

Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of *FOXP3*, a critical regulator of T-cell homeostasis

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Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX) is one of a group of clinical syndromes that present with multisystem autoimmune disease suggesting a phenotype of immune dysregulation. Clinically, IPEX manifests most commonly with diarrhea, insulin-dependent diabetes mellitus, thyroid disorders, and eczema. *FOXP3*, the gene responsible for IPEX, maps to chromosome Xp11.23-Xq13.3 and encodes a putative DNA-binding protein of the forkhead family. Recent data indicate that *FOXP3* is expressed primarily in the CD4⁺CD25⁺ regulatory T-cell subset, where it may function as a transcriptional repressor and key modulator of regulatory T-cell fate and function. This review describes the clinical features of IPEX and the structure, function, and known mutations of *FOXP3* that provide important insights into its role in maintenance of immune homeostasis.

Keywords

Immune dysfunction, polyendocrinopathy, enteropathy, autoimmunity, *FOXP3*, IPEX, Scurfy mouse, CD4⁺CD25⁺ regulatory T lymphocytes

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Abbreviations

BMT	bone marrow transplantation
IDDM	insulin-dependent diabetes mellitus
IPEX	immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance

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The immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance syndrome (IPEX; OMIM 304930) is a fatal, recessive disorder that presents most commonly in early childhood. Characteristic symptoms of the disorder include early-onset insulin-dependent diabetes mellitus (IDDM), severe enteropathy, eczema, variable autoimmune phenomena, and severe infections. The Human Genome Organization has recently established *IPEX* as the official designation for this condition, previously identified as X-linked syndrome of polyendocrinopathy, immune dysfunction, and diarrhea (XPID) [1] or X-linked autoimmunity-allergic dysregulation syndrome (XLAAD) [2].

The syndrome was first recognized in 1982 by Powell *et al.* [3•], who described a large family of five generations with several affected males; the syndrome subsequently was mapped to chromosome Xp11.23-Xq13.3 by linkage analysis [1,4]. IPEX shares many phenotypic features with the natural mutant mouse *scurfy* described more than 40 years ago [5]. *Scurfy* mice present with X-linked recessive inheritance of scaly skin; small, thickened ears; runting secondary to diarrhea and malabsorption; progressive anemia; thrombocytopenia; leucocytosis; lymphadenopathy; hepatosplenomegaly; and hypogonadism. *Scurfy* mice typically die by 3 to 4 weeks of age. The thymus is typically small for age but has normal cellular architecture. There is massive infiltration of activated lymphocytes into the liver and into lymph nodes and spleen, which have abnormal cellular architecture [6,7].

Previous studies in *scurfy* mice have demonstrated that the clinical phenotype is the result of immune dysregulation. CD4⁺ T cells from affected males are constitutively activated, expressing high levels of several activation markers and increased levels of cytokine production [8–10]. Both depletion and adoptive transfer experiments indicate that CD4⁺ T cells are primarily responsible for the disease phenotype in mice [9,11].

Foxp3, the gene responsible for *scurfy* syndrome, encodes a transcription factor belonging to the forkhead/winged-helix family [12••]. Considering the clinical similarities between the *scurfy* mouse and IPEX, the authors and others have identified the human orthologue

of the mouse *Foxp3* gene (*FOXP3*) as the causative gene for IPEX [2,13,14].

