NspI* array contains 262,264 single nucleotide polymorphisms, of which 128 are in the DiGeorge typically deleted region. Signal intensity at each feature was determined using a modification of the circular binary segmentation algorithm.^{17,18}

Spectratyping

RNA was prepared by using Dynabeads mRNA Direct Micro Kit (Invitrogen, Carlsbad, Calif) according to the manufacturer's instructions. Total RNA was converted to cDNA by standard reverse transcription. The quality of cDNA was measured by PCR amplification of serial dilutions of cDNA using T-cell receptor (TCR) constant region primers.¹⁹ Spectratyping was performed by PCR amplification of the complementarity determining region 3 (CDR3) using TCR V- β and C- β specific primers. The samples were run on an ABI 3100 sequencer using fragment analysis mode (Applied Biosystems, Foster City, Calif). The intensity data from the ABI 3100 were visualized by using proprietary software.

Cytogenetics and *in situ* hybridization analysis

Standard cytogenetic analyses were performed on cultured peripheral blood samples, and 20 G-banded metaphases were analyzed for both patients.

Fluorescence *in situ* hybridization (FISH) analysis was possible only on cells from patient 2. Testing for the engraftment was

performed with commercially available (Abbott-Vysis, Des Plaines, Ill) dual-color fluorescent probes specific for the centromeric regions of chromosome X and the sex-determining region on the Y-chromosome following the manufacturer's recommendations. Approximately 200 interphase and metaphase cells were analyzed by using a fluorescent microscope.

Patient 2 was not known to have a DGS-specific deletion at 22q11.2 at the time of diagnosis. To identify these cells posttransplant, FISH analyses with the TUPLE1 probe, known to be deleted in a majority of patients with DGS, were performed. One hundred metaphase and interphase cells were analyzed to calculate the percent cells with a deletion of the 22q11.2 (DGS) locus. This study was performed from the same population of cells used for the XX/XY engraftment study.

RESULTS

Patient 1

Clinical course. Patient 1 was a term female infant who presented in the neonatal period with hypocalcemic seizures.⁶ She had dysmorphic features (low-set ears, shortened philtrum, arched palate), and a heart murmur caused by pulmonary stenosis and a ventricular septal defect.

Initial immunologic evaluation showed a normal total lymphocyte count with 24% T cells and 17% B cells, but no lymphoproliferative responses to mitogens (Table II). Serum IgG and IgM were normal, and IgA was undetectable. Over the next few months, despite stable cardiac and metabolic status, the patient failed to thrive and developed chronic diarrhea and oral and genital candidiasis. At 4 months of age, serum IgG levels declined to 170 mg/dL, without functional antibody production. Circulating T cells declined to 1%, and lymphoproliferative responses to mitogens and antigens remained absent. Analyses for nucleoside phosphorylase and adenosine deaminase were both normal, and she had a normal 46 XX karyotype.

Because of the patient's worsening clinical and immunologic status, immune reconstitution was deemed necessary. Because a thymus was unavailable and the patient had an HLA-compatible brother, a BMT was performed at 28½ weeks of age. The patient received 1.2×10^9 nucleated marrow cells per kilogram from her brother without any pretransplant conditioning or posttransplant immunosuppression.

After transplantation, the patient experienced good weight gain and accelerated maturation with stable cardiac status. One year posttransplant, the patient developed *Shigella* gastroenteritis treated with oral trimethoprim-sulfamethoxazole. She later underwent several cardiac procedures to treat her congenital heart problems.

