

Brief report

Full hematopoietic engraftment after allogeneic bone marrow transplantation without cytoreduction in a child with severe combined immunodeficiency

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Bone marrow transplantation (BMT) for severe combined immunodeficiency (SCID) with human leukocyte antigen (HLA)-identical sibling donors but no pretransplantation cytoreduction results in T-lymphocyte engraftment and correction of immune dysfunction but not in full hematopoietic engraftment. A case of a 17-month-old girl with adenosine deaminase (ADA) deficiency SCID in whom full hematopoietic engraftment

developed after BMT from her HLA-identical sister is reported. No myeloablative or immunosuppressive therapy or graft-versus-host disease (GVHD) prophylaxis was given. Mild acute and chronic GVHD developed, her B- and T-cell functions became reconstituted, and she is well almost 11 years after BMT. After BMT, repeated studies demonstrated: (1) Loss of a recipient-specific chromosomal marker in peripheral

blood leukocytes (PBLs) and bone marrow, (2) conversion of recipient red blood cell antigens to donor type, (3) conversion of recipient T-cell, B-cell, and granulocyte lineages to donor origin by DNA analysis, and (4) increased ADA activity and metabolic correction in red blood cells and PBLs. (Blood. 2001;97:809-811)

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Introduction

Severe combined immunodeficiency (SCID) involves a failure of T cells to proliferate to various stimuli and a failure of B cells to produce specific antibodies.^{1,2} Adenosine deaminase (ADA) deficiency, an autosomal recessive genetic defect, produces a SCID phenotype.^{3,4} The enzyme defect leads to an abnormality in purine nucleoside metabolism that interferes with lymphocyte viability and function. ADA-deficient SCID is characterized by growth delay, candidiasis, respiratory infections, opportunistic infections, and, without specific therapy, early death.⁵ Low or absent ADA activity in erythrocytes or other cells is diagnostic.

Bone marrow transplantation (BMT) is effective therapy for SCID. The profound T-cell dysfunction associated with ADA-deficient SCID prevents graft rejection and permits BMT without conditioning with an HLA-matched sibling donor.^{1,2,6} Success rates are greater than 90%, with full recovery of T-cell and B-cell functions.⁶ Complete T-cell engraftment is routinely demonstrated, and approximately 50% of patients demonstrate engraftment of donor B cells.⁷ Without conditioning, host hematopoiesis persists with no evidence of myeloid or erythroid engraftment.^{5,7-9}

Study design

J.L., a 17-month-old girl, presented with fever, vomiting, cough, skin ulcers, a history of failure to thrive, recalcitrant thrush, chronic respiratory infections, and recurrent otitis media. Immunizations were current. Family history was negative for immunodeficiency. Physical examination revealed an ill-appearing white female with weight and height below the fifth percentile, a paucity of lymph nodes, and no hepatosplenomegaly.

Laboratory evaluation revealed normal hemoglobin and platelet counts. The white blood cell count was 1600/cmm (normal, 6000-17 000/cmm) and consisted of 10% neutrophils, 20% lymphocytes, 65% monocytes, and 4% eosinophils. Liver function test results were normal. Titers were negative for human immunodeficiency virus, cytomegalovirus, hepatitis B virus, herpes simplex virus, Epstein-Barr virus, adenovirus, poliovirus, *Toxoplasma*, and *Chlamydia trachomatis*.

ADA activity was deficient in erythrocytes and in peripheral blood mononuclear cells; purine nucleoside phosphorylase (PNP) activity was normal in both cell types (Table 1). Metabolic findings in erythrocytes were consistent with ADA deficiency, including elevated deoxyadenosine nucleotide (dAXP) of 0.491 mol/mL packed cells (normal, less than 0.002 μ M/mL), and reduced S-adenosylhomocysteine hydrolase activity of 0.42 nmol/h/mg protein (normal, 4.2 ± 1.9).³

A 3-year-old sister was HLA-identical (A2, A3, B35, B27, Cw2, Cw4, DRB1*01, and DRB1*04). Without myeloablative or immunosuppressive therapy, the patient was infused with 7.6×10^8 unfractionated nucleated marrow cells per kilogram from her sister. After BMT, the patient engrafted rapidly, remained well, and required no blood products (Figure 1). Biopsy-proven grade I skin graft-versus-host disease (GVHD) developed at day 39 after BMT and resolved with topical corticosteroids. Three months after BMT, she developed vomiting and diarrhea with elevated absolute neutrophil and lymphocyte counts (Figure 1). Liver function tests revealed the following values: aspartate aminotransferase, 270 IU/L (normal, 0-30 IU/L); alanine aminotransferase, 262 IU/L (normal, 7-56 IU/L); and γ -glutamyl transpeptidase, 123 IU/L (normal, 8-78 IU/L). The illness resolved rapidly, and liver function findings were normal 3 months later.