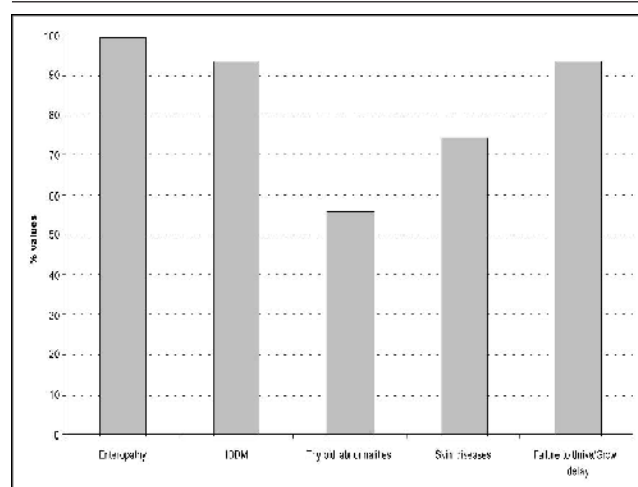
Clinical aspects of IPEX

The clinical features of IPEX have been summarized in recent reports [15,16] (Table 1). Although isolated cases with the characteristic phenotype of the IPEX syndrome have been documented over many years, they have not been linked to a single disorder [17,18]. Most of the affected males are brought for treatment for symptoms early in life, usually during infancy. Later onset of the syndrome was observed in the family described by Powell *et al.* [3•].

Among 20 kindreds with the IPEX phenotype that the authors have analyzed to date, a molecular diagnosis was established in 13 families by identification of specific mutations in the *FOXP3* gene [19]. The authors have observed that enteropathy, present in 100% of the patients, is the most common feature and usually manifests itself as watery or mucous-bloody diarrhea (Gambineri *et al.*, unpublished data, May 2003). Severe villous atrophy and mucosal erosion with lymphocytic infiltrates of the submucosa or lamina propria were observed in small bowel biopsies, often leading to the diagnosis of Crohn disease or ulcerative colitis. Endocrinopathies including IDDM and thyroid abnormalities were frequently observed. The thyroid abnormalities included abnormal serum triiodothyronine or thyroxine levels or both, and sometimes clinical evidence of hypothyroidism. Lymphocytic infiltrates observed in pancreas and thyroid suggest that the pathology of these organs is the result of an inflammatory destruction by a T-cell-mediated autoimmune mechanism rather than abnormal organogenesis [20,21]. Other common features of the IPEX phenotype are skin disorders—primarily eczema, but also erythroderma and exfoliative dermatitis, both erythroderma and exfoliative dermatitis occur at lower frequency than eczema.

Failure to thrive, almost always associated with the syndrome (Fig. 1), is caused by enteropathy and malabsorption [21–25], but may also be secondary to chronic illness. Increased risk of recurrent infections has been observed [3•,4,21–25] and may be correlated with a defect in immune regulation or may be a consequence of

Figure 1. Incidence of typical clinical manifestations of immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance in 13 patients



Incidence of various clinical manifestations in patients from 13 families with confirmed *FOXP3* mutations. IDDM, insulin-dependent diabetes mellitus.

the immunosuppressive therapy used to treat the disorder. The severity of infections typically varies from moderate infections of the upper respiratory or gastrointestinal tract to severe infections such as sepsis, peritonitis, and pneumonia. The most common infectious organisms are *Enterococcus* and *Staphylococcus* species. In addition, autoimmune hematologic disorders such as hemolytic anemia and thrombocytopenia frequently occur [21,26]. The authors have also observed autoimmune neutropenia in one patient (Torgerson, *et al.*, unpublished data, October 2002). Less common clinical features include glomerulopathy, arthritis, lymphadenopathy, and hepatosplenomegaly [3•,15,21].

Interestingly, heterozygous females appear to be healthy even though X-inactivation occurs randomly. X-chromosome inactivation analysis performed on one carrier female has demonstrated that normal and mutated *FOXP3* alleles are equally expressed in circulating T lymphocytes [27•], thereby masking the disease by a compensatory mechanism.

Laboratory findings

Patients with IPEX have normal complement levels and neutrophil oxidative burst. Serum IgG, IgM, and IgA levels are generally normal, although they may decrease over time because of a progressive loss of protein through the bowel caused by the enteropathy. In contrast, serum IgE levels are elevated in most patients, and eosinophilia is sometimes present.

Most patients generate a variety of autoantibodies against different organs, including thyroid, pancreatic islets, erythrocytes, platelets, and small intestine. Autoantibodies to a novel 75-kD gut and kidney-specific anti-

Table 1. Major clinical features of immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance

Watery/bloody diarrhea
Failure to thrive
Diabetes mellitus type 1
Hypothyroidism
Eczema
Hemolytic anemia
Thrombocytopenia
Lymphadenopathy
Hepatosplenomegaly
Recurrent infections

gen, AIE-75, have been described in patients with IPEX [23,28].

