

A Mystery Diagnosis: Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Recessive

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Abstract

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), a rare disease, has recently been shown to occur

more frequently than previously suspected. The clinical phenotype of IPEX is a direct result of an overactive immune system caused by the proliferation of autoaggressive T cells and

autoantibody-producing B cells. Without prompt diagnosis and treatment, the disease can be fatal within 1 year.

After reading this article, readers should be able to discuss the cause, symptoms, diagnosis, and treatments of IPEX.

Immunology exam 40901 questions and corresponding answer form are located after this CE Update on page **307**.

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is an X-linked recessive disease affecting the regulatory T cells. It is diagnosed in early childhood and can be rapidly fatal. IPEX was previously identified as X-linked syndrome, polyendocrinopathy, immune dysfunction with diarrhea (XPID) or X-linked autoimmunity-allergic dysregulation (XLAAD).¹ The rare X-linked disorder and its murine counterpart scurfy have provided new information about the role of regulatory T cells (Treg) in maintaining tolerance to self-antigens. Through the discovery of IPEX, researchers have found a mutation in the forkhead box protein 3 (FOXP3) gene. Further research of this protein has brought new insight into its importance in the development, function, and competitive fitness of Treg cells. Research is leading to the discovery of other genetic defects in the FOXP3 gene that cause different immune disorders. The research has also lead to improved treatment options for patients with IPEX including immunosuppressive drugs and bone marrow transplants.² Males who present with diarrhea, dermatitis, and endocrinopathy early in infancy require screening for IPEX and treatment with 1 of 3 experimental treatments. Bone marrow transplants, cord blood transfusion, and a combination therapy of low-dose cyclosporine A and steroids are the treatments of choice.

Etiology of IPEX

This disease is uncommon but retrospective research has provided data on early cases of autoimmune enteritis suggesting that the disease occurs more frequently than previously suspected.¹ There have been cases from as early as 1960 that were of unknown etiology. Retrospective research shows that the correct diagnoses for these cases are IPEX. Recent

research suggests that some of these cases may even be an IPEX-like syndrome caused by a deficiency in CD25+ cells.³

In 1982, Powell and colleagues described an X-linked syndrome in a family in which 8 males within 3 generations had several different combinations of diabetes mellitus, intractable diarrhea, hemolytic anemia, eczema, and/or thyroid immunity. These 8 males were all connected through the females of the family. They all had exaggerated responses to viral infections. Only 2 of the 8 males survived the first 10 years of life. When Powell looked back through the family history, 11 other males had died in infancy.⁵ The presence of this phenotype in multiple generations of the same family indicated that the disorder was genetic and X-linked. The specific or adaptive immune response is designed to protect the host from overwhelming bacterial, fungal, parasitic, and viral infections without accidentally attacking the host and creating an autoimmune disorder. A breakdown of self-tolerance occurs when the adaptive immune response is defective in some way. Primary immune deficiency disorders, such as autoimmune lymphoproliferative syndrome, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, and IPEX, are all examples of disorders occurring due to the breakdown of self-tolerance. The mechanisms of these diseases are currently being studied; however, IPEX and its murine homolog scurfy have had the most successful research findings in the past 6 years.² Understanding the symptoms and clinical findings of IPEX and the role of FOXP3 in the generation of Tregs will provide answers to cases of infant death that were once unexplained.

After the discovery that scurfy mouse is caused by a mutation in the FOXP3 gene, several IPEX families were tested for a similar mutation. The results showed mutations in the human ortholog, FOXP3,² meaning that IPEX was possibly caused by a similar, if not the same, mutation as the scurfy mouse. The FOXP3 gene codes for a protein that is 431

amino acids in length and contains a leucine zipper and zinc binding domain. The FOXP3 gene is located in the nucleus of Tregs. Treg cells express CD4, FOXP3, and CD25. All 3 of these are essential to the function of the Treg cells. If a mutation occurs in any of these 3 components, the Treg cell function will fail. Currently, there have been 20 different mutations of this gene associated with IPEX.¹ The gene is expressed primarily in the thymus, spleen, lymph nodes, and mainly in the CD4+CD25+ Treg cells.² FOXP3 belongs to the P subfamily of FOX transcription factors, which as a family are characterized by the presence of a winged-helix/FKH DNA binding domain. Roles for this family include transcriptional regulators in thymic development, lineage commitment, and function of lymphocytes, making this family of proteins essential in the regulation of the natural immune response.⁶

FOXP3 is necessary for the development and function of naturally occurring CD4+CD25+ regulatory T cells in mice.⁶ The function of the FOXP3 gene is not entirely known; however, researchers think that it is responsible for production of a protein which regulates T cell activation.⁷ Scurfy mice have a complete absence of Treg cells, whereas in humans, Treg cells are present but in decreased amount and functionality. The protein produced in the presence of nonmutated FOXP3 is referred to as scurf. In the absence of scurf or when DNA binding sites are modified, the T cells proliferate and become activated in an uncontrolled fashion. The T cells fail to undergo apoptosis, resulting in early-onset autoimmune disease.⁸ These patients have an extremely low count of T regulatory cells and an extreme overproliferation of effector T cells. When considering a proper therapy for patients with IPEX, it is easy to see that immunosuppressive therapy, such as cyclosporine A combined with steroids, would be an effective treatment.

