Brief Report

Treatment of the Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome (IPEX) by Allogeneic Bone Marrow Transplantation

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THE immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is a recessive disorder of early childhood.[1] Symptoms of the disease generally appear in infancy and include protracted diarrhea, ichthyosiform dermatitis, insulin-dependent diabetes mellitus, thyroiditis, and hemolytic anemia.[2-6] Nephropathy has been reported in two familial cases of enteropathy with insulin-dependent diabetes mellitus.[7] X-linked recessive inheritance accounts for the familial cases in the seven kindreds studied to date,[2-9] but sporadic cases in boys have also been reported.[6,10-12]

The results of immunologic investigations of IPEX[1] are inconsistent with any of the known X-linked immunodeficiency diseases.[13] Severe infections have been observed in patients with IPEX, but they may have been complications of immunosuppressive therapy.[4] Massive infiltration of T lymphocytes into the skin and gastrointestinal tract and high serum levels of autoantibodies against blood, thyroid, and pancreatic cells suggest that IPEX is an autoimmune disorder. Specific antibodies against a 75-kd antigen in intestinal and renal epithelial cells have been identified in one patient.[14] Mutations of the *FOXP3* gene have recently been identified in patients with IPEX, confirming that IPEX is a distinct X-linked immune disorder.[15-17] Scurfy mice, in which a disease similar to IPEX develops, have a mutation in an analogous gene.[18]

Supportive therapy with total parenteral nutrition, insulin, and blood transfusions is beneficial in patients with IPEX, and prolonged immunosuppressive therapy with steroids and cyclosporine has been attempted.[19,20] Nevertheless, the prognosis is very poor, and most reported cases have been fatal. Transient clinical improvement occurred in three children with continued parenteral nutrition and prolonged immunosuppressive therapy.[2,5,10,15] Given the immune pathogenesis of IPEX and the poor prognosis for patients with this condition, we performed transplantation of allogeneic bone marrow from an HLA-identical family member in a four-month-old boy with the disease.

CASE REPORT

We studied a child with IPEX born to parents from Morocco (Fig. 1). The mother had been pregnant seven times. She had had an unexplained spontaneous abortion during the fourth month of one pregnancy and had four healthy daughters who were 11, 16, 18, and 19 years of age at the time of this report. One son had died at 4.5 months of age from intractable diarrhea, thrombocytopenia, insulin-dependent diabetes mellitus, and ichthyosiform dermatitis. A biopsy of the duodenal mucous membrane from this child showed villous atrophy, and autopsy showed infiltration by lymphocytes and plasmocytes of the liver, small bowel, colon, and pancreas.

A second son was born at term and was normal in length and weight. At four weeks, persistent secretory diarrhea was reported; infectious and hormonal causes were ruled out. Duodenal biopsies (Fig. 2) showed a flattened mucous membrane, with partial-to-subtotal villous atrophy, and numerous T lymphocytes (CD3+CD25+) and plasma cells in the lamina propria. Epithelial necrosis of the crypts was commonly seen. Similar necrosis of the crypts was observed in the gastric and colonic mucous membranes. As the intractable diarrhea continued, the boy's weight fell below the third percentile; total parenteral nutrition was therefore initiated.

Insulin-dependent diabetes mellitus was diagnosed at 2.5 months of age on the basis of low serum insulin and C-peptide concentrations and glucose concentrations above 200 mg per deciliter (11 mmol per liter). Control of the blood glucose concentration was difficult to achieve with intravenous insulin. High serum titers of antibodies against glutamic acid decarboxylase (GAD65) and the pancreatic islets were detected (Table 1). Ichthyosis was observed two weeks after birth. It became a widespread exfoliative skin eruption that responded partially to emollients and hydrocortisone cream. A skin biopsy revealed extensive subacute eczematous lesions with lichenification.

Hemolytic anemia was diagnosed when the child was four weeks old. Autoantibodies were detected on the surface of red cells (IgG isotype Coombs' test), polymorphonuclear neutrophils (IgG isotype), and platelets (anti-IIb/IIIa IgG). The lowest values recorded for blood neutrophils, platelets, and hemoglobin were 1380 per cubic millimeter, 17,000 per cubic millimeter, and 6 g per deciliter, respectively. The patient required repeated transfusions of erythrocytes and platelets. Cholestatic hepatitis, with hyperbilirubinemia, also developed. Thyroid function was normal, as determined by measurements of serum triiodothyronine, thyroxine, and thyrotropin. Glomerular and tubular functions were normal. Serum antinuclear