At age 14 years, the patient was lost to follow-up for the next 9 years. During this period, she had a few respiratory infections and an uncomplicated case of varicella. In 2003, she had pneumonia requiring treatment with intravenous antibiotics. In 2005, she developed a community acquired pneumonia and was admitted at our institution with a loculated pleural effusion. At this point, we re-established contact with the patient. She was 24 years old then. On examination, she was a pleasant, well developed female

TABLE I. Hematopoietic cell transplantation in DGS

Author (date)	Age at transplant, sex	Transplant type	Pretransplant data	Posttransplant results*	Long-term follow-up
Goldsobel et al (1987) ⁶	28.5 wk, female	Bone marrow, HLA-DR mismatch Unconditioned	CD3 ⁺ cells: 28/uL IgG/A/M: 170/<2/90	CD3 ⁺ cells: 1398/uL IgG/A/M: 790/50/60	Alive at 24 y, XY karyotype, memory T-cell predominant, skewed TCR repertoire
Borzy et al (1989) ⁷	5 mo, female	HLA-identical bone marrow Unconditioned	CD3 ⁺ cells: 0/uL IgG/A/M: 198/<6/16	CD3 ⁺ cells: 1119/uL IgG/A/M: 1027/369/50	Alive at 21 y, XY karyotype, memory T-cell predominant, skewed TCR repertoire
Matsumoto et al (1998) ⁸	13 and 19 mo, male	HLA-identical bone marrow 2nd preconditioned (Busulfan/cyclophosphamide)	CD3 ⁺ cells: 5/uL IgG/A/M: 372/8/54	CD3 ⁺ cells: 864/uL IgG/A/M: n/a	Died 10 mo after BMT at home of unknown cause†
Bowers et al (1998) ⁹	7 wk, male	HLA-identical PBMCs Unconditioned	CD3 ⁺ cells: 45/uL IgG/A/M: n/a	CD3 ⁺ cells: 315/uL IgG/A/M: n/a	Unknown‡
Bensoussan et al (2002) ¹⁰	4 y, male	HLA-identical PBMCs Unconditioned	CD3 ⁺ cells: 166/uL IgG/A/M: n/a	CD3 ⁺ cells: >400/uL IgG/A/M: n/a	Alive at 16 y, mixed T-cell chimerism, reversed CD4/CD8 ratio, skewed TCR repertoire§
Ohtsuka et al (2004) ¹¹	3 mo, male	Cord blood stem cells No preconditioning	CD3 ⁺ cells: 5/uL IgG/A/M: n/a	CD3 ⁺ cells: 1690/uL IgG/A/M: n/a	Died at 253 d from systemic cytomegalovirus
Hoening et al (2004) ¹²	4 mo, male	HLA-identical PBMCs Unconditioned	CD3 ⁺ cells: 0/uL IgG/A/M: n/a	CD3 ⁺ cells: 500-1000/uL IgG/A/M: n/a	Alive at 2 y, T cells donor origin by chimerism analysis, TCR repertoire diverse
Al-Tamemi et al (2005) ¹³	6 mo, male	HLA-matched bone marrow Unconditioned	CD3 ⁺ cells: 150/uL IgG/A/M: 520/130/70	CD3 ⁺ cells: 1200/uL IgG/A/M: 800/125/75	Alive at 3.5 y, 95% CD3 ⁺ donor cell chimerism, memory T cell predominant, Normal CD4/CD8 ratio¶
Janda et al (2007) ¹⁴	6 and 7 mo, male	8/10-Matched bone marrow Unconditioned	CD3 ⁺ cells: 2/uL IgG/A/M: 540/40/60	CD3 ⁺ cells: 1600/uL IgG/A/M: 320/140/40	Alive at 26 mo, memory T-cell predominant, normal CD4/CD8 ratio

n/a, Not available.

*Posttransplant results are latest available data for each patient.

†Long-term follow-up data obtained from personal communication with Dr Tadashi Matsumoto (April 15-16, 2007).

‡Patient moved away, per personal communication with Dr Daniel Bowers (April 7, 2006).

§Long-term follow-up data obtained from personal communication with Dr Danielle Bensoussan (July 6, 2006).

||Long-term follow-up data being published elsewhere, per personal communication with Dr Manfred Hoening (May 4, 2007).

¶Long-term follow-up data obtained from personal communication with Dr Nada Jabado (June 28, 2007).

with a weight of 49.1 kg. She had mild cervical lymphadenopathy. Her cardiovascular and pulmonary examinations were consistent with her pneumonia and her corrected heart disease. She had moderate clubbing and mild cyanosis. The pleural effusion was drained by video-assisted thoracoscopic surgery. Recovery was uneventful. The patient has been well since then.

