Dermatologic and Immunologic Findings in the Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked Syndrome

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**Background:** The immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare genodermatosis associated with dermatitis, enteropathy, type 1 diabetes, thyroiditis, hemolytic anemia, and thrombocytopenia. IPEX results from mutations of FOXP3, a gene located on the X chromosome that encodes a DNA-binding protein required for development of regulatory T cells. If untreated, affected males die early in life from malabsorption and other complications. To our knowledge, this syndrome has never been described in the dermatology literature.

**Observations:** We studied an 11-year-old boy with IPEX. Mutation analysis revealed a G→A transition (1150G→H11022A) in exon 11, resulting in a putative substitution of Ala→Thr at residue 384, within the DNA-binding site. Histopathologic examination of an active skin lesion revealed psoriasiform dermatitis. The lesions improved with clobetasol ointment. The patient also displayed alopecia universalis, which had been present since age 18 months, accompanied by longitudinal ridging of the nails. Lymphocyte challenge tests revealed a profound inability to synthesize interferon γ (INF-γ) and dysregulated production of other cytokines.

**Conclusions:** IPEX is an often fatal genodermatosis associated with multiple autoimmune disorders. Cutaneous findings may include dermatitis, bullae, urticaria, alopecia universalis, and trachyonychia. Recognition of this life-threatening disorder is crucial for optimal treatment and genetic counseling.

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Tavirus and *Clostridium difficile* toxin were negative. Test results for antidiomysial antibodies were negative. A small bowel biopsy specimen demonstrated partial villous atrophy and generalized disaccharidase deficiency (values in micromole per minute per gram of protein): lactase, 3.6 (normal >14.1); sucrase, 16.4 (normal >25.5); maltase, 60.2 (normal >85); and palatinase, 1.8 (normal >4.3). Pancreatic enzyme concentrations in the duodenal fluid after challenge with intravenous secretin revealed normal concentration of trypsin and very low activities of lipase, amylase, and chymotrypsin. Exogenous pancreatic enzymes failed to control the diarrhea. Total parenteral nutrition was initiated at age 8 months, when the patient began a 3-month hospitalization. The diarrhea waxed and waned despite treatment with prednisone (0.5-2 mg/kg body weight per day) alone and in combination with cyclosporine.

On admission, he was found to have leukocytosis (white blood cell count, 17,000/µL), eosinophilia (11% of white blood cells), neutropenia (absolute neutrophil count, 378/µL), and elevated immunoglobulin level (IgG, 1150 mg/dL [normal, 442-890 mg/dL]; IgA, 120 mg/dL [normal, 31-77 mg/dL]; IgM, 150 mg/dL [normal, 19-55 mg/dL]; and IgE, 33 U/mL [normal, 1-17 U/mL]). Within 1 week, eosinophils comprised 26% of the total leukocyte count and neutrophils were completely absent. Antineutrophil antibodies were detected. A bone marrow biopsy specimen was remarkable for increased myeloid precursors, with maturation arrest at the band stage. The neutropenia persisted following 2 courses of intravenous immunoglobulin at a dose of 1 g/kg body weight given 1 month apart as well as a 2-week course of daily prednisone with doses up to 2 mg/kg body weight per day. While the prednisone dose was being tapered, therapy with granulocyte colony-stimulating factor was initiated at 10 µg/kg per day, and neutrophil counts increased dramatically. A lymphocyte profile was normal and the CD4-CD8 ratio was 3 (normal, 2.4-2.8). The diarrhea subsided as the neutropenia improved.

At age 2 years, the patient developed insulin-dependent (type 1) diabetes mellitus and was found to have a high anti-islet cell antibody titer. At age 3 years, screening laboratory studies detected an elevated thyrotropin level (9.3 mIU/L [normal, 0.2-6.0 mIU/L]) and antithyroid microsomal antibodies (1:400 [normal, 1:100]). The serum thyroxine level was normal, and the patient was clinically euthyroid. No antiadrenal antibodies were detected. Results of lymphocyte proliferation studies on mitogens (ie, phytohemagglutinin, concanavalin A, and pokeweed) and antigens (ie, tetanus, diphtheria, streptolysin O, and dermatophytin O) were normal except for a depressed response to streptolysin O.

