

Letter to the Editor

Emergency treatment for ζ chain-associated protein of 70 kDa (ZAP70) deficiency

To the Editor:

ζ Chain-associated protein of 70 kDa (ZAP70) is a cytoplasmic tyrosine kinase associated with signaling events downstream of the T-cell receptor (TCR). After TCR stimulation, ZAP70 is recruited to the T-cell plasma membrane, binding phosphorylated CD3 ζ and undergoing a conformation-induced increase in catalytic activity.¹⁻³ Once activated, ZAP70 proceeds to phosphorylate a number of signal transduction proteins, including SLP-76, Cbl, Vav, and others, thus activating several TCR-mediated pathways.⁴

A major clue to the critical role of tyrosine kinases in general and ZAP70 in particular in the development and activation of lymphocytes came with the discovery of naturally occurring mutations of the ZAP70 gene in patients with CD8 lymphocytopenia.⁵⁻⁷

Like other types of severe combined immunodeficiency or combined immunodeficiency, these patients can present in infancy with repeated, opportunistic, life-threatening infections^{5,6} and sometimes with autoimmune manifestations⁸⁻¹⁰ or malignancy.¹¹ Evaluation of the immune system reveals consistent CD8 lymphocytopenia with normal numbers of circulating CD4⁺ cells. Yet these cells are dysfunctional because they do not respond to mitogens or antigens.^{5,6} Natural killer cell numbers and B-cell development are preserved.

Hematopoietic stem cell transplantation (HSCT) is the only known curative treatment for this condition. Protocols including full conditioning, usually including busulfan and cyclophosphamide, were successful in fully reconstituting a normal immune system and leading to long-term cure.¹²

We present a patient of Mennonite descent who was born at term after an uneventful cesarean section. He was thriving and developing well until 4 months of age, when he began experiencing repeated episodes of what was described as "bronchiolitis." Respiratory symptoms improved with oral steroid treatment. He subsequently had chronic cough with periodic acute exacerbations of wheezing. In addition, he was noted to gradually fall off his birth weight, which was at the 95th percentile. Serum IgG levels were low, but IgA, IgM, and IgE levels were normal (Table I).⁷ Results of multiple nasal and pharyngeal swabs were negative for respiratory syncytial virus, influenza, and parainfluenza viruses.

At the age of 7 months, he was admitted to a community hospital for exacerbation of wheezing with increased breathing difficulties and higher oxygen requirements. Chest radiographic analysis showed areas of hyperventilation with an interstitial ground-glass pattern. He was treated with antibiotics, as well as inhaled and oral steroids, with no improvement. Therefore he was transferred to a pediatric intensive care unit, where he was intubated and mechanically ventilated. Chest radiographic analysis showed bilateral alveolar infiltrates, and he was given a diagnosis of acute respiratory distress syndrome.

Cultures obtained from his endotracheal tube grew *Klebsiella pneumoniae*, *Pneumocystis jiroveci*, and parainfluenza type 3. Results were negative for *Mycoplasma pneumoniae*, *Legionella* species, and mycobacteria.

Laboratory evaluation revealed a normal blood count and normal numbers of circulating CD3⁺, CD4⁺, CD19⁺, and CD56⁺ cells but markedly diminished CD8⁺ cell numbers.

T-cell receptor excision circle (TREC) levels were only 86 (normal level, >400 copies/0.5 μ g of DNA). *In vitro* response to the T-cell mitogen phytohemagglutinin (PHA) was markedly decreased, but responses to the combination of 12-O tetradecanoylphorbol 13 acetate (PMA) and ionomycin were comparable with those seen in control subjects (Table I). This pattern of lymphocyte proliferation, together with the profound CD8 lymphocytopenia and ethnic origin of the patient, suggested ZAP70 deficiency. We have therefore sequenced the ZAP70 gene and identified g.24417G>A, the typical mutation found in Mennonites.⁶

The patient was subsequently referred to our institution for HSCT. He was transferred to the pediatric intensive care unit, but in spite of good antimicrobial therapy, his condition continued to deteriorate. His oxygen requirements increased, and respirator support was maximized. We requested urgent study of tissue typing in the family and identified a full HLA-matched (10/10) sibling.

The patient was gradually stabilized in the pediatric intensive care unit after requiring high-frequency ventilation, dopamine, and broad-spectrum antimicrobial agents. He gradually returned to conventional ventilation but started to spike daily fevers. His antibiotics were switched, and antifungal medication was started. His respiratory condition deteriorated, and he had pneumomediastinum, as well as subcutaneous emphysema. He required high-frequency ventilation again.