Engraftment. Engraftment of patient 1 was confirmed with peripheral blood karyotyping. Twenty-four years after BMT from her brother, the patient's karyotype was

found to be 46, XY. There was no evidence of female cells (XX) in any of the metaphases analyzed. However, when ROMA was performed by using DNA isolated predominantly from granulocytes, there were 2 X chromosomes present, and chromosome 22 showed the typically deleted region at 22q11.2 (Fig 1).

Immunologic studies. A summary of the immunologic studies performed at the time of our last evaluation is presented in Tables II and III. For comparison purposes, we also include the results of 2 previous determinations,

TABLE II. Patient 1 data

Test	1 Month pretransplant*	1 Year posttransplant*	23 Years posttransplant
CD3 cells/ μ L (%)	28 (1%)	1398 (39%)	747 (69%)
CD4	0	502 (14%)	421 (39%)
CD8	56 (2%)	502 (14%)	332 (31%)
PHA SI (CPM mitogen/CPM media alone)	0	91	117
IgG (mg/dL)	280	790	2220†
IgA (mg/dL)	3	50	251†
IgM (mg/dL)	60	60	137†
IgE (IU/mL)	15	8	11
Diphtheria antibody (U/mL)	ND	6.52	ND
Pneumococcal antibody (prevaccine to postvaccine)	ND	ND	5/12 to 10/12 serotypes
Varicella zoster antibody status	ND	ND	Positive
TREC analysis	ND	ND	Undetectable
Karyotype	46 XX	46 XX, XY chimera	46 XX, XY chimera

ND, Not done.

*Data obtained from original publication.⁶

†Normal adult ranges for IgG, 690-1660; IgA, 80-400; IgM, 37-318.

1 before and another 1 year after transplantation. In 2006, 23 years after undergoing an HLA-matched BMT, she had a normal white cell count of 7300 cells/ μ L, with a differential of 66% neutrophils and 21% lymphocytes. T lymphocytes were within the normal range with a conserved CD4:CD8 ratio. T-cell function was preserved, as evidenced by the strong proliferative responses to both mitogens and antigens (tetanus SI 24 vs 63 in control; candida SI 24 vs 89 in control). She had a high serum IgG, normal levels of IgA and IgM, and protective antibody titers to tetanus toxoid and varicella. She responded to the pneumococcal polysaccharide vaccine with an increase from 5/12 to 10/12 protective serotypes. Advanced T-cell subsets showed a predominance of the memory T-cell phenotype in the CD4⁺ population, with a CD4⁺CD45RO⁺CD45RA⁻ cell percentage of 93% versus 64% in a control patient. The patient's CD8⁺ cells also showed an increased percentage of memory cells compared with the control. There is no established reference range for the distribution of memory or naive T cells in normal hosts, but the differential increase in the memory phenotype is likely to reflect the patient's depressed/absent thymic function. Consistent with this lack of thymic function was the observation that there were no detectable TRECs.

Patient 2

Clinical course. Patient 2 was a term infant female with multiple dysmorphic features (low-set ears, narrow palpebral fissures, micrognathia, and a high-arched palate) who presented with hypocalcemic seizures at 3 days of life.⁷ A heart murmur present was caused by an atrial septal defect and an aortic arch anomaly. She developed candidal infections, cough, diarrhea, and failure to thrive.

As shown in Table IV, her initial immunologic evaluation revealed a virtual absence of lymphocytes. CD20⁺ cells were 2387 cells/ μ L (78%).⁷ Serum IgG and IgM were low-normal for age, and IgA was undetectable.

