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Brief Report

TREATMENT OF X-LINKED SEVERE COMBINED IMMUNODEFICIENCY BY IN UTERO TRANSPLANTATION OF PATERNAL BONE MARROW

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SEVERE combined immunodeficiency is a congenital syndrome due to various genetic abnormalities that cause susceptibility to infection, failure to thrive, lymphoid hypoplasia, very low levels of T lymphocytes, and hypogammaglobulinemia.[1,2] Untreated, the disorder is usually fatal within the first year of life. We report the successful treatment of a fetus with the X-linked variant of severe combined immunodeficiency by the in utero transplantation of paternal bone marrow that was enriched with hematopoietic cell progenitors.

CASE REPORT

The patient, 11 months old at this writing, is the second son of a 28-year-old woman known to carry a mutation found in X-linked severe combined immunodeficiency. Her first son died at seven months of age of severe combined immunodeficiency, confirmed by autopsy and molecular analysis. Studies of his DNA identified a splice-donor-site mutation in the gene for the common γ chain of the interleukin-2 receptor (*IL2RG*) in complementary DNA at position 868(+5) in intron 6.

The woman became pregnant again. Analysis of DNA obtained at 12 weeks' gestation by chorionic-villus sampling showed that the fetus was an affected male. After extensive nondirective counseling the family decided in favor of prenatal treatment.

Bone marrow was harvested under general anesthesia from the 30-year-old father of the fetus. After enrichment of the bone marrow with CD34+ cells (hematopoietic cell progenitors), the fetus received three transplants of 14.8 million, 2.0 million, and 1.8 million cells (114 million, 8.9 million, and 6.2 million cells per kilogram of estimated fetal weight), respectively, by percutaneous, ultrasound-guided, intraperitoneal injection at 16, 17.5, and 18.5 weeks' gestation. At delivery by cesarean section, the infant appeared normal except for a mild macular rash. A biopsy of the rash revealed no evidence of graft-versus-host disease, such as infiltrating lymphocytes, apoptotic keratinocytes, or vacuolar changes of the basal epithelium. The rash resolved with a seven-day course of methylprednisolone at a dose of 1 mg per kilogram of body weight per day intramuscularly.

METHODS

Ethical Considerations

A decision to continue the pregnancy independently of the option of in utero transplantation was made by the parents after consultation with specialists in genetics and pediatric immunology. Both parents subsequently gave informed consent for the in utero transplantation procedures. The protocol and consent forms were reviewed and approved by the Human Investigation Committee of Wayne State University.

Prenatal Genetic Evaluation

Identification of the *IL2RG* mutation and analysis of DNA extracted from the biopsy of chorionic villi were performed according to published techniques.[3-6]

Donor-Cell Processing

Paternal bone marrow was obtained by aspiration from the posterior iliac crest and placed in RPMI-1640 medium with preservative-free heparin. The mononuclear cells were separated and divided into three aliquots; the first was processed immediately, and the other two were cryopreserved.

After separation by Ficoll–Hypaque density-gradient centrifugation, mononuclear cells were incubated with biotinylated monoclonal antibody against CD34 in RPMI with 0.1 percent human serum albumin. The cells were washed and passed through a Ceprate avidin–biotin immunoabsorption column (Cellpro, Seattle). The CD34+ cells that bound to the column were removed by gentle agitation. Incubation with the antibody was repeated, and the cells were passed through a second Ceprate column. After each step of enrichment, aliquots were taken for phenotypic assessment, assays to monitor loss of progenitor cells, and bacterial and fungal cultures.

Injection Procedure

Injections were performed transabdominally with a 22-gauge 3.5-in. (9-cm) spinal needle under real-time ultrasound guidance. The maximal volume injected was 1 ml.

Detection of Donor-Cell Engraftment

Analysis of cord-blood mononuclear cells and fractionated cells for *IL2RG* sequences was performed as previously described.[3-5] Mononuclear cells were typed with fluorescein-isothiocyanate–conjugated monoclonal antibodies against HLA class I antigens.[4] The father was classified as A3, A68, B7, B8, Bw6; the mother as A31, A30, B35, Bw6; and the patient as A3, A30, B35, B8, Bw6. Therefore, the donor-specific HLA class I antigen HLA-B7, identified by monoclonal antibody against the antigen from hybridoma HB-59 (American Type Culture Collection, Rockville, Md.), was used to identify donor cells in the infant.