Total lymphocyte counts and T/B lymphocyte subsets are generally within normal ranges [4,24,25,29,30•], although an increase in CD4⁺ T cells was reported in a Japanese patient [26]. *In vitro* lymphocyte proliferation to mitogens and specific antigens is normal or slightly decreased. Only one patient with increased responses to mitogens and enhanced interleukin-2 production has been reported [31]. In a single family, one affected male had low T-cell responses to mitogens, a second showed hypogammaglobulinemia and poor response to immunization with diphtheria and tetanus toxoid, and a third was found to have normal antibody responses to protein antigens but defective response to pneumococcal polysaccharides [4], demonstrating that there is not a significant commonality in the immune aberration of patients with IPEX. This variability in the immune defect may represent the effect of modifying genes or, alternatively, may be caused by environmental influences, including immunosuppressive therapy.

Treatment of IPEX

Immunosuppressive agents such as cyclosporin A and FK506, either alone or in combination with steroids, constitute the best available choice for treatment of patients with IPEX [4,32,33]. However, because of the toxicity and the fact that none of these drugs seems to maintain long-term remission of symptoms, bone marrow transplantation (BMT), currently the only effective cure for the disease, is indicated.

To date, three patients have received matched BMT; a fourth patient was transplanted with cord blood stem cells but died after 3 months because of transplant-associated complications (Whitesell, personal communication, June 2001). Two of three patients transplanted with marrow from a matched sibling are still living and are asymptomatic except for IDDM (Lederman, personal communication, March 2003). The third patient achieved a complete remission after BMT, including resolution of IDDM. Interestingly, his symptoms resolved during his extensive conditioning regimen consisting of anti-T-lymphocyte globulin, busulfan, and cyclophosphamide, and he remained asymptomatic for 23 months posttransplant, although only 20 to 30% of the T cells were of donor origin. The patient died of hemophagocytic syndrome 29 months after BMT [30•].

Structure and mutations of FOXP3

The 11 exons of *FOXP3* encode a new member of the forkhead family. The protein consists of 431 amino acids in humans and 429 amino acids in mice, with a high level of evolutionary conservation (86% identity). *FOXP3* is expressed at high levels in thymus, spleen, and lymph nodes. Analysis of the subpopulations of purified lymphocytes show that *FOXP3* is predominantly