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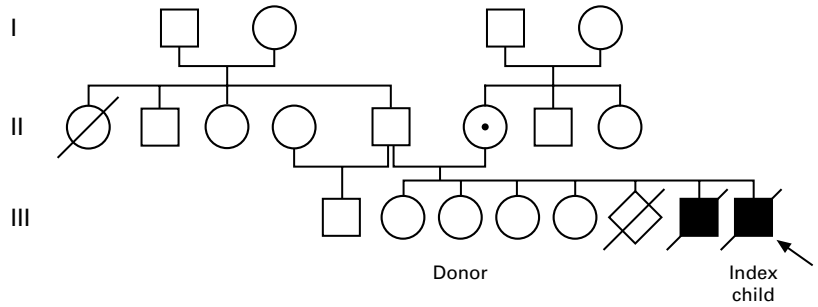

Figure 1. Pedigree of the Patient's Family.

Squares represent male family members, circles female family members, the diamond the child who died in utero, and slashes deceased family members. Solid squares represent patients with clinical IPEX, the circle with a dot a female carrier, and the arrow the child who underwent transplantation. A disease-causing *FOXP3* mutation was identified in the child, who received a bone marrow transplantation from a sister, and his mother was found to be heterozygous for the mutation. The HLA-identical sister, the donor, carried two wild-type copies of *FOXP3*.

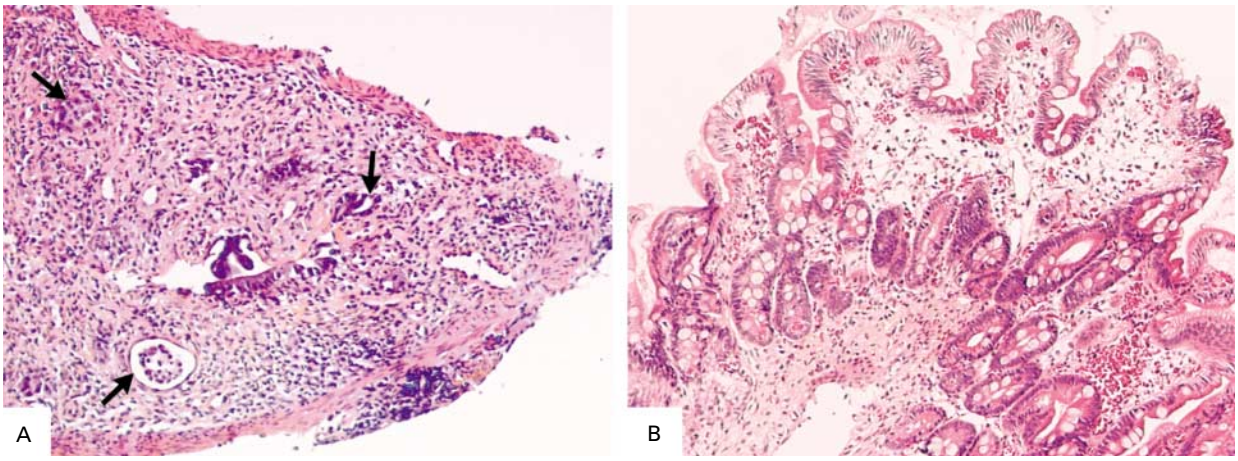

Figure 2. Histopathological Findings at Low Magnification.

A duodenal-biopsy specimen obtained at the time of diagnosis shows a flattened mucosa with absent or necrotic crypts (arrows, Panel A), and a duodenal-biopsy specimen obtained six months after bone marrow transplantation shows a normal mucosa (Panel B).

antibodies were detected. Antibodies against the 75-kd gastrointestinal autoantigen were not detected.[14]

The numbers of CD19+, CD3+, CD4+, and CD8+ lymphocytes in the blood were within normal ranges; the in vitro T-lymphocyte response to phytohemagglutinin and the serum IgG, IgA, and IgM concentrations were also within normal ranges at four months of age. In contrast, serum IgE concentrations were very high (up to 1750 IU per milliliter [4200 μg per liter]). Titers of serum antibodies against antigens used for vaccination (tetanus toxoid, poliovirus) were normal.

IPEX was suspected clinically, and the diagnosis was confirmed by the presence of a mutation in the *FOXP3* gene.[15] The mutation was a substitution of guanine for thymine at nucleotide position 1113 (exon 10), resulting in the substitution of cysteine for phenylalanine at amino acid position 371 (F371C). The patient's mother was a heterozygous carrier of the F371C mutation.

Immunosuppressive treatment was initiated (250 mg of methylprednisolone per square meter of body-surface area per day for a total of 1250 mg per square meter per week, followed by 2 mg of methylprednisolone per kilogram of body weight per day plus 25 mg of methylprednisolone per kilogram per week and 0.3 mg of tacrolimus per kilogram per day).

