Mechanistic Associations of a Mild Phenotype of Immunodysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome

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Background & Aims: **The syndrome of immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) is a rare disorder resulting in the expression of multiple autoimmune and allergic features. Early onset enteropathy and type 1 diabetes (T1D) are the most common clinical features. The IPEX syndrome is caused by mutations of the FOXP3 gene, which is essential for the development of regulatory T cells (Treg). We describe 2 unrelated patients with IPEX syndrome with a mild clinical phenotype and with novel FOXP3 mutations and the phenotypic and functional characterization of their Treg cells.** ***Methods:*** **The FOXP3 gene was analyzed by sequencing amplimers from genomic DNA. Treg cells were characterized by evaluating the number of CD4+CD25+ T cells and their functional ability to suppress the proliferation of autologous CD4+CD25− effector T cells stimulated with anti-CD3 and anti-CD28 antibodies.** ***Results:*** **A 7-year-old boy and a 24-year-old man presented with autoimmune enteropathy characterized by early onset persistent diarrhea not associated with T1D or other endocrinopathies. These 2 patients carry novel FOXP3 mutations that do not abrogate the function of the forkhead domain. They have normal numbers of CD4+CD25+ T lymphocytes, however, these show severely defective suppressive function in vitro.** ***Conclusions:*** **Our 2 patients show that IPEX patients may present with early onset enteropathy and long-term survival without T1D or other endocrinopathies. This milder phenotype may be associated with FOXP3 mutations that do not abrogate the function of the forkhead domain.**

The syndrome of immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) is characterized by multiple autoimmune and allergic features.[1–3] Early onset enteropathy, with villous atrophy and infiltrating inflammatory cells, is the most common manifestation. It is difficult to treat and is the primary cause of death. Early onset type 1 diabetes (T1D), with autoantibodies to islet antigens, usually is present and other autoimmune endocrinopathies are common, together with thrombocytopenia, hemolytic anemia, and arthritis.[1,4] Asthma and eczema, with increased serum immunoglobulin (Ig)E levels, are frequent. Most reported patients with IPEX have died in infancy or early childhood.

IPEX is caused by mutations of the FOXP3 gene encoding a transcription factor, named *scurfin*, of the forkhead family.[5] Scurfin is a DNA-binding protein that suppresses cytokine gene expression in activated T lymphocytes, possibly through direct repression of NFAT.[6] In human beings, 13 FOXP3 mutations have been reported.[4] Ten disrupt the forkhead domain, 2 affect the leucine zipper domain, and 1 affects the first polyadenylation signal. The forkhead domain is required, both in humans and mice, for FOXP3 function.[6] The impact of mutations in the leucine zipper domain and in the polyadenylation site on expression and function of FOXP3 still is unknown.

FOXP3-deficient mice have a disease similar to IPEX.[7] In mice, FOXP3 is essential for the development of $CD4^+CD25^+$ regulatory T cells (Treg) and transplantation of Tregs from a normal strain rescues the clinical phenotype.[8] Human Treg cells, also included in the $CD4^+CD25^+$ population, inhibit proliferation and cytokine production of effector T cells and have been implicated in several immune-mediated disorders.[9] We report

Abbreviations used in this paper: ANA, antinuclear antibody; bp, base pair; cDNA, complementary DNA; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin IPEX, immunodysregulation, polyendocrinopathy, enteropathy, X-linked; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; T1D, type 1 diabetes; Treg, regulatory T cells.

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2 unrelated patients with IPEX and a mild clinical phenotype characterized by early onset enteropathy, absence of T1D or other endocrinopathies, and prolonged survival. These patients carry novel FOXP3 mutations that do not disrupt the forkhead domain and show a functional defect of $CD4^+CD25$ Treg cells.

Case Reports

Patient 1

A 22-year-old man was referred because of recurrent diffuse joint pain associated with the presence of antinuclear antibodies (ANAs). The mother died at 42 years of age of intestinal lymphoma. The patient had 3 brothers. One brother died at 1 month of age from intractable diarrhea; diabetes was not reported. The other 2 brothers, both asymptomatic, had biopsy examination–proven celiac disease and responded to a gluten-free diet. One brother died at 14 years of age in an accident; the other brother, aged 26 years, was lost to follow-up evaluation.

