Severe Food Allergy as a Variant of IPEX Syndrome Caused by a Deletion in a Noncoding Region of the *FOXP3* Gene

TROY R. TORGERSON,* AVRIEL LINANE,* NICOLETTE MOES,‡,§ STEPHANIE ANOVER,* VÉRONIQUE MATEO,‖ FRÉDÉRIC RIEUX–LAUCAT,‖ OLIVIER HERMINE,¶ SHASHI VIJAY,* ELEONORA GAMBINERI,# NADINE CERF–BENSUSSAN,§ ALAIN FISCHER,‖,** HANS D. OCHS,* OLIVIER GOULET,‡,§ and FRANK M. RUEMMELE‡,§

*University of Washington & Children's Hospital, Department of Pediatrics, Division of Immunology, Rheumatology, & Infectious Diseases, Seattle, Washington; ‡AP-HP, Hôpital Necker-Enfants Malades, Department of Pediatrics, Pediatric Gastroenterology, Paris; §Université Paris Descartes, Faculté de Médecine René Descartes, Site Necker, INSERM, U793, Paris; ‖Université Paris Descartes, Faculté de Médecine René Descartes, Site Necker, INSERM, U768, Paris; ¶Université Paris Descartes, Faculté de Médecine René Descartes, Site Necker, CNRS, UMR 8147, Paris, France; #Department of Pediatrics, "A. Meyer" Children's Hospital, University of Florence, Florence, Italy; and **AP-HP, Hôpital Necker-Enfants Malades, Department of Pediatrics, Pediatric Immunology, Paris, France

Background & Aims: **Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX; OMIM 304930) syndrome is a congenital syndrome characterized by autoimmune enteropathy, endocrinopathy, dermatitis, and other autoimmune phenomena. In the present work, we aimed to uncover the molecular basis of a distinct form of IPEX syndrome presenting at the edge of autoimmunity and severe allergy.** ***Methods:*** **The *FOXP3* gene was sequenced, FOXP3 messenger RNA (mRNA) was quantified by real-time polymerase chain reaction (PCR), and protein expression in peripheral blood lymphocytes was analyzed by flow cytometry after intracellular staining. In coculture experiments ($CD4^+CD25^-$ and $CD4^+CD25^+$ cells), the functions of regulatory T cells were analyzed. Expression of interferon γ and interleukin 2 and 4 mRNA within the inflamed intestinal mucosa was quantified by real-time PCR.** ***Results:*** **Here, we describe a distinct familial form of IPEX syndrome that combines autoimmune and allergic manifestations including severe enteropathy, food allergies, atopic dermatitis, hyper-IgE, and eosinophilia. We have identified a 1388-base pair deletion (g.del−6247_−4859) of the *FOXP3* gene encompassing a portion of an upstream noncoding exon (exon −1) and the adjacent intron (intron −1). This deletion impairs mRNA splicing, resulting in accumulation of unspliced pre-mRNA and alternatively spliced mRNA. This causes low *FOXP3* mRNA levels and markedly decreased protein expression in peripheral blood lymphocytes of affected patients. Numbers of $CD4^+CD25^+FOXP3^+$ regulatory T cells are extremely low, and the $CD4^+CD25^+$ T cells that are present exhibit little regulatory function.** ***Conclusions:*** **A new mutation within an upstream noncoding region of FOXP3 results in a variant of IPEX syndrome associating autoimmune and severe immunoallergic symptoms.**

An old but initially speculative immunologic concept claiming the existence of suppressor T lymphocytes[1,2] was substantiated with the discovery that mutations of the transcription factor FOXP3 result in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome[3–5] and that FOXP3 is required for the generation of $CD4^+CD25^+$ regulatory T lymphocytes (T_{REG}).[6–8] A growing body of experimental and in vivo evidence in humans and animals has demonstrated a crucial role for FOXP3-expressing $CD4^+$ T cells as potent suppressors of self-reactive T-cell activation and proliferation, presumably via direct cell-cell interaction.[9,10] Some evidence points to a role for T_{REG} cells in the control of immune responses to exogenous antigens, such as dietary food antigens,[11,12] but much less is known about this aspect of their function.

The *FOXP3* gene maps to Xp11.23 and encodes a 431-amino acid (48 kilodalton) protein, also named *scurfin*. FOXP3 has significant homology to members of the Forkhead/winged-helix transcription factor family[13] and plays a central role in the generation of T_{REG} cells. FOXP3 functions as transcriptional repressor, thus allowing negative control of T-cell activation via DNA sequences containing FOXP3 binding sites located adjacent to nuclear factor of activated T-cell regulatory sites in cytokine promoters such as interleukin (IL)-2 or granulocyte-macrophage colony-stimulating factor enhancer.[14–16]

IPEX syndrome is a severe, systemic autoimmune disorder that typically presents in infancy with various autoimmune symptoms including protein-loosing enteropathy; early onset, insulin-dependent diabetes mellitus; and other endocrinopathies, dermatitis, and autoim-

Abbreviations used in this paper: IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; T_{REG}, regulatory T lymphocytes.

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mune cytopenias.[17–20] Effective therapeutic options for IPEX syndrome patients are limited. In addition to supportive measures, such as total parenteral nutrition, insulin injections, thyroid hormone substitution, and red blood cell transfusions, various immunosuppressive regimens have been utilized including high-dose steroids, cyclosporin A, tacrolimus, sirolimus, and rituximab with variable success.[19–23] Because IPEX syndrome results from an absent or dysfunctional lymphocyte subset, bone marrow transplantation offers a potentially curative treatment option for boys suffering from this devastating disorder.[24–26] Results thus far have been mixed, but efforts are underway to optimize conditioning regimens to improve further this mode of therapy.