Eleven months after BMT, lichenoid skin lesions developed that were consistent with chronic GVHD; they completely resolved after 11 months of oral and topical corticosteroid therapy. A dermatofibrosarcoma protuberans was completely excised from the patient's left forearm 8 years after

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Table 1. Adenosine deaminase and purine nucleoside phosphorylase activity in red blood cells and peripheral blood mononuclear cells

		Erythrocytes		Mononuclear cells	
		ADA	PNP	ADA	PNP
Patient	Before BMT	0.98	1259	0.71	3062
Patient	After BMT	17.6 ± 6.6	1585 ± 104	1176 ± 1082	5970 ± 2861
Donor		18.4	1744	937	5179
Normal levels		63 ± 41	1336 ± 441	1197 ± 516	3007 ± 824

ADA indicates adenosine deaminase; PNP, purine nucleoside phosphorylase; BMT, bone marrow transplantation.

Enzyme activities are expressed as nmol/h/mg protein. Pre-BMT patient and donor values are from single samples. Normal values and post-BMT data for the patient are given as mean ± SD. Post-BMT patient samples were obtained on 4 occasions—at 16 months, 26 months, 47 months, and 127 months after transplantation.

BMT. At long-term follow-up, the patient is well, has no sequelae from BMT, and shows no evidence of immunodeficiency.

Two-color flow cytometric analysis for lymphocyte phenotyping,¹⁰ natural killer cell assays,¹¹ cell proliferation assays,¹² and red blood cell antigen typing¹³ were performed as described. GW-banding procedures¹⁴ demonstrated a 1qh+ polymorphism in the recipient's phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBLs) and unstimulated BM. For DNA studies, T cells and B cells were isolated with antibody-coated magnetic beads and granulocytes enriched by Ficoll separation. DNA was extracted from each fraction, and amplification of short tandem repeats was performed.¹⁵ ADA activity, PNP activity, and dAXP levels were measured as previously described.^{16,17} ADA genotype was determined with DNA extracted from fibroblasts cultured from the dermatofibrosarcoma protuberans through single-strand conformational polymorphism analysis of ADA exons 4 and 5. The procedures used for exon 5 have been reported.¹⁸ For exon 4, a genomic segment (base pair [bp] 24 787-25 481) was first amplified using the following: primer 1, (+) 5'-GTA TGC AGT TCC AAA GTA GAG CTG; primer 2, (-) 5'-CAG TTA TGA AGT TAG AGC AGG ACC. The product was then subjected to nested polymerase chain reaction of bp 24 900 to 25 261 using the following: primer 3, (+) 5'-gcg gaa gct tGG ATG TCA TTT GCT CCT G (5' end-labeled with $\gamma^{32}\text{P}$ -dATP); primer 4, (-) 5'-gcg cga att cCA TCT TTC TGA GGC CAT G (lowercase denotes nongenomic 5' extensions). The final exon 4 polymerase chain reaction product was then sequenced.

Results and discussion

Allogeneic BMT is effective treatment for various inherited defects of the pluripotent hematopoietic progenitor cell (HPC). An immu-

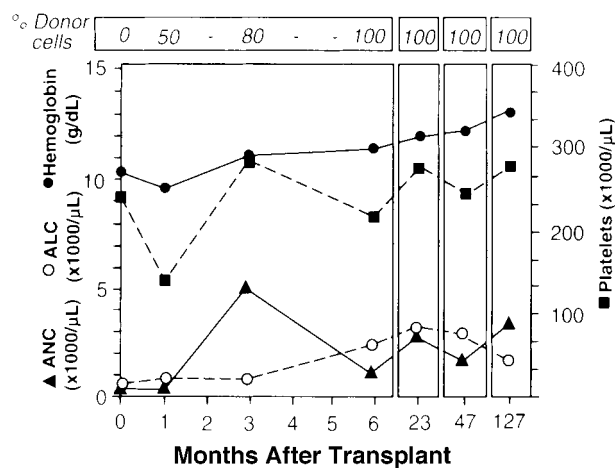


Figure 1. Graphic representation of hematologic parameters and engraftment status at selected times after bone marrow transplantation. Blood components (absolute lymphocyte count [ALC], absolute neutrophil count [ANC], platelets) and hemoglobin values are shown in the lower panel. Percentage donor cells analyzed by cytogenetics in the recipient after transplantation are shown in the top panel. A specific 1qh+ polymorphism was detected in the recipient before BMT. At 1 and 3 months after BMT, analysis of PHA-stimulated PBLs demonstrated mixed chimerism. Full donor engraftment was found at 6 months, and all subsequent findings of PBLs and BM (at 1 and 8 years) have remained 100% of donor origin.