At 18 days of age, patient 1 developed diarrhea and severe malnutrition. Parenteral nutrition and a subsequent diet with a peptide formula reportedly were associated with improvement and he was maintained on a gluten-free diet. At 5 years, after reintroduction of a full gluten-containing diet, abdominal pain, diarrhea, and failure to thrive relapsed. An intestinal biopsy examination showed subtotal villous atrophy and mononuclear cell infiltrate in the lamina propria (Figure 1*A*). Immunohistochemistry subsequently was performed and revealed an intraepithelial $CD3^+CD8^+$ cell count of 42/100 epithelial cells (upper limit of normal, 40) (Figure 1*B*). High titers of antigliadin IgG and antiendomysial antibodies were found. Celiac disease was diagnosed. After an initial clinical improvement with a gluten-free diet, diarrhea rapidly relapsed and failure to thrive persisted. Two years later a second intestinal biopsy examination showed similar findings (not shown). IgE levels were high (200 mU/mL; normal values for age, $<$60 mU/mL), reportedly in the absence of allergy. Diarrhea and failure to thrive persisted. At 15 years, the patient developed arthralgia and transient arthritis. Antienterocyte antibodies were detected for the first time in this patient (immunofluorescence on human duodenum; Dr. E. Bosi, San Raffaele Research Institute, Milan, Italy). A diagnosis of autoimmune enteropathy was made and treatment with glucocorticoids and azathioprine was started with partial improvement. At 18 years the patient developed well-defined, scaly, erythematous plaques on the trunk and limbs. A skin biopsy examination showed paracheratosis, Poitrier-like epidermal microabscesses, and mononuclear cell infiltrate in perivascular aggregates, consistent with psoriasiform dermatitis (Figure 1*E*).

Chronic enteropathy remained active until admission to our unit despite continuous treatment with glucocorticoids, immunosuppressants (cyclosporin A, tacrolimus), and infliximab, alone or in combination, and intermittent parenteral nutrition. At admission, the patient was receiving prednisone, cyclosporin A, and nightly parenteral nutrition. His height was 164 cm (25th–50th percentile), and his weight was 48 kg (10th–25th percentile). He was in fair general condition, presenting with weakness and mild signs of dehydration. Maculopapular lesions were present over the limbs and trunk. The liver was mildly enlarged. The white blood cell count was 10,570/mm^3, hemoglobin level was 12.1 g/dL, platelet count was 219,000/mm^3, erythrocyte sedimentation rate (ESR) was 28 mm/h (normal, $<$20 mm/h), and the C-reactive protein level was 39 mg/L (normal, $<$5 mg/L). Metabolic acidosis (pH 7.30; bicarbonate, 13.3 mEq/L) and renal failure (serum creatinine level, 2.59 mg/dL; urea nitrogen level, 42 mg/dL) were present. Blood electrolyte, glucose, and liver enzyme levels were normal. ANAs were positive (1:160; homogeneous pattern). Antitransglutaminase antibodies were negative. The patient was treated with 3 intravenous methylprednisolone pulses (1 g/day) and parenteral rehydration. He was discharged 1 week later in good general condition with prednisone (1 mg/kg/day in 1 daily dose), tacrolimus (.04 mg/kg/day), azathioprine (2 mg/kg/day), and nightly parenteral nutrition. His weight was 50 kg, creatinine level was 1.45 mg/dL, and bicarbonate level was 22.0 mEq/L.

Patient 2

A 5-year-old boy was referred to our unit because of pain in the wrist and the left proximal interphalangeal joint of the second finger and diffuse arthralgia. His 12-year-old brother was healthy. Severe chronic diarrhea had appeared at 14 months of age. An intestinal biopsy examination showed dysmorphic villi with no atrophy and a marked mononuclear cell infiltrate in the lamina propria (Figure 1*C*) and an intraepithelial $CD3^+CD8^+$ cell count of 60/100 epithelial cells (upper limit of normal, 40) (Figure 1*D*). Antigliadin and antiendomysial antibodies were negative. The patient did not improve on a gluten-free diet. IgE levels were 74 mU/mL (normal value for age, $<$10 mU/mL). Antienterocyte antibodies were negative. ANAs were present (1:5120, homogenous pattern). The patient was diagnosed with an unspecified autoimmune enteropathy and treatment with prednisone, azathioprine, and exclusive intravenous nutrition