Figure 1. Clinical photographs of the patient’s skin and nails. A, Chronic scaly erythematous plaques were present on the flexural and extensor surfaces of the legs. B, Scaly, erythematous, eroded plaques on the penis and surrounding skin. C, Longitudinal ridging was present on numerous nails. D, Nonscarring alopecia of the scalp, eyebrows, and eyelashes.
In the years that followed, the patient was hospitalized multiple times for exacerbations of diarrhea, hypalbuminemia, and uncontrolled diabetes. A gastrosomy tube was placed. Autoimmune neutropenia resolved, but intermittent periods of mild to moderate lymphopenia occurred. The thyroiditis resolved spontaneously, and thyroid hormone replacement was never required.

When the patient presented to us at age 9 years, a skin biopsy was performed on a lesion that had developed within the preceding week, revealing psoriasiform dermatitis (Figure 2). Based on the clinical history, a diagnosis of IPEX was suspected. Sequencing of the FOXP3 gene (see “Mutation Identification” below) confirmed the diagnosis. Serum was negative for antibodies to the AIE-75 intestinal antigen. Pimecrolimus (0.1%) cream applied twice daily for 1 month was ineffective, but a recurrence of psoriasiform hyperplasia of the epidermis with overlying confluent parakeratosis. Exocytosis of mature lymphocytes into the epidermis with associated spongiosis was also noted. Immunocytochemical stains for CD4, CD8, and CD3 (not shown) were positive in approximately 98% of the cells, consistent with a predominantly T-cell infiltrate. Approximately 60% to 70% were positive for CD8, and 30% to 40% were positive for CD4. Periodic acid–Schiff stain results were negative for fungal organisms.

Figure 2. Skin biopsy specimen of a new skin lesion on the left posterior thigh (hematoxylin-eosin, original magnification ×10). The biopsy findings are consistent with psoriasiform dermatitis. Sections showed irregular psoriasiform hyperplasia of the epidermis with overlying confluent parakeratosis. Exocytosis of mature lymphocytes into the epidermis with associated spongiosis was also noted. Immunocytochemical stains for CD4, CD5, and CD8 (not shown) were positive in approximately 98% of the cells, consistent with a predominantly T-cell infiltrate. Approximately 60% to 70% were positive for CD8, and 30% to 40% were positive for CD4. Periodic acid–Schiff stain results were negative for fungal organisms.

Methods

Consent

Written informed consent was obtained from the patient’s mother to obtain blood specimens for genetic testing and cytokine studies. Written informed consent was also obtained from the 2 control patients who donated blood specimens for cytokine studies.

Mutation Identification

Genomic DNA was extracted from peripheral blood mononuclear cells using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, Calif) according to the manufacturer’s recommendations. Each of the 11 exons of the human FOXP3 gene (including at least 45 base pairs of intronic sequence of each exon/intron boundary) were amplified for analysis by the polymerase chain reaction (PCR) using 7 primer pairs. Polymerase chain reaction was performed using a PTC-100 thermal cycler (MJ Research Inc, Waltham, Mass) with the following conditions: approximately 75 ng of genomic DNA template, 1 cycle at 94°C for 30 seconds, 57°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. Polymerase chain reaction products were purified using the QIAquick PCR purification kit (Qiagen). Sequencing was performed on fluorescent-labeled DNA using the ABI PRISM big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, Calif), and chromatograms were generated on an Applied Biosystems (Foster City, Calif) Model 377 DNA sequencer according to the manufacturer’s instructions. The A of the first initiation codon (ATG) is counted as nucleotide number 1.