For dual-color immunofluorescence analyses, mononuclear cells from the patient were stained simultaneously with the fluorescein-isothiocyanate–conjugated antibody against HLA-B7 and a phycoerythrin-conjugated monoclonal antibody against CD2, CD3, CD4, CD8, CD14, CD19, CD38, or CD56 (Becton Dickinson, Mountain View, Calif.)[6] or a biotin-conjugated antibody

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against CD34 (Caltag, San Francisco). The antibodies conjugated with fluorescein isothiocyanate and phycoerythrin were used at saturating concentrations. Conjugated monoclonal antibodies with irrelevant specificities served as negative controls. Cells were analyzed with a FACScan flow cytometer (Becton Dickinson). To detect donor-derived hematopoietic progenitor cells in the recipient's bone marrow, bone marrow cells obtained from the infant at three months of age that were positive for CD34 were selected with the CD34 Isolation Kit according to the manufacturer's instructions (MACS, Miltenyl Biotec, Baraisch-Gladbach, Germany).[7] The enriched population was analyzed by dual-color flow cytometry after staining with an allophycocyanin-conjugated monoclonal antibody against CD34 (avidin–allophycocyanin, Becton Dickinson) and a fluorescein-isothiocyanate–conjugated antibody against HLA-B7 or a phycoerythrin-conjugated monoclonal antibody against CD38.

Proliferation Assays

Proliferative responses of cord- and peripheral-blood mononuclear cells to phytohemagglutinin, pokeweed mitogen, and concanavalin A were measured by standard methods.[8] The mixed-lymphocyte reaction was performed according to a previously described technique.[9]

RESULTS

Enrichment of Donor Marrow with CD34+ Cells

From the harvest of 12.4 billion paternal cells (1.9 percent of which were CD34+ cells), a total of 18.6 million cells were transplanted in three aliquots. After enrichment, the transfused parental cells were at least 98.5 percent CD34+ cells and at most 0.5 percent CD3+ cells.

Engraftment

Phenotypic analysis by flow cytometry of the recipient's cord blood at birth, five months after the last transplantation, with the use of HLA-B7 as a donor-specific marker, demonstrated that all the patient's T lymphocytes were of donor origin, whereas his B lymphocytes (Fig. 1), monocytes (Fig. 1), and natural killer cells (data not shown) were of host origin. This pattern of "split" chimerism in mononuclear cells of the blood was also found at 3 and 6 months of age (8 and 11 months, respectively, after the last transplantation). The *IL2RG* sequences in cord-blood cells had the donor's genotype in the T cells but the mutant genotype in mononuclear cells and granulocytes (Fig. 2).

A population of paternal hematopoietic stem cells in the recipient's bone marrow was found by flow cytometry when he was three months of age. Approximately 3 percent of the separated CD34+ bone marrow cells were HLA-B7+. Of the CD34+, CD38− cells in this enriched population, over 17 percent were HLA-B7+ (data not shown; CD38 is a differentiation marker that appears later than CD34).

Hematologic Findings

At birth, the numbers of B cells and CD8+ T lymphocytes were normal (1312 B cells per cubic millimeter, 41 percent of total lymphocytes; and 896

