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Brief report

Successful bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning

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Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare, fatal autoimmune disorder caused by mutations in the *FOXP3* gene leading to the disruption of signaling pathways involved in regulatory T-lymphocyte function. Lifelong multiagent immunosuppression is necessary to control debilitating autoimmune manifestations such as colitis and food allergies. Allogeneic hematopoietic stem cell transplantation (HSCT) can restore T-cell regulatory function but has been previously associated with poor outcome. We describe successful HSCT in 4 patients with IPEX syndrome using a novel reduced-intensity conditioning regimen that resulted in stable donor engraftment, reconstitution of FOXP3$^+$ T regulatory CD4$^+$ cells, and amelioration of gastrointestinal symptoms. (Blood. 2007;109:383-385)

Introduction

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a life-threatening disorder associated with protracted diarrhea, severe food allergies, ichthyosiform dermatitis, endocrine insufficiency, and hemolytic anemia.[1-3] Mutations of the *FOXP3* gene result in loss of functional regulatory T cells and fatal autoimmune manifestations. Chronic immunosuppression is used to control symptoms.[4,5] Allogeneic hematopoietic stem cell transplantation (HSCT) restores T-cell regulatory function but has been associated with poor outcome.[6] We describe successful HSCT in 4 boys with IPEX syndrome after reduced-intensity conditioning consisting of alemtuzumab (Campath-1H; Genzyme, Cambridge, MA), fludarabine, and melphalan.

Patients, materials, and methods

Details regarding patients, pretransplantation immunosuppression, clinical indications for transplantation, and *FOXP3* mutation analysis are summarized in Table 1. Additional manifestations included elevated serum IgE levels, diabetes mellitus, hypothyroidism, hypoadrenalism, and growth failure, necessitating hormone replacement. Before transplantation, patient 3 underwent *Mycobacterium chelonae abscessus*–induced thigh abscess resection and had vancomycin-resistant *Enterococcus faecium* infection. Patients 1, 2, and 3 had recurrent *Clostridium difficile* colitis.

Patients 2 and 3 underwent second transplantations 6 and 33 months after the first grafts were rejected. Patient 2 previously received myeloablative conditioning with full-dose busulfan, fludarabine, antithymocyte globulin (ATG), and unrelated cord transplantation matched at 4 of 6 loci. Patient 3 received reduced intensity conditioning with 200 cGy total body irradiation (TBI), fludarabine, and a cord transplant from an HLA-matched sibling.

All recipients received alemtuzumab 48 mg (if they weighed more than 10 kg) or 33 mg (if they weighed less than 10 kg; patients 2 and 4) on days −21 to −19), fludarabine 150 mg/m^2 on days −8 to −4, and melphalan on day −3. Two patients received melphalan 140 mg/m^2, and 2 others received melphalan 70 mg/m^2. Dose de-escalation strata were planned in this transplantation protocol for nonmalignant disorders, as previously described.[7] All patients received bone marrow, 3 from unrelated donors and 1 from the same matched sibling. The median total nucleated cell (TNC) dose was 9.3×10^8/kg (range, 3.1-12.6 $\times$ 10^8/kg), and the CD34$^+$ cell dose was 9.25×10^6/kg (range, 5.04-34.7 $\times$ 10^6/kg). Prophylaxis for graft-versus-host disease (GVHD) consisted of cyclosporine or tacrolimus (levels maintained until day 100), methotrexate (10 mg/m^2 on day 1; 7.5 mg/m^2 on days 3 and 6), and methylprednisone (1 mg/kg daily on days 7-28). Supportive care included prophylaxis for *Pneumocystis* infection, acyclovir for herpesvirus positivity, weekly monitoring for cytomegalovirus (CMV) reactivation, and prophylaxis for fungal infection until day 100.

Donor chimerism was determined by molecular analysis of blood or bone marrow. Immune reconstitution monitoring included lymphocyte subpopulations, serum immunoglobulin levels, and lymphocyte proliferation to phytohemagglutinin (PHA). For detection of FOXP3$^+$ cells, whole blood was stained with CD4 and CD25 (Becton Dickinson, San Jose, CA), permeabilized with FOXP3 kit reagents (eBioscience, San Diego, CA), and intracellularly stained with CD152 (PharMingen, San Diego, CA) and FOXP3 (eBioscience) antibodies. Immunofluorescence-positive cells were detected with a FACSCalibur flow cytometer (Becton Dickinson). The proportion of FOXP3$^+$ cells was determined with the use of low side scatter gating for CD4$^+$, CD25^{bright}, and CD152$^+$ cells.