There was a transient clinical response, but then the child's condition worsened. At four months of age, he was therefore considered for allogeneic transplantation of bone marrow from his healthy, 18-year-old, HLA-identical sister, who did not carry the F371C mutation. The conditioning regimen consisted of intravenous rabbit anti–T-lymphocyte globulin (Pasteur-Merieux, Lyons, France; 10 mg per kilogram per day on days 14 to 10 before transplantation), oral busulfan (Glaxo Wellcome, Paris; 5 mg per kilogram per day on days 9 to 6 before transplantation), and intravenous cyclophosphamide (Asta Medica, Paris; 50 mg per kilogram per day on days 5 to 2 before transplantation).

A total of 435×10^7 bone marrow mononuclear cells per kilo-

TABLE 1. CLINICAL AND BIOLOGIC CHARACTERISTICS OF THE PATIENT BEFORE AND AFTER BONE MARROW TRANSPLANTATION.*

CHARACTERISTIC	AT DIAGNOSIS (SEPT. 1998)	1 MO AFTER TRANSPLANTATION (DEC. 1998)	6 MO AFTER TRANSPLANTATION (MAY 1999)	16 MO AFTER TRANSPLANTATION (MARCH 2000)	23 MO AFTER TRANSPLANTATION (OCT. 2000)†
Weight (g)	3,340	5,100	7,460	9,140	10,140
Height (cm)	50	59	68	80	83
No. of stools/day	10	5	4	3	2 or 3
Ichthyosis	Present	Absent	Absent	Absent	Absent
Hemoglobin level (g/dl)	8.9	9.9	10.9	12.5	12.4
Antierythrocyte antibodies	1:8	–	–	–	–
No. of neutrophils (per mm^3)	1,380	6,500	3,700	4,600	4,800
Antineutrophil antibodies	+++	ND	–	–	–
No. of platelets (per mm^3)	17,000	331,000	530,000	504,000	273,000
Antiplatelet antibodies	++	–	–	–	–
Autoantibodies					
Anti–pancreatic islets	1:16	–	–	ND	–
Anti-GAD65	+++	+	–	ND	–
75-kd antienterocytes	–	ND	–	ND	–
Anti–interleukin-2	ND	–	–	ND	–
Antinuclear	1:80	–	–	ND	–
Antitissue‡	ND	ND	–	ND	–

*ND denotes not determined. Plus signs indicate increasing titers of antibodies, and minus signs no antibodies. GAD65 denotes glutamic acid decarboxylase.

†The child died 29 months after transplantation (April 2001).

‡Antitissue autoantibodies include antimitochondrion, anti–endoplasmic reticulum, and anti–smooth-muscle antibodies.

gram and 217×10^6 CD34+ cells per kilogram were infused. For prophylaxis against graft-versus-host disease, the patient received cyclosporine intravenously (until day 21) and then orally (until day 180), so that the serum concentration of the drug was maintained at 150 to 200 μg per liter. Intravenous acyclovir (Glaxo Wellcome), at a dose of 1600 mg per square meter per day, was given for prophylaxis against cytomegalovirus infection until day 60. The blood products used tested negative for cytomegalovirus and were irradiated.

RESULTS

On day 11 after transplantation, the polymorphonuclear neutrophil count in the blood was more than 500 per cubic millimeter. Analysis of sex chromosomes by fluorescence in situ hybridization on day 19 showed that 95 percent of white cells were of the female XX genotype. There was no evidence of venous occlusion, graft-versus-host disease, or infection.

Diarrhea improved as early as three days before bone marrow transplantation, and stools were normal one month after transplantation. Two weeks later, enteral feeding was successfully reinstated with an elemental diet without cow's-milk proteins or gluten. Two months after bone marrow transplantation, solid foods were slowly introduced into the diet. Stools remained normal in number and appearance. Parenteral nutrition was discontinued one month later. Six months after bone marrow transplantation, the patient's diet was normal and was associated with a normal intestinal transit time and no apparent gastrointestinal problems. Duodenal, jejunal, and colonic biopsies showed normal tissues with recovered villous architecture and mucous membranes; only rare lymphocytes were detectable in the lamina propria (Fig. 2).

The conditioning regimen was associated with a progressive improvement in blood glucose control, and insulin therapy was stopped seven days before bone marrow transplantation. Autoantibodies directed against pancreatic islet cells and GAD65 were no longer detected by day 180. Blood products (red cells and platelets) were not required after day 21. Moderate hepatitis occurred from day 42 to day 50. Six months after bone marrow transplantation, mild eczematous lesions were observed on the face and forearms, and the diagnosis was confirmed by a skin biopsy. These skin lesions improved rapidly when treated with a corticosteroid cream for three to four weeks. After a new series of vaccinations (against tetanus and poliomyelitis) were administered six months after bone marrow transplantation, the patient's T-lymphocyte responses in vitro to phytohemagglutinin and vaccine antigens were within the normal range.