Here, we present a kindred with a distinct clinical presentation of IPEX syndrome with no detectable endocrinopathy but an impressive allergic phenotype manifested by severe food allergy and eczema. Molecular analyses revealed a unique *FOXP3* mutation involving a large deletion in an upstream, noncoding region of the FOXP3 gene. This deletion, encompassing the 3′ half of the untranslated −1 exon and approximately 1000 base pairs (bp) of the adjacent intron, leads to markedly reduced *FOXP3* messenger RNA (mRNA) levels and absence of regulatory T cells. These cases provide a unique insight into the critical role of naturally arising $CD4^+CD25^+FOXP3^+$ T_{REG} cells in controlling immune responses to exogenous antigens as well as in maintaining self-tolerance.

Clinical Features

Patients IV.1 and IV.2

Patient IV.1, (Figure 1) the index case, followed at Necker-Enfants Malades Hospital, Paris, was born in 2000 after an uneventful pregnancy to unrelated, healthy white parents. No abnormalities were observed during the neonatal period while he was exclusively breastfed. At 3 weeks of age, infant formula was introduced, and, within 1 week, he developed massive watery-bloody diarrhea requiring total parenteral nutrition, daily albumin supplementation, and repeated blood transfusions. The severe protein-loosing enteropathy was accompanied by the appearance of an erythematous, eczematous skin rash. Laboratory evaluation revealed no signs of glucose intolerance, thyroid abnormalities, Addison's disease, or hematologic abnormalities, and there was no evidence of renal or lung involvement. Immunologic testing at the age of 6 weeks showed normal peripheral blood lymphocyte counts and subsets (3800/μL: $CD3^+$, 91%; $CD4^+$, 65%; $CD8^+$, 21%; CD19, 5%) with reduced natural killer (NK) T cells ($CD3^+CD56^+$, 0.6%) and normal levels of IgG (200 mg/dL), IgM (15 mg/dL), and IgA (26mg/dL). In contrast, IgE levels were strikingly elevated at >3000 μ/mL and associated with eosinophilia (950 cells/μL). At the age of 2 months, high titer antienterocyte antibodies

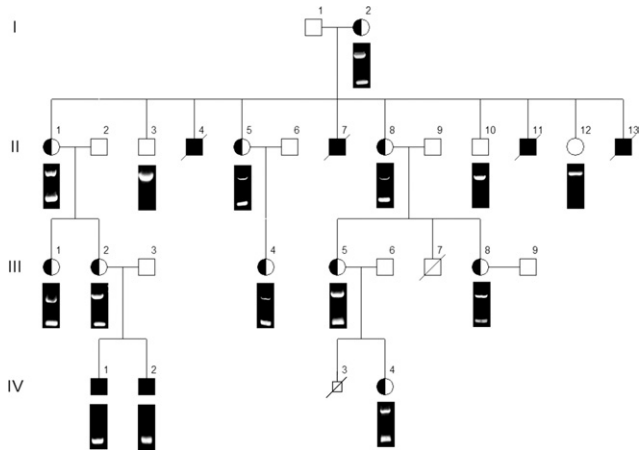

Figure 1. Pedigree demonstrating X-linked inheritance in the identified kindred. Individual family members were tested for the presence of the identified deletion mutation using PCR with forward and reverse primers flanking the deletion in genomic DNA. These primers generate an 1805-base pair fragment from a normal allele and a 417-base pair fragment from an allele harboring the identified 1388-base pair deletion. An ethidium bromide-stained gel demonstrating the fragments amplified from genomic DNA is shown beneath each of the individuals who were tested. Note that the proband (*IV.1*) and his brother (*IV.2*) demonstrate only the mutant allele, whereas normal, unaffected males demonstrate only the wild-type allele (*II.3* and *II.10*). All but one of the females tested were found to be carriers of the mutation.

(immunofluorescence study) were demonstrated. Antiautoimmune enteropathy (AIE) 75-kilodalton antibodies[27] (directed against intestinal epithelial cell antigens) were initially negative but became strongly positive within the first 4 months of life. No other auto-antibodies were detected, including antinuclear, antiliver, antikidney, antismooth muscle, antimitochondrial, antimicrosomal, or antithyroglobulin antibodies. Initial endoscopic evaluation of the gastrointestinal (GI) tract, performed at 7 weeks of age, revealed gastric hyperemia and villous atrophy in the duodenum. Histologic analysis showed an intense lympho-plasmocellular infiltrate with a marked eosinophilic component in the lamina propria of the stomach as well as the duodenum (Figure 2). The epithelial layer was disorganized with a high rate of apoptosis among the enterocytes. Duodenal biopsy specimens were characterized by severe to total villous atrophy and a massive T-cell infiltration in the lamina propria, with no or only moderate increase of intraepithelial lymphocytes. The colonic mucosa revealed superficial ulcerations and a major mononuclear cell infiltrate that was rich in eosinophils. Neither bacteria nor viral pathogens could be identified in the intestinal/colonic mucosa or in peripheral blood.

Patient IV.2, the younger brother of the index case, was born at term in 2002 after an uneventful pregnancy and delivery. Because of the older brother's medical history, the mother's diet was restricted beginning during the third trimester of pregnancy to exclude cow milk protein