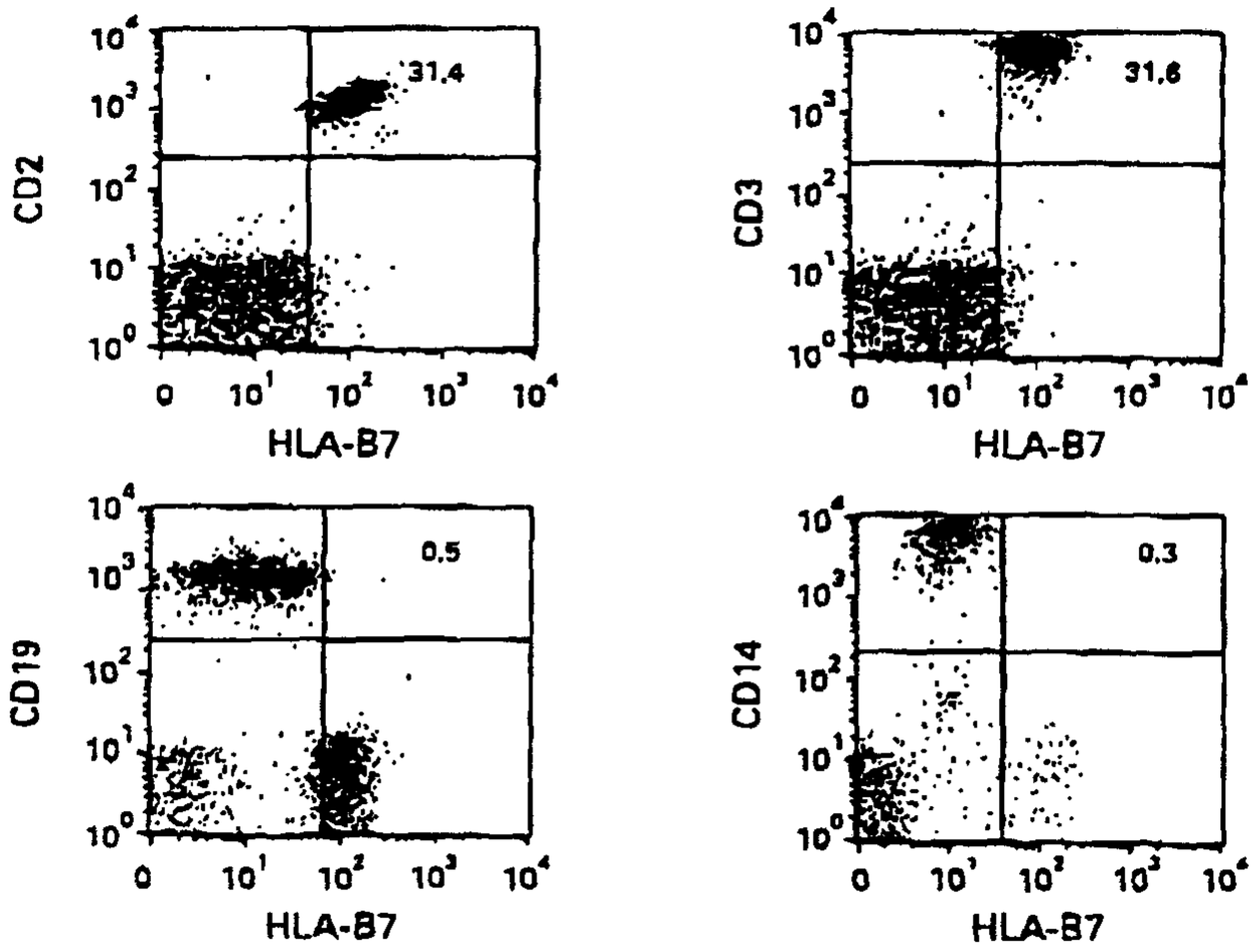

Figure 1. Analysis of Cord Blood by Dual-Color Flow Cytometry.

Donor cells are HLA-B7+. The percentages of cells positive for both the lineage marker and HLA-B7 are indicated in the upper right quadrants. Essentially all the CD2+ and CD3+ cells (T cells) are HLA-B7+, whereas all the CD19+ cells (B cells) and CD14+ cells (monocytes) are HLA-B7−.