Immunologic Studies

At the time the diagnosis of IPEX was confirmed by sequence analysis, peripheral blood was collected from the patient and a healthy control, diluted 10-fold with Iscove’s Modified Dulbecco’s Medium (Life Technologies, Rockville Md) supplemented with 0.1% fetal calf serum (HyClone; Logan, Utah), then dispensed into 24-well culture dishes (Becton-Dickinson Biosciences, Bedford, Mass) in 1-mL aliquots. The cultures were incubated at 37°C in a 5% carbon dioxide atmosphere in the presence of lipopolysaccharide (LPS [1 µg/mL]; Sigma Chemicals, St Louis, Mo) and IFN-γ (1000 U/mL; R&D Systems, Minneapolis, Minn) or concanavalin A (ConA [10 µg/mL]; Sigma Chemicals) and phytohemagglutinin-P (PHA [1 µg/mL]; Sigma Chemicals) or antihuman CD3 antibodies (anti-

37.2 EU/mL [normal <20.0 EU/mL]), and were negative for anti–Saccharomyces cerevisiae mannan IgG and anti-OmpC.

At age 10 years, the patient experienced worsening of his chronic diarrhea, accompanied by severe anemia (hematocrit, 14%). The cause of the anemia was not determined, but autoimmune destruction and gastrointestinal bleeding were excluded. An upper gastrointestinal tract radiography with small-bowel follow-through revealed circumferential wall thickening and mucosal irregularity of the terminal ileum, suggestive of Crohn disease. Infliximab was administered at a dose of 6 mg/kg body weight and administered again 2 and 4 weeks later. After 1 month, stool frequency decreased and consistency increased. These remained stable for over 6 months. At age 11 years, the patient developed additional intermittent episodes of increased stool frequency, which have been managed with parenteral nutrition.
CD3 (1 µg/mL; BD PharMingen, Franklin Lakes, NJ) and antihuman CD28 antibodies (anti-CD28 [250 ng/mL]; BD PharMingen). Cell-free culture supernatants were collected 72 hours later and analyzed by standard sandwich enzyme-linked immunoabsorbent assay for human interleukin (IL) 6, IL-8, IL-12 (p70), INF-γ, and monocyte chemoattractant protein 1 (MCP-1), prostaglandin E2 (PGE2), IL-12, and IFN-γ content. Production of each mediator is expressed as a percentage difference compared with normal peripheral blood cells treated similarly. Results are the average of 2 experiments.

Blood from the patient with IPEX was compared with blood from a healthy control (Figure 3). In the absence of exogenous stimulation there was virtually no production of any mediator. Production of IFN-γ was minimal, regardless of stimulant. Levels of IL-12 were markedly depressed in blood stimulated with LPS and IFN-γ. Production of IL-4 is not shown, since we were unable to obtain consistent IL-4 production in his sample or in the healthy control. High levels of MCP-1, a type II cytokine closely linked to IL-4 production, were generated after stimulation with LPS and IFN-γ. Production of IL-6 and IL-8 was elevated and normal, respectively, when blood cells were stimulated by LPS and IFN-γ, whereas both were depressed after stimulation with ConA/PHA and anti-CD3/anti-CD28. Production of prostaglandin E2, a proinflammatory and immunoregulatory mediator, was moderately depressed in blood cells activated with LPS and IFN-γ, markedly depressed in cells stimulated with ConA/PHA, and essentially normal in cells stimulated with anti-CD3/anti-CD28.

### MUTATION IDENTIFICATION

Sequencing of the patient's genomic DNA revealed a G→A transition (1150G>A) in exon 11, resulting in a substitution of Ala→Thr at residue 384, within the DNA-binding domain of FOXP3. An identical mutation was subsequently detected in 1 allele in the patient's mother and sister, accompanied by a normal allele. No mutation was identified in the patient's maternal grandmother.

### IMMUNOLOGIC STUDIES

A previous report revealed PHA-derived T lymphoblasts from a patient with IPEX were poorly inducible for messenger RNA (mRNA) for IFN-γ, whereas mRNAs encoding the type II cytokines were augmented. In our patient, peripheral blood cells were activated in vitro for 72 hours with stimulants known to activate T cells (ConA/PHA and anti-CD3/anti-CD28) as well as monocytes (LPS and IFN-γ) to assess selected type 1 (IL-12 and IFN-γ) and type II (IL-4 and MCP-1) cytokines, as well as modulators of innate immunity (IL-6, IL-8, and prostaglandin E2).