Twenty-three months after bone marrow transplantation, the patient resumed normal feeding and was progressing very well, with normal hematologic and immunologic function, feeding, stools, glucose concentrations, and skin (Table 1). Stable mixed chimerism was found at 16 and 23 months after transplantation in red cells (CcK− and CCK+ red cells) by

blood typing and in leukocytes (28 percent XX at 23 months), polymorphonuclear cells (15 percent XX), resting CD3+ T lymphocytes (30 percent XX), phytohemagglutinin–interleukin-2–driven CD3+ T-lymphocyte blasts (25 percent XX), and CD19+ B cells (3 percent XX) by fluorescence in situ hybridization analysis. The patient's weight, height, and head circumference 23 months after bone marrow transplantation were normal.

While the child was still in remission, fever, splenomegaly, and lymphadenopathy developed, along with hyponatremia, elevated concentrations of liver enzymes, and thrombocytopenia. Most peripheral-blood T lymphocytes were activated. Hemophagocytosis was found in the bone marrow. The results of an extensive search for infectious agents, including cytomegalovirus and Epstein–Barr virus, were negative. Despite treatment with broad-spectrum antibiotics and high-dose methylprednisolone (5 mg per kilogram per day), the child died. The death occurred 29 months after bone marrow transplantation, when the child was nearly 3 years of age. An autopsy was not performed.

DISCUSSION

IPEX is usually fatal; there are only three known long-term survivors of the disease.[2,5,10,15] Tissue infiltration by lymphocytes, the presence of serum autoantibodies, and the mutations in *FOXP3* that is expressed in lymphocytes in patients with IPEX strongly suggest that the pathogenesis of the disease involves the immune system, prompting the use of immunosuppressive therapy for this condition.[19,20] In one case, cyclosporine had partial, transient efficacy.[10] Azathioprine failed to induce a remission in one child.[21] Combination therapy with tacrolimus, betamethasone, and verapamil was transiently beneficial in one patient, but the patient died.[22] Our results show that bone marrow transplantation should be considered as a potentially curative option in patients with IPEX.

In our patient, bone marrow transplantation was followed by a complete remission. Interestingly, the conditioning regimen itself controlled most of the clinical and biologic features of the disease, including the diarrhea, hyperglycemia, and dermatitis. Engraftment probably facilitated a sustained remission, and the patient remained in clinical and biologic remission for more than two years after bone marrow transplantation. Given that there is a germ-line mutation in IPEX, it is unlikely that autologous bone marrow transplantation would produce a sustained remission. In our patient, irreversible insulin-dependent diabetes had not yet occurred; this is important, because a lack of pancreatic islets has been found at autopsy in several patients with IPEX. Interestingly, partial donor chimerism was sufficient to maintain a complete remission, suggesting that a minority of healthy donor leukocytes is sufficient to control the autoimmune process. Bone marrow transplantation in other children with IPEX would be required to assess the stability and efficacy of transplantation.

FOXP3 is expressed primarily in CD4+ T lymphocytes. Studies in scurfy mice, which have a similar mutation, have shown that CD4+ T-helper lymphocytes are important in the development of the lesions.[23,24] These observations suggest that mouse scurfy and human IPEX are disorders of T-helper–lymphocyte regulation. Our success with bone marrow transplantation is consistent with these findings and demonstrates that hematopoietic cells have an essential role in the pathogenesis of IPEX.

The occurrence of diabetes mellitus and eczematous ichthyosis distinguishes IPEX from other inherited autoimmune conditions such as complement deficiencies,[25] autoimmune lymphoproliferative syndrome,[26] and autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy.[27,28] Transplantation of haploidentical bone marrow has been successfully attempted in two children with the autoimmune lymphoproliferative syndrome caused by complete Fas deficiency.[29,30] Together with our results, these data suggest that allogeneic bone marrow transplantation may be a therapeutic option in other severe inherited autoimmune conditions.

We have no clear explanation for the development of a rapidly progressive hemophagocytic syndrome, which proved fatal, in our patient. There was no evidence of an infectious agent, and whether the hemophagocytic syndrome was a complication of the bone marrow transplantation, was related to the fact that the child had mixed hematopoietic chimerism, or both, is unclear. Hemophagocytosis is not a known feature of IPEX. Bone marrow transplantation, in our opinion, remains a potentially curative option for patients with IPEX, even though the possibility of long-term complications, such as hemophagocytosis, should be carefully considered.

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