Symptoms

Symptoms of this disorder appear in early infancy between birth and approximately 7 months of age. The symptoms include ichthyosiform dermatitis, protracted diarrhea, hemolytic anemia, insulin-dependant diabetes mellitus (IDDM), and thyroiditis.⁹ IPEX also manifests with thrombocytopenia, eczema, and hypothyroidism.¹ IPEX has 3 main symptoms which are present in almost every case: when IPEX patients are diagnosed they will have some form of gastrointestinal disease, thyroid or pancreatic disease, and some type of dermatitis. These symptoms gave rise to the 3 components of the syndrome's name: immune dysregulation, polyendocrinopathy, and enteropathy.

The gastrointestinal disease symptom of IPEX presents as intractable or protracted diarrhea and is often hallmarked by villous atrophy and lymphocytic infiltrates of the intestinal mucosa. When an infant switches from breast feeding to regular formula, the diarrhea can worsen. The change to regular formula is accompanied by food allergies in most patients. Patients with severe diarrhea often do not respond well to nutritional manipulation, so they require total parenteral nutrition.⁸

Autoimmune endocrinopathies involving the thyroid and pancreas are characteristic of IPEX and occur early in life. Type 1 diabetes mellitus is the most common endocrine disorder. IPEX patients show early signs of IDDM during the first year of life.⁹ In some cases, glucose intolerance is present at birth. IPEX is so severe that more than 80% of the patients, including those with a diabetes protective HLA haplotype, develop IDDM.¹¹ Patients who have IDDM often have anti-

islet cell antibodies and chronic interstitial inflammation with lymphocytic infiltrates in the pancreas. In the general population, males are twice as likely as females to develop IDDM and research is suggesting that the reason for this may be that the IDDM gene is also X-linked and possibly in the FOXP3 gene.¹² Thyroiditis is common and can lead to hypothyroid or hyperthyroid states. Hypothyroidism is the most common presentation with decreased thyroid hormones and antithyroid antibodies. The pancreas and thyroid are the only endocrine organs that are known to be affected by the IPEX syndrome.²

The final component of the 3 main symptoms of IPEX is dermatitis. Dermatitis can present in the form of eczema, ichthyosiform dermatitis, or an erythematosus rash. The dermatitis is most commonly reported as a mild or moderate eczematous rash. In recent research of older patients with IPEX, psoriasiform and pemphigoid nodularis are appearing more frequently.

In addition to the 3 basic symptoms of IPEX, patients with the FOXP3 mutations typically have other autoimmune disorders. The most common are hemolytic anemias, autoimmune thrombocytopenia, and autoimmune neutropenia. These 3 combined affect nearly 50% of IPEX patients. Renal disease is another common symptom, ranging in severity from mild hematuria and proteinuria to rapidly progressive glomerulonephritis.² IPEX patients also face increased susceptibility to infections. In 50 patients studied by Powell and colleagues, infections including sepsis, meningitis, pneumonia, and osteomyelitis were observed. The common pathogens observed in these patients were *Enterococcus*, *Staphylococcus*, cytomegalovirus, and *Candida*.⁴ Autoimmune hepatitis is a problem for 20% of the patients as well as neurologic and developmental delay.²

Diagnosis

The diagnosis of IPEX can take several months and delays crucial immunosuppressive treatment. The diagnosis of IPEX depends heavily on the family history, the elimination of other diagnoses of similar presentation, and the clinical presentation. Physicians are responsible for the elimination of other possible diseases prior to an official diagnosis of IPEX. Transient neonatal diabetes, pancreatic hypoplasia, autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy are examples of disorders that must be ruled out by the physician.¹⁴ Laboratory findings in IPEX show elevated IgE levels in the patients' serum and 60% have shown elevated IgA levels in the serum.¹ It is also necessary to detect anti-enterocyte antibodies in the patients' serum, which is a common finding in IPEX syndrome.¹⁵ In patients with IPEX CD4+CD25+, T cells are present, but most patients with FOXP3 mutations have decreased or absent Treg cells.¹ Other than the Treg cells, the other T and B cells appear normal. Patients that present with intractable diarrhea should have a biopsy of the intestinal mucosa performed to determine if villous atrophy is a symptom. The laboratory is developing new ways to identify IPEX through the use of endoscopic biopsy. The endoscopic biopsies are taken of the duodenal, colonic mucosa, and gastric mucosa. These biopsies are used to determine the degree of villous atrophy.¹⁵