In 1982, Powell et al described a family with an X-linked syndrome characterized by early onset of diarrhea, diabetes mellitus, dermatitis, and early death. Since then, additional kindreds have been identified, and other observed features include hypothyroidism, hemolytic anemia, enteritis due to antenterocyte antibodies, increased incidence of infections, hemolytic anemia, food allergy, elevated IgE levels, and nephropathy.

Recently, mutations of FOXP3, which encodes scurf, a DNA-binding protein involved in the generation of regulatory T cells, were identified in several IPEX families. Prior to this discovery, a naturally occurring mutation of FOXP3 was identified in the scurfy mutant.
Scurfy T cells display increased levels of FOXP3, and gene knockout experiments have demonstrated that FOXP3 is necessary for their development. Furthermore, regulatory T cells are the predominant site of FOXP3 expression, and gene knockdown experiments have demonstrated that FOXP3 is necessary for their development. Scurfy T cells display increased levels of cellular proteins associated with activation, including CD69, CD25, CD80, and CD86. Increased immune activity leads to increased production of IL-2, IL-4, IL-5, IL-6, IL-10, IFN-γ, and tumor necrosis factor α. Scurfy T cells are less sensitive than healthy controls to cyclosporine and to inhibitors of tyrosine kinases such as herbimycin A and genistein. Thus, defective FOXP3 in scurfy mice leads to loss of regulatory T cells, increased immune activity, and loss of natural safeguards against autoimmunity. It is likely that similar mechanisms account for the spectrum of autoimmune disease seen in humans with IPEX.

The missense mutation Ala384Thr identified in our patient affects the forkhead/DNA binding domain of scurfy. The same missense mutation has been identified in 2 other unrelated patients with IPEX. Most mutations identified to date affect the forkhead domain or result in loss of product. Schubert et al have demonstrated that overexpression of scurfy in a mouse model attenuates activation-induced IL-2 production and cell proliferation. In addition, they demonstrated forkhead binding sequences adjacent to the NFAT (nuclear factor of activated T cells) regulatory sites in the promoters for IL-2 and granulocyte-macrophage colony-stimulating factor. It is therefore possible that IPEX patients fail to down-regulate immune function because of lack of protein or because the mutated forkhead domain cannot properly bind to DNA.

Immunosuppressive agents seem to be beneficial in the treatment of IPEX. Long-term control of gastrointestinal symptoms with cyclosporine was early as 1990. In a Japanese child with IPEX, diarrhea was transiently controlled with cyclosporine though long-term control was achieved only with combined betamethasone and tacrolimus treatment.

Allogeneic bone marrow transplantation has been used with mixed results. Interestingly, the symptoms of IPEX in transplantation patient 1 remained controlled even when donor engraftment declined to 28%. Patients 5 and 6 also remained clinically stable despite major declines in donor engraftment. It is possible that even limited numbers of normal regulatory T cells are sufficient to attenuate the remaining abnormally reactive T cells of host origin. This may explain the absence of disease in female heterozygotes who have random X chromosome inactivation. Indeed, in the scurfy mouse, normal T cells have been shown to be curative. We have chosen to defer bone marrow transplantation in the case of our patient owing to his high overall functional status.

Dermatitis has been reported frequently in IPEX patients, and skin biopsies have been performed in several patients. One case described spongiotic psoriasiform epidermal hyperplasia accompanied by parakeratosis, thick suprapapillary plates, dilated blood vessels, a superficial perivascular infiltrate of lymphocytes and histiocytes, and mild exocytosis. A skin biopsy specimen from a 4-year-old boy demonstrated nonspecific lymphohistiocytic dermal infiltrates; treatment with topical and parenteral corticosteroids and topical cyclosporine were only minimally beneficial. Three years later, this same patient developed 0.5- to 1.0-cm bullae; a skin biopsy specimen demonstrated subepidermal blisters. Immunofluorescence showed deposits of IgG along the roof of the blister as well as deposits of C3 along the roof and the floor of the blister in a granular pattern, consistent with bullous pemphigoid. The blisters improved substantially following treatment with dapsone, cyclosporine, and prednisone.