Approval was obtained from the institutional review boards of all participating centers listed in this study. Informed consent was obtained in accordance with the Declaration of Helsinki.

Results and discussion

Alemtuzumab infusion was well tolerated, and no patients experienced toxicity during conditioning. Myeloid (absolute neutrophil

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Table 1. Demographic details on HSCT recipients

Demographics	Patients			
	1	2	3	4
Age, y	7	1.4	4	0.5
Race	White	African American	White	White
FOXP3 mutation analysis	A>G splice junction mutation in intron 9	303_304 del TT	1271 G>A, C424Y	1226 A>G, D409G
Clinical indications for HSCT	Colitis, food allergies, eczema, FTT, AIHA, RAD	Colitis, food allergies, eczema, FTT, AIHA, TPN	Colitis, food allergies, eczema, MGN	Colitis, TPN, AIHA
Pre-HSCT IS	Imuran, CSA, prednisone	CSA, rituximab	FK506, MMF, prednisone	FK506, rituximab, prednisone, alemtuzumab
Stem-cell source*	8 of 8 URD, BM	7 of 8, A-antigen mismatched URD, BM	8 of 8, MSD, BM	8 of 8, URD, BM
Cell dose TNCs $\times 10^8$/kg/ CD34, $\times 10^6$/kg	12.6/34.7	6.0/5.8	3.1/5.04	5.3/12.7
Melphalan dose, m^2	70	140	70	140
Day of myeloid engraftment	12	16	13	12
Day of platelet engraftment	13	26	26	23

Myeloid engraftment is defined as having an ANC greater than 0.5×10^9/L; platelet engraftment, as having a platelet count greater than 50×10^9/L.

URD indicates unrelated donor; MSD, matched sibling donor; TNC, total nucleated cell; IS, immunosuppression; FTT, failure to thrive; AIHA, autoimmune hemolytic anemia; TPN, total parental nutrition; RAD, reactive airway disease; CSA, cyclosporine; MMF, mycophenolate mofetil; MGN, membranous glomerulonephritis.

*Allele matched by high-resolution typing.

count [ANC] greater than 0.5×10^9/L) and platelet (count greater than 50×10^9/L) engraftment occurred at medians of 12.5 (range, 12-16) and 24.5 (range, 13-26) days, respectively (Table 2). Colitis and food allergies resolved after HSCT. Endocrine dysfunction persisted, necessitating continued hormone replacement. Hypotension and malaise developed in patient 3 and were corrected with mineralocorticoid therapy. Patient 2, who was younger, had a 50% decrease in insulin requirement to 0.4 U/kg daily (anti–GAD antibody levels before and after HSCT were 115.9 and 1.6 U/mL, respectively; normal range, 0-1.5 U/mL). Transplantation outcomes and immune reconstitution are summarized in Table 2. Immune reconstitution was robust after 6 months except when immunosuppression was continued for chronic GVHD. $FOXP3^+$ cells were lowest in patient 3; his female sibling donor was not tested for FOXP3 mutation heterozygosity, which might have accounted for the lower numbers of $FOXP3^+$ cells. Alemtuzumab levels measured by enzyme-linked immunosorbent assay (ELISA; BioAnaLab, Cambridge, United Kingdom) in patient 3 were high (206-220 ng/mL) until day 30, in contrast to other patients with nonmalignant disorders who underwent transplantation with a similar regimen (levels undetectable on day 0; S.S., unpublished observations, August 2005). This is likely because of the lower $CD52^+$ cell counts in IPEX patients after pretransplantation immunosuppression. Alemtuzumab was undetectable on day 100.

Acute respiratory distress developed in patient 2 after pulmonary hemorrhage of unclear etiology on day 7, but the patient recovered completely after extracorporeal membrane oxygenation for 72 hours. Infectious complications after HSCT included *Clostridium difficile* colitis (n = 3) 4 to 16 weeks after HSCT, *Enterococcus faecalis* (patient 2) between 8 and 12 weeks, *Histoplasma capsulatum, Enterococcus faecium,* and *Staphylococcus hominis* infections (patient 3) 5 months after HSCT, and CMV reactivation (patient 1) at 6 months. No infections were encountered more than 6 months after HSCT.