Age is not a reason to rule out IPEX as a possible diagnosis. Normally, patients present with intractable diarrhea at approximately 4 months of age. Research has shown that the

location of the mutation on the FOXP3 gene determines the severity of the syndrome. Most of the symptoms can be treated individually, allowing patients to live longer and remain undiagnosed. Retrospective research is uncovering new cases with a wide range of ages. The presence of a rash or psoriasiform dermatitis, IDDM, and/or hypothyroidism supports a diagnosis of IPEX. When these symptoms are present, hemolytic anemia, thrombocytopenia, or neutropenia may also be present. If IPEX is suspected based on clinical presentation and family history, the diagnosis will be confirmed by mutation analysis of the FOXP3 gene.²

Experimental Treatments

Therapeutic options to control the life-threatening symptoms caused by IPEX are limited. Most treatments are ineffective, but 2 experimental approaches show the most promise. The most successful treatments are immunosuppression combined with steroids and bone marrow transplant. In addition to these treatments, supportive measures are sometimes necessary. Parenteral nutrition, insulin injections, red blood cell and platelet transfusion, and prophylactic intravenous gamma globulin are needed to aid the treatments. Most treatments are successful in the short term; however, more long-term success is needed.¹⁶

Chronic immunosuppression has had positive results in some patients with IPEX, but has been ineffective in others. Patients administered regimens that include a combination of steroids and the calcineurin suppressive agents, cyclosporine A (CsA) or tacrolimus, have shown high levels of improvement; however, long-term use is limited due to its direct renal toxicity.¹⁶ Cyclosporine A given alone leads to immediate results but will not prevent a fatal outcome. When given CsA combined with prednisolone, patients show long-term results with no toxicity present. The treatment is given in the following doses: 2 mg/kg of CsA and 0.5 mg/kg of prednisolone. Complete remission was possible and maintained through the daily low dose of CsA and prophylactic intravenous gamma globulin.¹⁷ Cyclosporine A treatment produced similar results in the study conducted by Powel and colleagues, where 1 patient survived long-term.

Success has been seen with tacrolimus and dexamethasone; however, this combination leads to several side effects. One patient has survived long-term but he suffers from osteoporosis, renal tubular disease, growth failure, and a steroid induced cataract.¹⁴ To overcome the renal toxicity of these treatments, research has been conducted with sirolimus as an effective treatment. Sirolimus, unlike tacrolimus and CsA, does not block calcium fluctuation. Sirolimus is a less-toxic T-cell immunosuppressor that targets and inhibits a serine-threonine kinase, blocking lymphocyte maturation in the late G1 phase. It is believed that it acts on the effector T cells, leaving the regulatory T cells relatively unaffected and fully functional, creating a balance in Treg cells versus effector T cells. Sirolimus was shown to successfully control the gastrointestinal and dermatologic symptoms of IPEX while reducing the inflammatory reactions in all 3 patients in the study.¹⁶ Many of the skin lesions of IPEX patients can be treated with topical steroids and immunomodulatory agents. The lesions have improved with these treatments.¹³

Allogenic bone marrow transplants, hematopoietic stem cell transplants, and submyeloblastic cord blood transplants

have all been used in the treatment of IPEX. Bone marrow transplants were originally thought of as the essential treatment, but the use of these treatments has shown mixed results. In most cases, the conditioning regimen itself controlled most of the clinical and biologic features of the disease. The engrafted bone marrow is believed to be responsible for the complete remission that is seen in some patients. Since IPEX is caused by a germ-line mutation, autologous bone marrow transplants would not lead to a full remission.⁹ The mixed results of bone marrow transplants suggest that further experience is needed to make this an effective treatment. Bone marrow transplants should be performed early in the course of the disease. Treatments should start at the initial onset of symptoms. The discovery of the genetic basis of IPEX allows early infant screening of males at risk. If the infants have the mutation in the FOXP3 gene, then presymptomatic bone marrow transplants can be performed to prevent the clinical manifestations.¹⁴

Conclusion

IPEX is a rare disease that has been proven to occur more frequently than previously suspected. The clinical phenotype of IPEX is a direct result of an overactive immune system caused by the proliferation of autoaggressive T and autoantibody-producing B cells.¹⁶ Without prompt diagnosis and treatment, the disease can be fatal within 1 year. Mutation of the transcription factor FOXP3 results in the absence or dysfunction of Tregs and leads to the IPEX phenotype.² Patients should be screened for IPEX using immunohistochemical staining of the gastrointestinal mucosa for the FOXP3 protein and villous atrophy.¹⁸ Several experimental treatments are still undergoing research to determine which is the most effective. Patients should be treated by bone marrow transplant, cord blood transfusion, and/or immunosuppressive drugs like cyclosporine A, tacrolimus, or sirolimus; however, all have mixed results. New research is underway to determine a more effective treatment for IPEX patients. LM

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