We believe that in patients with profound T-cell deficiency who have a robust number of circulating T cells and residual immune function, HSCT, even from HLA-identical donors, should include conditioning. Indeed, there is little evidence of successful engraftment without conditioning in cases with combined immunodeficiency in general, including ZAP70 deficiency (Dr F. Cohen, personal communication). Conversely, we and others have shown excellent long-term immune reconstitution after HSCT, including a conditioning regimen.¹² Therefore we planned to use a myeloablative HSCT protocol, including conditioning with busulfan and cyclophosphamide, in the patient described here. However, because of his poor clinical state at the age of 9 months, we decided to administer 5×10^8 nucleated cells/kg of donor bone marrow with no delay. Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine only. Over a period of 1 week after HSCT, he had a maculopapular skin rash on his legs, arms, palms, and soles. His liver function test results increased, and his conjugated bilirubin level increased to 31 μ mol/L. He was therefore given 1 mg/kg methylprednisolone daily. Over the second week after HSCT, he started to improve, his rash disappeared, his liver function test results decreased, his respirator settings were improving, and he was afebrile. Eighteen days after HSCT, he was extubated and tolerated oral feeding well and therefore was transferred to a regular ward.

Thirty-five days after HSCT, the patient was discharged from the hospital in excellent clinical condition, with the exception of periodic skin GvHD. T-cell chimerism of peripheral blood with interphase fluorescence *in situ* hybridization determined at 2, 4, and 7 months after transplantation demonstrated 35%, 7%, and 3% XX-positive cells (donor), respectively. In addition, karyotyping of PHA blasts 10 months after HSCT showed less than 2% female donor cells. Bone marrow aspirate also showed barely detectable donor cells. Peripheral lymphocyte counts decreased

TABLE I. Immune evaluation before and after transplantation

	At presentation	After first transplantation	After second transplantation	Normal range
Lymphocyte markers (cells/ μ L)				
CD3 cells	1071	3627 (d+42); 827 (m+6)	1105 (m+20)	900-4500
CD4 cells	1044	3388 (d+42); 715 (m+6)	673 (m+20)	500-2400
CD8 cells	27	252 (d+42); 72 (m+6)	295 (m+20)	300-1600
Natural killer cells	183	197 (d+42); 678 (m+6)	968 (m+20)	100-1000
CD19 cells	774	2579 (d+42); 525 (m+6)	1174 (m+20)	200-2100
Mitogen stimulation and thymic output				
PHA response (% control)	0.2	42 (m+8); 8 (m+11)	109.7 (m+12)	≥ 50
PMA + ionomycin (% control)	85			≥ 50
TREC (copies/0.5 μ g of DNA)	86	251 (m+6)	3274 (m+12)	≥ 400
Immunoglobulins and antibodies				
IgG (g/L)	1.8	7.5 (on IVIG)	7.7 (m+20)	4.5-14.3
IgA (g/L)	0.4	0.6	0.5 (m+20)	0.2-1.0
IgM (g/L)	0.4	0.6	1.2 (m+20)	0.2-1.8
Anti-tetanus antibody (IU/mL)			>7 (m+20)	≥ 0.01
Donor chimerism (% in whole blood)		35 (d+86); 3 (m+11)	100 (d+18); 100 (m+20)	

Time from first or second transplantation is expressed as days (d+) for the first 90 days and months (m+) thereafter. The *in vitro* mitogenic response to PHA and to the combination of PMA and ionomycin was measured as previously described.⁷

dramatically (Table I). Clinically, the patient continued to have repeated episodes of skin GvHD and stopped gaining weight. He continued to require intravenous immunoglobulin replacement monthly, and his TREC levels were severely reduced to 251 copies/0.5 μ g of DNA, all ominous signs of graft failure. At the age of 24 months, he received a second HSCT using his donor's frozen HLA-identical bone marrow cells at a dose of 5×10^8 nucleated cells/kg. This time, he received full myeloablative conditioning with busulfan and cyclophosphamide, as well as GvHD prophylaxis with cyclosporine and prednisone. The transplant course was uneventful, with no major infections or GvHD. Neutrophil engraftment occurred on day +13, and immunosuppression was stopped 5 months after the second HSCT. Chimerism studies showed 100% donor cells from day +18 after HSCT, consistently so up to the last estimation at 20 months after bone marrow transplantation. He is now 2 years after his second HSCT and growing and developing very well. He is fully reconstituted with normal *in vitro* responses to mitogens, normal TREC levels, and normal immunoglobulin and antibody levels (Table I).

In conclusion, profound T-cell immunodeficiency is a medical emergency. Preparation for HSCT should start with no delay as soon as this diagnosis is suspected. In less than ideal conditions, donor bone marrow can be infused as an urgent measure with a transient but lifesaving effect. Then a second more definitive HSCT can be carefully planned and executed under ideal circumstances.

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