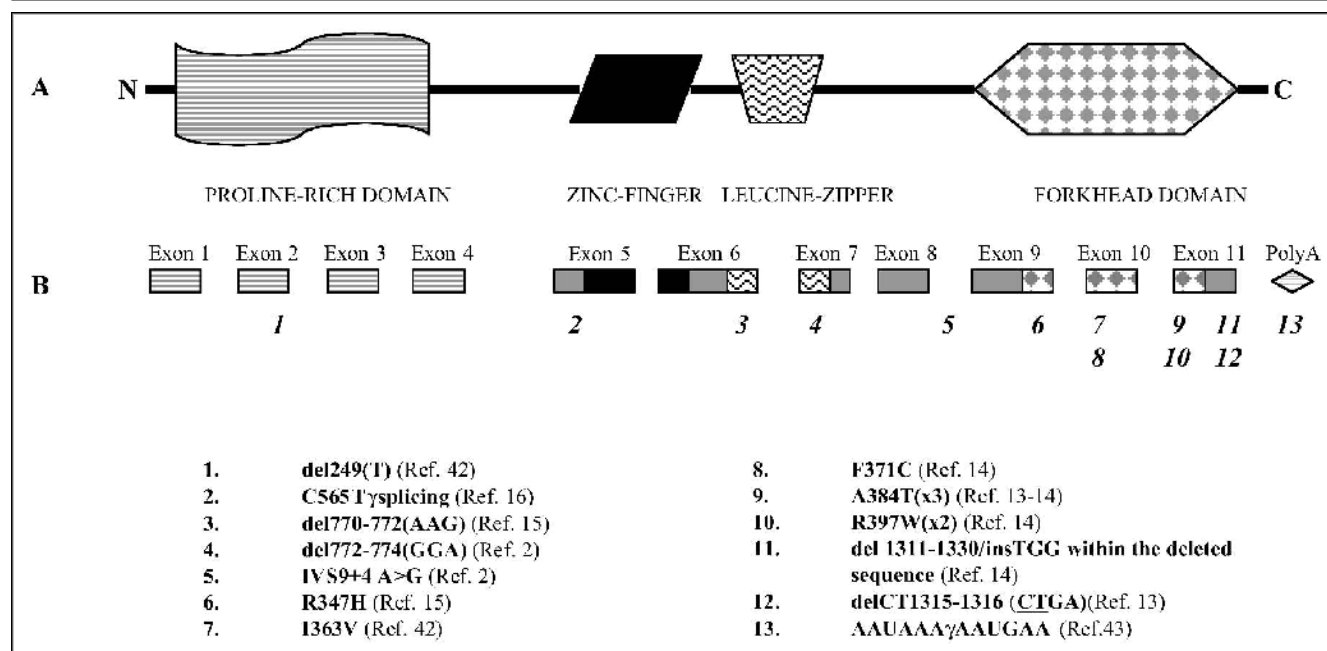
expressed in CD4⁺CD25⁺ regulatory T lymphocytes but also in CD4⁺CD25⁻ T cells at low levels, with little to no expression in CD8⁺ and B220⁺ cells [12••,34••].

All forkhead proteins possess a winged-helix domain of approximately 110 amino acids involved in nuclear localization and DNA binding [35–37]. In most members of this family, the forkhead domain is located near the amino terminus, but in *FOXP3*, the forkhead domain is uniquely positioned at the C terminus (amino acid residues 337–420) [12••]. Other interesting structural domains include a proline-rich region at the amino terminus and a C2H2 zinc finger and leucine zipper domain in the central portion, conserved structural motifs involved in protein–protein interactions, but with unknown significance in *FOXP3*.

Forkhead domain-containing proteins are involved in diverse biologic processes. Several members play key roles in cell lineage commitment and developmental differentiation [38]. The overexpression of scurf (Foxp3 protein) in mice leads to a significant repression of immune function, suggesting a possible role as a repressor of transcription in T cells [39••,40••]. Two other members of the Foxp subfamily, Foxp1 and Foxp2, have been shown to act as transcriptional repressors in the lung [41]. The identification of putative forkhead binding sequences adjacent to critical nuclear factor of activated T-cell sites in many cytokine promoters suggests a possible competition between nuclear factor of activated T cells and Foxp3 for the control of the expression of genes involved in T-cell activation [39••].

The 13 mutations of *FOXP3* reported to date, shown in Figure 2, are distributed throughout the gene. All five missense mutations identified are located within the forkhead domain [13–15,42]. The other mutations reported are outside the forkhead domain but within the coding region, and include one deletion in exon two at nucleotide 249 [42], causing frameshift and early termination; two different deletions of 3 bp each involving nucleotides 770–772 and 772–774, respectively, within exon seven, presumably altering the function of the leucine-zipper domain [2,15]; and two deletions that remove the stop codon, creating products with C-terminal extensions [13,14]. Other mutations include a splice site mutation affecting the 5' end of exon five [16] and an A→G transition at position +4 of intron eight that causes exon nine deletion, resulting in frameshift and early termination [2].