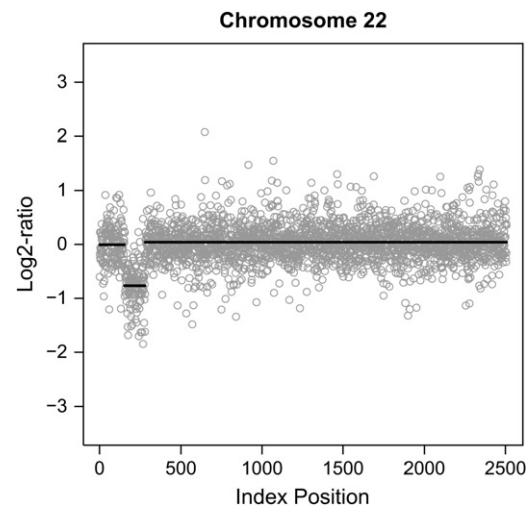


FIG 1. ROMA results from DNA isolated from whole blood showing the typically deleted 22q11.2 region in patient 1.

Adenosine deaminase and nucleoside phosphorylase levels were normal. She had a normal 46, XX karyotype.

The patient continued to have persistent infections and failure to thrive. At 5 months of age, she received a transplant of 4.3×10^8 nucleated bone marrow cells/kg from her HLA-identical brother (1.94×10^9 total). There was no pretransplant conditioning or posttransplant immunosuppression.

The candidiasis resolved after transplantation, and there was no clinical or laboratory evidence of graft-versus-host disease. Inguinal lymph nodes were palpable at 4 months and cervical nodes at 7 months post-BMT. The patient had a few episodes of otitis media and a mild case of respiratory syncytial virus bronchiolitis. Ten months later, she had repair of her atrial septal defect, followed by improved weight gain and appetite. A thymus was not seen at the cardiac surgery.

Over the period of the next 20 years, the patient had no serious infections. She had uncomplicated varicella at

TABLE III. Current memory versus naive T-cell subsets

Test	Patient 1	Patient 2	Control
CD4 ⁺ CD45RO ⁺ CD45RA ⁻	93%	96%	64%
CD4 ⁺ CD45RO ⁺ CD45RA ⁺	4%	2%	7%
CD4 ⁺ CD45RO ⁻ CD45RA ⁺	2%	2%	29%
CD8 ⁺ CD45RO ⁺ CD45RA ⁻	43%	67%	35%
CD8 ⁺ CD45RO ⁺ CD45RA ⁺	16%	13%	7%
CD8 ⁺ CD45RO ⁻ CD45RA ⁺	41%	20%	58%

TABLE IV. Patient 2 data

Test	2 Months pretransplant*	17 Months posttransplant*	21 Years posttransplant
CD3 cells/ μ L (%)	0	1606 (34%)	1119 (65%)
CD4	0	368 (8%)	431 (25%)
CD8	398 (13%)	1134 (24%)	637 (37%)
PHA stimulation (CPM)	300	105,100	ND [‡]
IgG (mg/dL)	198	387	1027 [†]
IgA (mg/dL)	<6	<6	369 [†]
IgM (mg/dL)	16	40	50 [†]
IgE (IU/mL)	<2.4	<2.4	<2
Tetanus SI (CPM in stimulated/CPM in unstimulated)	ND	5.0	ND
TREC analysis	ND	ND	Undetectable
Karyotype	46 XX	46 XX,XY chimera	46 XX,XY chimera

ND, Not done.

*Data obtained from original publication.⁷[†]Normal adult ranges for IgG, 690-1660; IgA, 80-400; IgM, 37-318.[‡]At last evaluation in 1999, the patient had PHA stimulation of 172,336 CPM versus control, which had 138,596 CPM.

age 7 years. As of her last evaluation in June 2007, she continues to be in good health.

Engraftment. Engraftment of patient 2 was assessed by FISH analysis for sex-chromosome mismatch identification and the 22q11.2 deletion (Fig 2). Quantitative analysis revealed 21% of the cells with 2 X chromosome-specific signals (XX cells) in her peripheral blood. The same percentage of cells were also positive for the 22q11.2 deletion, suggesting that all DGS deleted cells were female cells (recipient's).

