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 thymic[3-5] 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 mismatch[6]; the other was HLA-identical[7]). 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 *in situ* 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/μL.

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