nosuppressive and myeloablative preparative regimen is usually necessary to suppress the host's immune system to prevent rejection of the infused stem cells. The only exception to the above experience is in BMT for SCID, when a matched sibling or a T-depleted haploidentical stem cell donor is available. Because of the host's severe immune dysfunction, the donor graft is not rejected.² Only T-cell progenitors, which have a selective growth advantage over endogenous host T cells, engraft.^{5,7} After BMT all erythroid and myeloid precursors remain of recipient origin.¹ After unconditioned BMT for SCID with an HLA-matched sibling donor, T-cell function is restored in a few weeks and B-cell function in a few months.⁶ Normalization of B-cell function does not necessarily reflect donor B-cell engraftment.^{5,9} In many cases, B-cells remain partially or completely of host origin, but they function normally in the presence of donor T cells. Natural killer cells may also be of donor, host, or mixed origin after unconditioned BMT.^{5,8}

We report a patient with classic ADA-deficient SCID, heteroallelic for 2 previously reported ADA missense mutations, R101Q (exon 4) and R156C (exon 5).¹⁸⁻²⁰ Before BMT she had lymphopenia, absent B cells, an inverted CD4/CD8 ratio, absent natural killer activity, and markedly depressed *in vitro* mitogen response to pokeweed, concanavalin A, and phytohemagglutinin. Her bone marrow had normal cellularity with depressed T- and B-cell numbers for her age. After BMT, all the above evaluations gradually normalized. In addition, we found multiple markers confirming complete erythroid and myeloid engraftment.

Recipient and donor were both blood group A Rh-positive. The recipient (Jk^a negative, E positive) had complete conversion to donor erythrocyte antigens (Jk^b positive, E negative) when studied at 4 and 26 months after BMT. Anti-B was weakly detected before BMT and strongly detected 26 months after BMT.

Before BMT, all mitoses in the BM demonstrated the 1qh+ polymorphism. At 1 and 3 months after BMT, analysis of PHA-stimulated PBLs demonstrated mixed chimerism. Full donor engraftment was found at 6 months, and all subsequent findings of PBLs and BM (at 1 and 8 years) have remained of 100% donor origin (Figure 1).

For DNA studies, 9 short tandem repeat markers were analyzed. Informative loci (D3S1358, FGA, TH01, TPOX, CSF1PO, D13S317, and D7S820) collectively demonstrated that all cell types (T, B, and granulocyte-enriched) in the specimen studied were of donor origin after BMT.

After BMT, ADA activity in erythrocytes and blood mononuclear cells increased to the level found in the donor (Table 1). Erythrocyte dAXP level was less than 0.01 nmol/mL packed cells in all samples obtained after BMT. All the above studies demonstrated complete T, B, erythroid, and myeloid cell engraftment from 6 months after BMT without any evidence of mixed chimerism.

After BMT the host HPC ceased to function in our patient. Because no episode of pancytopenia or marrow aplasia occurred, transient mixed chimerism of host and donor hematopoiesis must

have developed. Over time the host stem cells ceased to function, and only the donor stem cells and their progeny persisted. The most appealing speculation for this observation is the development of donor T-cell–mediated GVHD reaction that targeted and destroyed the host HPC without other manifestations of significant GVHD. Approximately 3 months after transplantation, the child had an episode of vomiting, diarrhea, and elevated liver enzyme levels. At that time a significant increase in hemoglobin, platelet, absolute neutrophil, and absolute lymphocyte counts were found that persisted into long-term follow-up (Figure 1). The patient's cytogenetic studies also changed during this period, from 50% to 80% donor cells, with 100% donor cells present at 6 months and later (Figure 1).

It has been reported that severe GVHD with pancytopenia and aplasia develop in infants with SCID after in utero engraftment with maternal cells.²¹ In addition, transfusional GVHD in immunodeficient infants is characterized by the development of severe GVHD and aplastic anemia, as is transfusional GVHD in immunocompetent patients after open heart surgery.^{22,23} In all the above examples, donor T-lymphocytes mediate host hematopoietic stem cell destruction and lead to the development of marrow aplasia and severe pancytopenia. However, unlike our experience, fatal GVHD developed in all the reported recipients.

An alternative hypothesis is that because ADA-sufficient cells (donor) have a selective growth advantage over ADA-deficient

cells (host), donor HPC engraftment would occur. However, in all reported cases of BMT in ADA SCID, host erythropoiesis and myelopoiesis persist.

Our observation is certainly rare. The Buckley et al⁹ survey of 87 patients with SCID who underwent either HLA-haploidentical or HLA-identical BMT without a preparative regimen or GVHD prophylaxis found no case of complete hematopoietic engraftment, though red cell typing was not specifically noted. Haddad et al⁸ recently reported that 2 of 8 patients with SCID with B cells had evidence of some donor-derived monocytes after HLA-haploidentical, T-cell–depleted BMT without a preparative regimen. None of the 5 HLA-identical BMT patients had donor-derived monocytes. We suggest that investigators following up patients with SCID who underwent transplantation without a preparative regimen look for evidence of partial or complete myeloid and erythroid engraftment in their patients. Identification and further study of this phenomenon may lead to a better understanding of allogeneic tolerance.

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