Table 2. Outcome and immune reconstitution after HSCT

	Patients			
	1	2	3	4
Follow-up, mo	25	19	11	6
Donor chimerism, %	100	100	89	84.6
GVHD, acute/chronic	Grade 2 gut/extensive skin	0/0	0/0	0/0
Immunosuppression	FK506/photopheresis	No	No	Tapering prophylaxis
Lansky score	80	100	100	100
$FOXP3^+$ cells				
%*	52	77	72	33
Absolute no./mm^3†	29	99	17	30
Absolute lymphocyte numbers, mm^3¶				
CD4	446	2005	649	218
CD8	1757	792	193	44
B cells	124	1322	669	218
Lymphocyte proliferation to PHA, % normal control	26.5	100.2	78.6	35
Immunoglobulin level, mg/dL				
IgG‡	681	502	769	1300
IgM§	149	42	48	79
IgA‖	54	96	121	18.3

Ranges: *76%-91%; †43-149/mm^3; ‡608-1572 mg/dL; §52-352 mg/dL; ‖45-312 mg/dL.

¶Patients 1 and 2 were measured at 1 year; patient 3, at 9 months; and patient 4, at 6 months.

In patient 1, grade 2 acute GVHD of the gut developed 5 months after HSCT and was resolved. At 9 months, extensive chronic GVHD involving skin and joints developed, and the patient has shown good response to treatment with tacrolimus and extracorporeal photopheresis. To date, GVHD has not developed in any other recipient. Patients 2 and 3 discontinued immunosuppression 10 and 5 months after HSCT. Patient 4 is being tapered from immunosuppression therapy.

IPEX is a lethal disorder of childhood caused by mutations of *FOXP3*, loss of function of regulatory T cells and of scurfin, a *FOXP3* gene product protein critical for controlling T-cell activation.[5] Inactivity of scurfin or modification of target DNA-binding sites results in T-cell proliferation, defective apoptosis, and autoimmunity in mice and humans. Without extensive immunosuppression, the disease progresses to death in early childhood as a result of hemorrhage, sepsis, colitis, or diabetic complications.[6]

HSCT in affected mice results in 20% to 50% survival rates even with mixed-donor chimerism.[8] Transplantation experience in children with IPEX is limited. Of 4 previously reported transplantations with sibling donors, 3 patients died of hemophagocytosis (1 patient) and disease progression (2 children).[3,6] The fourth patient survived myeloablative transplantation that resulted in mixed-donor chimerism at 12 months (70% and 30% T and B cells, respectively).[9] Improvement in clinical symptoms paralleled donor engraftment and normalization of T-cell function. Another report of myeloablative HSCT in 3 patients described 2 survivors with clinical improvement.[10] No previous reports have been published of successful reduced-intensity HSCT in children with IPEX.

The reduced-intensity conditioning regimen described here was well tolerated during administration, even after previous HSCT and myeloablative conditioning. It was successful in achieving donor-cell engraftment with BM as the stem-cell source in all patients after 2 previously rejected UCB grafts. In all recipients, colitis and allergies subsided after transplantation. Except for one patient with acute and subsequently chronic GVHD of the skin and joints, transplantation-related complications were mainly early infections and were self-limiting. Infections likely receded after early immune reconstitution. Immune functions had significantly recovered after 9 months except in patient 1 (Table 2). $FOXP3^+$ cells also recovered to normal numbers in patients 2 and 3 and were present in patient 4. Lower numbers in patient 1 were likely the result of chronic GVHD therapy.

Successful engraftment and amelioration of symptoms in all 4 patients suggested that HSCT should be considered early for all patients with IPEX syndrome. The potential advantages of early HSCT would be the avoidance of disease-related organ toxicity, infection risk associated with chronic immunosuppression, and possible prevention of autoimmune endocrine organ destruction. This reduced-intensity conditioning approach made use of alemtuzumab early before transplantation to specifically immunoablate recipients and to facilitate donor engraftment. It was well tolerated and supported stable engraftment and early immune reconstitution, and it may reduce the late development of toxicities associated with standard myeloablative conditioning regimens.

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Authorship

Contribution: A.R. helped to compile the manuscript; N.K., A.F., S.M.D., and J.D. enrolled patients at their participating institutions; S.M.L. performed the FOXP3 staining on T cells; and S.S. developed the study.

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