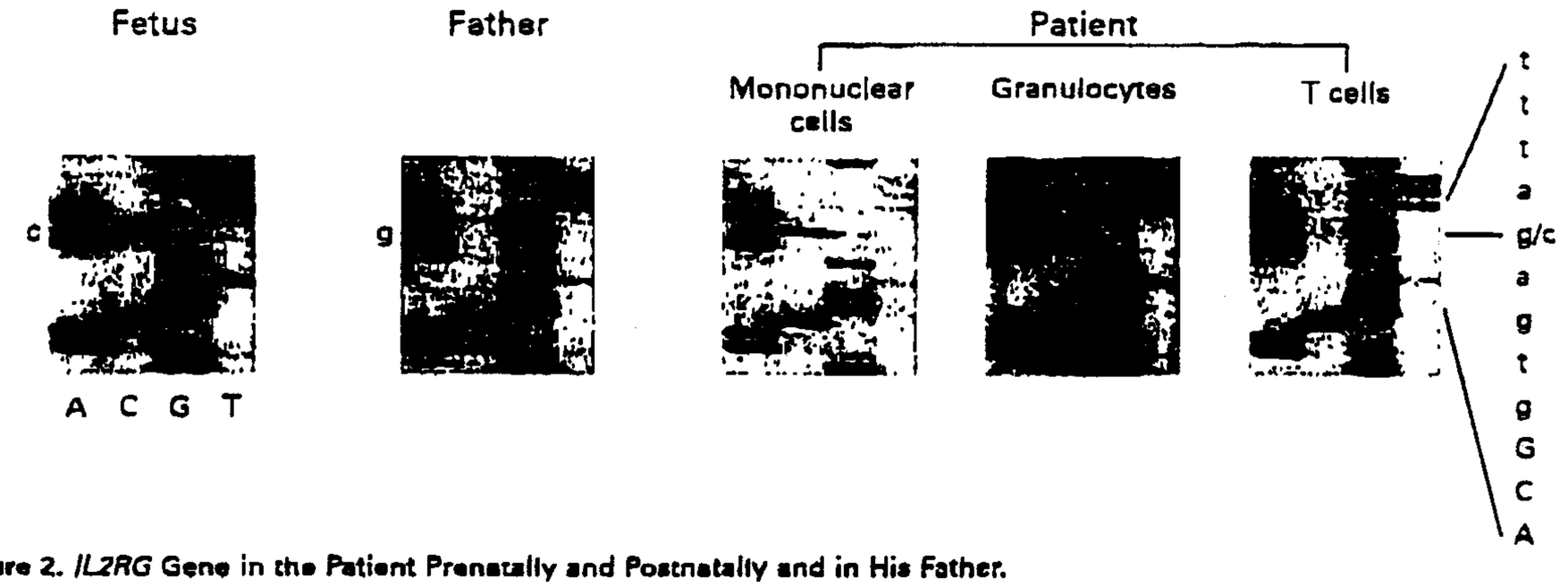

Figure 2. *IL2RG* Gene in the Patient Prenatally and Postnatally and in His Father.

The uppercase and lowercase letters shown at the right denote the DNA bases corresponding to the exon 6 and intron 6 splice regions, respectively. The mutation in this genotype of severe combined immunodeficiency is a 5′ splice-site transversion of *g* to *c*, which follows the end of exon 6. It can be seen in the cells from the fetus, which were obtained by chorionic-villus sampling. The sequence in the patient's father (the bone marrow donor) has the wild-type g at this position. The cord-blood mononuclear cells from the patient had predominantly the mutant sequence, but granulocytes had only the mutant sequence, and T cells had only the donor genotype.

CD8+ T lymphocytes per cubic millimeter, 28 percent of total lymphocytes) and have remained normal since then. The total lymphocyte count and the numbers of CD3+ and CD4+ T lymphocytes were all low at birth but progressively increased and became normal for age at five months of age (10 months after the last transplantation) (8200 lymphocytes per cubic millimeter; 5248 CD3+ lymphocytes per cubic millimeter, 64 percent of total lymphocytes; and 3034 CD4+ lymphocytes per cubic millimeter, 36 percent of total lymphocytes). These values remained normal at 11 months of age.

Cellular Immune Function

Serial measurements of in vitro responses of the patient's lymphocytes to plant mitogens were generally more than 10 times greater than those of controls (medium alone). At one month of age the response of the patient's cells to phytohemagglutinin was 17,342 disintegrations per minute (dpm); to pokeweed mitogen, 5322 dpm; and to concanavalin A, 12,847 dpm (control, 560 dpm after three days of incubation). The response of the cells to mitogen has fluctuated, but since the age of six months it has equaled or exceeded that of normal subjects (values at six months of age: phytohemagglutinin, 87,333 dpm; pokeweed mitogen, 8566 dpm; and concanavalin A, 47,636 dpm).

Humoral Immune Function

Serum concentrations of IgM have been normal since birth. IgG concentrations progressively fell to a physiologic nadir at four months of age and then rose into the low-normal range. IgE concentrations have increased to within the normal range, but IgA remains undetectable. At seven months of age, after three rounds of vaccination, the patient had detectable IgG antibodies against diphtheria toxoid (titer, 1:640), tetanus toxoid (titer, 1:1280), and haemophilus (0.4 μg per milliliter).

Immunologic Tolerance

The patient's mononuclear cells, obtained when he was three months of age, did not respond to the father's mononuclear cells in a mixed-lymphocyte reaction (value, 2193 counts per minute [cpm]; control value, 2028 cpm) and had a partial response to the mother's mononuclear cells (7048 cpm), but were fully responsive to mononuclear cells from three unrelated persons (15,111, 24,294, and 22,844 cpm).

Clinical Course

The patient has remained in excellent health since birth. He has undergone surgery for an incarcerated inguinal hernia and strabismus without complication. He has had two upper respiratory tract infections and a single episode of otitis media, all of which resolved normally. His growth and development are normal at 11 months of age (75th percentile for height and weight).