Immunologic studies. At the time of her last evaluation in June 2007 (21 years posttransplantation), the patient had a normal white blood cell count with a differential of 79% neutrophils and 21% lymphocytes. The results of the rest of the immunologic work-up are shown in Tables III and IV. Her lymphocyte counts were in the low-normal range, but with an inverted CD4:CD8 ratio and a clear predominance of the memory phenotype in the CD4 subset compared with adult controls. TRECs were undetectable. Serum immunoglobulins were normal with intact specific antibody responses.

V- β analysis. The T-cell repertoires in both patients were analyzed by using CDR3 spectratyping of peripheral blood T cells. In this approach, the repertoire is measured on the basis of V- β gene family utilization and further resolved on the basis of the CDR3 length for each family. In normal individuals, the distribution of the CDR3 lengths is predominantly Gaussian, with an occasional

skew in the intensity caused by recent immune activity (Fig 3, upper panels). As shown in Fig 3, the repertoire was characterized by extensive skewing.

Review of other cases

Since our original publications, other patients with complete DGS have been treated with HCT (Table I). There are 9 cases reported in the literature.⁶⁻¹⁴ We contacted the authors and were able to assess the current status of all patients except 1.⁹ Overall survival is greater than 75%, similar to that reported with thymic transplantation.⁵ One male patient in France¹⁰ is doing well 16 years posttransplantation of PBMCs. He has mixed T-cell chimerism, a reversed CD4:CD8 ratio, and a skewed TCR repertoire. Another boy in Canada¹³ is now 3.5 years post-BMT and is in good health. He has 95% donor cells, a normal CD4:CD8 ratio, and a memory T-cell predominance. Two patients have died. The first one,⁸ in Japan, died suddenly from unknown causes 10 months posttransplant. The other patient, also from Japan, died from overwhelming cytomegalovirus infection less than a year after transplantation. Of note, this patient received a cord blood transplant, likely to be deficient in antigen-experienced memory cells. Two other patients transplanted more recently (one in Germany and another in the Czech Republic) have also engrafted and are doing well. These 2 cases^{12,14} have similar features to our patients—that is, unconditioned transplants, memory T-cell predominance,

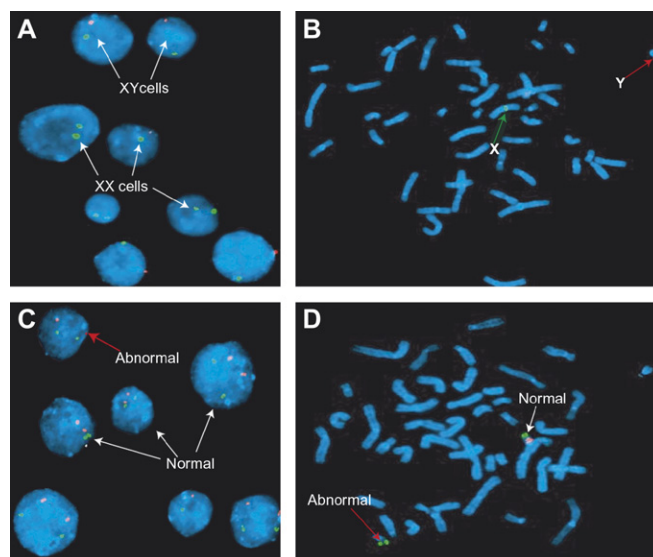


FIG 2. FISH analysis for sex-chromosome mismatch identification and the 22q11.2 deletion in patient 2. **A** and **B**, Analysis of engraftment by sex chromosome mismatch. **A**, Interphase nuclei with chromosome X (green) and Y-specific (red) fluorescence probes. Recipient cells are XX (2 green dots), whereas donor cells are XY (1 red, 1 green). **B**, A male metaphase cell (donor) positive for the XY probe (arrows). **C** and **D**, 22q11.2 deletion as detected by FISH with the TUPLE1 probe. **C**, Interphase nuclei with the TUPLE1 gene locus labeled in red and an internal control locus aryl sulfatase A (ARSA) labeled green showing normal (donor's origin; 2 red and 2 green signals) and abnormal (recipient's origin; TUPLE 1 deleted, 1 red, 2 green signals) cells. **D**, A female metaphase cell positive for TUPLE1 deletion in the abnormal chromosome 22 (red arrow).

and T-cell chimerism. Taken together, these results demonstrate the effectiveness of HCT in fostering immune competence in complete DGS.