The fixed, nummular plaques observed in our patient have not been described in other patients with IPEX. The lesions failed to respond to occasional use of an ul-
trapotent topical corticosteroid but responded when one was applied regularly. Likewise, to our knowledge, this is the first report describing the presence of alopecia universalis in a patient with IPEX, although at least 1 other IPEX patient is known to have developed this condition in childhood (H.O., unpublished observation, July 2001). We suspect that the cause of the patient’s alopecia is autoimmune, and his nail findings are consistent with those seen in alopecia areata. Our patient has also had intermittent urticaria, although this has been dormant in recent years.

Using a panel of serologic markers that have been associated with IBD, we found positive perinuclear antineutrophil cytoplasmic antibodies and IgA anti–S. cerevisiae mannan antibody. According to reference data supplied by the test manufacturer, the pattern of markers seen in our patient is observed in fewer than 3% of all patients with confirmed IBD and has not been observed in healthy controls. Of patients with IBD who displayed this pattern, 49% have Crohn disease and 51% have ulcerative colitis. The decreased disaccharidase activity observed while the patient had severe villous atrophy is most likely due to loss of villi.

Our patient’s neutropenia was accompanied by the presence of antineutrophil antibodies, a finding that has not yet been reported in IPEX. The cause of his recent anemia is puzzling; many features suggest hemolytic anemia, but Coombs testing results were always negative.

When activated in vitro, our patient’s mononuclear cells failed to produce IFN-γ and IL-12 (type I cytokines) and produced a pattern of other lymphokines that differed from healthy controls, suggesting a fundamental cytokine dysregulation in IPEX. Chatila et al.11 have studied the expression of cytokine mRNA in 2 patients with IPEX and showed that expression of IFN-γ mRNA was diminished with respect to controls, whereas expression of mRNA for the TGFβ2 cytokines IL-4, IL-5, IL-10, IL-13 was increased. This skewing of the immune system of IPEX patients toward the TH2 pathway, as exemplified in our patient who failed to produce the TGFβ1 cytokine IFN-γ, is consistent with the clinical findings of dermatitis, food allergy, and elevated IgE levels.11

IPEX shares similar clinical features with autoimmune polyendocrine disease type 1 (APS 1, Mendelian Inheritance in Man 240300), also known as autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy, an autosomal dominant disorder that results from mutations in the autoimmune regulator (AIRE) gene on chromosome 21, which also codes for a DNA-binding protein. Patients with autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy often develop multisystem autoimmune endocrine disease accompanied by alopecia universalis, and diarrhea. These patients typically present in childhood with chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenal insufficiency.30

In conclusion, a dermatologist who sees a patient with dermatitis or alopecia universalis accompanied by other autoimmune disease should consider that these may be features of a single genodermatosis rather than disparate findings. Proper diagnosis will guide therapy, which may include systemic immunosuppression or stem cell transplantation. Testing for mutations of FOXP3 will firm the diagnosis in affected patients and facilitate genetic counseling of female family members concerning carrier status.

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REFERENCES


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**News and Notes**

The National Registry for Ichthyosis and Related Disorders is seeking enrollment of all patients with inherited disorders of keratinization (except ichthyosis vulgaris). Serum testing for X-linked recessive ichthyosis, as well as molecular diagnosis of selected disorders, is available without charge. We are eager to assist with research efforts, and we welcome proposals. Information and enrollment forms can be downloaded from our Web site. Please contact us to enroll your affected patients or discuss research interests: phone: 1-800-595-1265; e-mail: info@skinregistry.org; Web site: www.skinregistry.org.