DISCUSSION

The genetic basis of X-linked severe combined immunodeficiency is a mutation of *IL2RG*, the gene encoding the common cytokine-receptor γ chain.[10,11] This mutation, by inactivating the common γ chain, renders the T cells of boys with X-linked severe combined immunodeficiency unresponsive to several cy-

tokines. The result is a block in T-cell development and a severe deficiency of mature T cells. B cells, although present in normal or even increased numbers, are dysfunctional.[12]

X-linked severe combined immunodeficiency can be diagnosed prenatally by molecular techniques.[13,14] This allows planning for bone marrow transplantation in the first weeks or months of life.[15,16] Results are excellent (almost 100 percent) with an HLA-identical donor (15 to 30 percent of cases), but survival is 60 to 80 percent if a parent whose HLA antigens match half of those of the child's is the donor.[15-17] The success of bone marrow transplantation can be hindered by preexisting infection, graft failure, graft-versus-host disease, and the usual delay (three to four months) before immunologic reconstitution is complete.

The biology of X-linked severe combined immunodeficiency gives transplanted normal T lymphocytes a growth advantage and may allow postnatal transplantation without myeloablation. This selective advantage may explain the state of split hematopoietic chimerism after postnatal bone marrow transplantation, in which all T lymphocytes are of donor origin,[3,13,18,19] whereas all other lineages are of host origin. In our patient we found split chimerism after prenatal bone marrow transplantation.

The rationale for prenatal transplantation of hematopoietic stem cells is based on the ontogeny of the hematopoietic system.[20-23] In early gestation the fetus is immunologically immature, and space is available in the developing bone marrow for engraftment of hematopoietic stem cells. In normal sheep, transplanted allogeneic or xenogeneic hematopoietic stem cells engraft early in gestation, without immunosuppression or the need for myeloablation.[24-27] These results indicate the capacity of donor hematopoietic cells to compete with host cells for growth in a normal hematopoietic environment. In patients with a disease that provides a selective growth benefit for normal cells, such as T cells in X-linked severe combined immunodeficiency, prenatal transplantation of normal cells could be particularly advantageous. The impressive levels of donor-cell engraftment we found in our patient support the rationale for such transplantation.

Clinical experience with in utero hematopoietic stem-cell transplantation is limited.[28] In most cases engraftment has not been achieved. Touraine and colleagues[29-32] have reported the successful treatment of one patient with bare lymphocyte syndrome and another patient with autosomal severe combined immunodeficiency by in utero transplantation of hematopoietic cells from fetal liver. Multiple prenatal and postnatal fetal liver transplantations were performed, and published evidence of engraftment in these two patients is limited.

The risks of in utero hematopoietic stem-cell transplantation must be considered. The fetus is particularly susceptible to graft-versus-host disease, the induction of which depends on the number of T cells in the graft.[33,34] We have demonstrated the engraftment of adult hematopoietic cells enriched with CD34+ cells without the occurrence of graft-versus-host disease in a xenogeneic human–sheep model.[35-38] The enrichment increased the number of hematopoietic stem cells while reducing the number of T lymphocytes. To minimize the number of transplanted T cells, we passed the father's bone marrow cells through anti-CD34 immunoabsorption columns twice. An additional concern was the procedure itself. The risk of loss of pregnancy with chorionic-villus sampling is 0.5 to 0.75 percent.[39] The risk of loss of pregnancy from a single prenatal intraperitoneal transfusion, based on extensive experience in the treatment of fetal anemia, is approximately 1 percent.[40] The predicted additive procedural risk for our patient was therefore less than 4 percent.

The presence of donor-derived CD34+,CD38− cells in the patient's bone marrow strongly suggests the engraftment of donor hematopoietic stem cells, early progenitors, or both. Furthermore, the number of CD3+ cells we transplanted, as compared with the number already present in the patient, would require a massive increase in the number of donor lymphocytes, which is unlikely in the absence of graft-versus-host disease. The presence of multipotent progenitor cells of donor origin in a patient with severe combined immunodeficiency and split chimerism after postnatal bone marrow transplantation has been documented by others.[41]

There are many potential advantages to prenatal transplantation, including the ability to engraft unmatched donor cells without immunosuppression or ablation of the recipient's bone marrow. Early gestational transplantation allows immunologic reconstitution to begin before the onset of clinical manifestations of the disease, and the development of donor-specific tolerance could allow the recipient to receive postnatal transplants from the same donor. The risks of in utero transplantation appear to be low, and failure of engraftment does not preclude standard postnatal therapy. The success of this case supports the cautious application of in utero transplantation to other selected congenital hematologic diseases.

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