Phenotypically, every patient with one of these *FOXP3* mutations has classic IPEX, and the symptomatic differences are unremarkable. The only mutation that has been associated with an attenuated phenotype affects the first polyadenylation signal downstream of the stop codon [43•]. This mutation, found in the family originally described by Powell *et al.* [3•], presumably desta-

Figure 2. Schematic structure of FOXP3 protein and mutations within FOXP3 gene reported in the literature

(A) Structure of the FOXP3 protein schematically represented with all significant domains. (B) Genomic organization of the FOXP3 gene, including the regions encoding each protein domain. Mutations identified within the FOXP3 gene are indicated below the exons involved. The nucleotide number 1 is the first nucleotide of exon one.

bilizes the mRNA, causing decreased levels of normal FOXP3 protein in T cells, and therefore providing a possible explanation for the variable and sometimes mild phenotype among the affected males of this family. Moreover, several patients from this family have reportedly developed relatively late-onset symptoms and have had episodic rather than persistent diarrhea. Some of them survived past childhood and developed autoimmune diseases (sarcoidosis, arthritis, glomerulonephritis, ulcerative colitis, and neuropathy) that are rarely associated with the classical IPEX syndrome. Many of the deaths within this family occurred after immunizations or viral infections, highlighting the role of specific immune activation in hastening the disease. This phenomenon has been well documented in mice, where development of *scurfy* phenotype in affected males was shown to be dependent on antigen-specific activation through the T-cell receptor in T-cell receptor transgenic animals [44].

FOXP3: a critical determinant of regulatory T-cell function

Data from the *scurfy* mouse model have yielded new insight into the important role played by Foxp3 in maintenance of immune homeostasis. As has been shown recently, Foxp3 is expressed primarily in the CD4⁺CD25⁺ regulatory T-lymphocyte population, suggesting that Foxp3 plays a role in regulatory T-cell development and function [34••]. The importance of CD4⁺CD25⁺ regulatory T cells for the maintenance of immune homeostasis has been demonstrated by depletion of this subpopula-

tion from healthy mice. These animals spontaneously develop various T-cell-mediated autoimmune diseases, such as thyroiditis, gastritis, and IDDM. Reconstitution of the depleted mice with CD4⁺CD25⁺ T cells promptly suppresses the development of autoimmune diseases [45,46•]. Interestingly, experiments in *scurfy* mice using adoptive transfer, bone marrow transplantation, and T-cell subset depletion support the concept that mutation of *Foxp3* causes loss of tolerance as a direct consequence of a regulatory T-cell malfunction [9,11]. It has recently been shown that retroviral gene transfer of *Foxp3* into naïve T cells will confer a regulatory T-cell phenotype on these cells, suggesting that *Foxp3* is a key regulatory gene for the development and maintenance of regulatory T cells [34••].

Conclusions

The clinical syndrome IPEX, characterized by autoimmunity and variable immunodeficiency, is caused by immune dysregulation. Enteropathy, endocrinopathy, hemolytic anemia, thrombocytopenia, eczema, and recurrent infections are characteristic findings of IPEX. The dramatic phenotype and the rapidity of onset of the symptoms make the syndrome unique among the immune dysregulation and lymphoproliferative disorders.

T-cell-immunosuppressive drugs such as cyclosporin A and FK506 are the most effective treatment for patients with IPEX. However, because of their toxicity and their limited efficacy for long-term remission of symptoms, BMT is the treatment of choice.

FOXP3, the gene responsible for IPEX, is a member of the forkhead family of transcription factors. Putative functional domains that may be important for FOXP3 activity include a winged-helix domain (nuclear localization and DNA binding), a proline-rich region, and zinc-finger and leucine-zipper domains. The majority of known IPEX mutations are localized within or near the forkhead domain, suggesting that they may interfere with DNA-binding. Other mutations were found within the leucine-zipper domain in conserved residues previously shown to be important for the function of FOXP3, suggesting an important role for this domain. The remaining mutations are scattered throughout the gene and may affect mRNA splicing and stability. Further structural and functional analysis of these domains will allow a better understanding of the role of FOXP3 in the maintenance of tolerance and the effect of *FOXP3* mutations on its function.

Recent studies demonstrating the important role of FOXP3 in regulatory T-cell development and function have provided new insight into the molecular basis of IPEX.

The recognition that FOXP3 controls regulatory T cells will impact understanding of autoimmune disorders and may lead to novel therapeutic concepts.

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