DISCUSSION

Because these patients had been lost to follow-up for more than a decade, the clinical course and laboratory features in the intervening years could not be precisely documented. Their health status in our recent evaluation was satisfactory. One of them has reportedly not had any infectious complications, whereas the other has had several bronchopulmonary infections that resolved with proper antibiotic treatment. In contrast with what has been reported in patients undergoing thymic transplantation,⁵ neither of our patients has developed features of autoimmune disease. Like most other patients with DGS, both present with a mild developmental delay and speech impairment, but they lead normal, independent lives.

Both of these patients with complete DGS received a single dose of donor bone marrow and, 20 years later, remain engrafted and in good immunologic health. In patient 1, engraftment was assessed on whole blood, in which mature neutrophils outnumber the lymphoid cells. All dividing lymphocytes carry the donor Y chromosome, whereas DNA microarray analysis showed predominance of host XX cells, presumably myeloid and B cells. In case 2, isolated PBMCs showed 21% XX and 79% XY donor cells. The percentage of the cells positive for the 22q11.2 deletion was about the same as that of the XX cells. These

results indicate that the patients are hematopoietic chimeras. Antibody function is intact as evidenced by the normal levels of immunoglobulins and responses to pneumococcal vaccine and varicella infection. We interpret these latter findings as evidence of normal B-cell function and, more importantly, effective T-cell help.

Our studies indicate that postthymic donor cells and not naive stem cells are responsible for the immune reconstitution. In these and other reported cases of patients with DGS who underwent hematopoietic cell transplants, the majority of the lymphocytes are donor memory T cells, a conclusion fortified by their limited V- β repertoire, the phenotypic RO marker, and the virtual absence of TRECs. Although these findings may be considered an immunologic disadvantage, the antigen-experienced memory T cells have the ability to confer protective immunity rapidly to a wide variety of pathogens. Because T cells are pivotal effectors in the control of viral infections, this may constitute an advantage of BMT over thymic transplantation, where infection with herpes viruses reportedly is a major risk factor for death.^{4,5}

Studies in patients after HCT have shown that memory T cells are long-lived and do not revert to the naive phenotype.²⁰ These mature T cells proliferate to restore T-cell numbers rapidly in lymphopenic hosts but do exhibit a restricted TCR repertoire. This observation has led to the assumption that recovery of full immunity after transplant depends on the generation of naive T cells through thymic maturation. However, gaps in the T-cell repertoire may not result in a clinically significant immune defect. Emerging evidence indicates that T-cell and B-cell

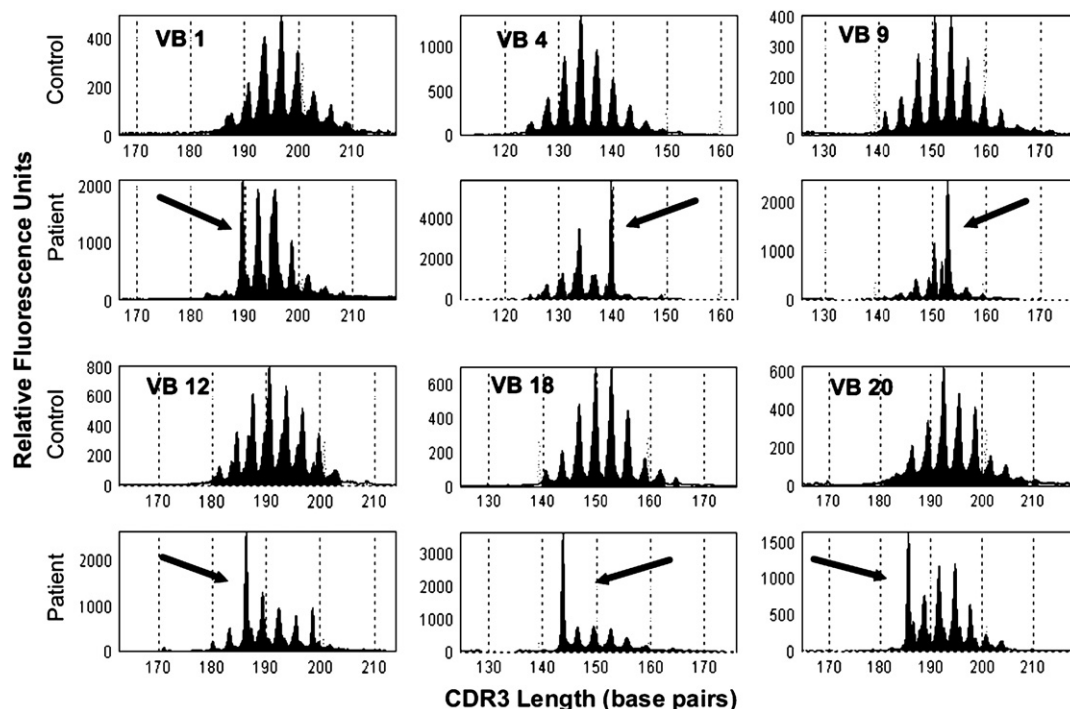


FIG 3. Spectratyping of patient 1. Example of spectratype analysis of TCR β CDR3 lengths from PBMCs obtained from a control individual and patient 1. The V- β family analyzed is identified in every pair of panels. Arrows point to skew in the distribution.

receptors are polyspecific and can recognize multiple distinct ligands.²¹ Thus, mature memory cells may respond to a wider spectrum of microbial antigens, including neoantigens such as vaccines—as evidenced by the response of our patients to many pneumococcal serotypes and varicella infection.

Rapid immune reconstitution is critical for survival in patients undergoing HCT. Of the many parameters postulated to influence this process, the source of the hematopoietic graft (ie, bone marrow, peripheral stem cells, cord blood) has consistently been shown to affect the rate of immune recuperation in all transplantation settings.²² Patients with complete DGS constitute a very special case and therefore, the advantages and disadvantages of each of these sources has to be considered in the context of absent thymic function. The lack of thymus in complete DGS may render purified stem cell transplants ineffective because maturation to naive cells may not occur. Yet intriguing new findings by Clark et al²³ indicate that, *in vitro*, human skin matrix can support the development of human stem cells into T cells, suggesting that the thymus may not be unique in that function.

Peripheral blood transplants and BMTs result in more rapid reconstitution than do isolated stem cell transplants or thymic transplantation, and thus may be preferable in patients at risk of or with active infection. Of the 9 patients in the current series, 8 received transplants from these sources (3 PBMCs and 5 bone marrow). Six of them are alive and well with a mean survival time of 11 years at the

time of this report, indicating the effectiveness of either of these sources in the treatment of complete DGS. The main limitation in this regard is the availability of an HLA-matched donor. Umbilical cord blood (UCB) has been successfully used in the treatment of patients with various primary immunodeficiencies.²⁴ UCB has a high content of naive T cells, which have a longer lifespan than their memory counterparts. This may be an important factor in preventing premature immune senescence in complete DGS, in which *de novo* T-cell generation is by definition unattainable. However, naive T cells require a longer time to mature into effector memory cells and thus do not provide immediate defense against most microbial agents. Furthermore, the delayed immune reconstitution reported after UCB transplant²⁵ could limit its applicability when protracted infection is of concern.

Therapeutic options are limited for the small percentage of patients with 22q11.2 deletion who have complete DGS. Thymic transplantation has shown promising results in restoring immune function, providing a normal T-cell repertoire with the presence of naive T cells, although the total T-cell numbers remain reduced.⁵ This procedure is complex and of restricted availability. For patients with active infection or coexistent severe illness, thymic transplantation may be contraindicated.⁵ We conclude that BMT is a suitable alternative, particularly in cases in which an HLA-matched donor is available, and when the patient's condition precludes a long recovery period. In these cases, BMT may provide sufficient T-cell

immunity for prolonged survival and is, we believe, the treatment of choice for most such patients.

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