REVIEW ARTICLE

Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome

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Immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX, OMIM 304790) is a rare, recessive disorder resulting in aggressive autoimmunity and early death. Mutations in *FOXP3* have been identified in 13 of 14 patients tested. Research in the mouse model, scurfy, suggests that autoimmunity may stem from a lack of working regulatory T cells. We review published reports regarding the genetics, clinical features, immunology, pathology, and treatment of IPEX. We also report three new patients who were treated with long term immunosuppression, followed by bone marrow transplantation in two. IPEX can be differentiated from other genetic immune disorders by its genetics, clinical presentation, characteristic pattern of pathology, and, except for high IgE, absence of substantial laboratory evidence of immunodeficiency. While chronic treatment with immunosuppressive drugs may provide temporary benefit for some patients, it does not cause complete remission. Remission has been observed with bone marrow transplantation despite incomplete engraftment, but the long term outcome is uncertain.

The syndrome of immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) is a rare X linked recessive disorder of immune regulation resulting in the expression of multiple autoimmune disorders. Protean features include early onset type 1 diabetes mellitus (T1DM), severe enteropathy, eczema, anaemia, thrombocytopenia, and hypothyroidism. IPEX is usually lethal in infancy or childhood. Since there are reports of similar patients under many different names, the Human Genome Organization (HUGO) recently established "IPEX" as the official designation for this condition. Mutations in the *FOXP3* gene in Xp11.23, encoding a protein called scurfin, were recently identified in several patients, thus establishing a genetic aetiology.

The clinical and genetic features of the syndrome and the options for management have not been extensively reviewed. The purpose of this paper is to describe three additional patients with IPEX and their associated mutations, and to review published reports regarding the clinical and molecular features of the disease.

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NEW IPEX CASES

Three cases of IPEX were diagnosed and managed at the Children's Hospital Medical Center in Cincinnati, Ohio between 1986 and 2001.

Case 1

The clinical diagnosis of IPEX was made at 13 years of age after a review of the past medical history and an immunological evaluation. This patient developed T1DM at approximately 3 months of age and was insulin dependent for his entire life. Symptoms of diarrhoea and failure to thrive began during the first year of life and led to a biopsy confirmed diagnosis of coeliac disease at 18 months of age. The patient initially showed improvement with a gluten free diet, but subsequently relapsed and his enteropathy was reclassified as ulcerative colitis, and later as autoimmune when anti-enterocyte antibodies were detected. During the last five years of his life the patient showed no increase in stature or substantial weight gain. He was maintained on intravenous nutrition and partial elemental enteral feeds, prednisone, CSA, and later tacrolimus, the latter treatment showing more substantial benefit. During the year before his bone marrow transplant (BMT), he had experienced intractable hypertension with encephalopathy, probably resulting in a residual left posterior temporal lesion identified by MRI scan of the brain, and life threatening central line sepsis.

The patient's maternal grandfather had a life long history of ulcerative colitis and severe autoimmune haemolytic anaemia, both of which contributed to his death at the age of 36 having fathered a healthy daughter and son. The patient was the oldest of six living children. His parents, two sisters, and three brothers are in good health; his mother experienced one miscarriage, gender unknown.

Believing that the patient suffered from a genetically determined X linked immunoregulatory disorder, allogeneic BMT from his 11 year old HLA matched sister was proposed. Pre-transplant evaluation showed only mild atrophy of the pancreas on abdominal CT scan. The patient underwent pre-transplant conditioning with IV cyclophosphamide, 1200 cGY total body irradiation, and rabbit antithymocyte globulin. The allogeneic marrow was partially depleted of T cells. Post-transplant prophylaxis for graft *v* host disease (G*v*HD) consisted of low dose steroids and CSA.

Abbreviations: IPEX syndrome, immunodysregulation, polyendocrinopathy, enteropathy, X linked syndrome; T1DM, type 1 diabetes mellitus; CSA, cyclosporin A; BMT, bone marrow transplant; G*v*HD, graft *v* host disease; EBV, Epstein-Barr virus; CMV, cytomegalovirus; TCR, T cell receptor; NFAT, nuclear factor of activated T cells; AIE, X linked autoimmune enteropathy

Table 1 FOXP3 sequence variations found in genomic DNA from IPEX patients

References	Family	Mutation (cDNA)	Predicted consequence	Comment
This report	1	1040G>A	R347H	Winged helix I
	2	IVS9 + 459 A>G	None	Known variant (GenBank AF235097)
	3	748delAAG	ΔK250	Predicted coiled coil domain
		543C>T	S181S	Known variant (GenBank AF235097)
16	1	1189C>T	R397W	
	2	del1290-1309/insTGG	GPter> VGKGGWTNRGQTGGRQRWWGQG	Adds tail to Wing-2 of winged helix domain
	3	1113T>G	F371C	Exon 10
	4	1150G>A	A384T	Winged helix
15, 16	1	No coding region mutation, but A>G in first polyadenylation signal	Reduced levels of normally coded mRNA	Attenuated phenotype
15	1	1150G>A	A384T	Winged helix
15	2	1293delCT	ter432T (+ 25 residues)	Adds tail to Wing-2 of winged helix domain
12	100	IVS9 + 4 A>G	Exon 9 skipped; frameshift at codon 273, stop at 286.	Eliminates winged helix domain
	200	600delGGA	ΔE201	3-heptad, myc-like zip motif
17	1	227delT	Frameshift, stop at 128	Eliminates all structural domains
	2	A1087G	I363V	Winged helix II

Institutional human subjects approval was obtained. See reference 16 for methods.

Engraftment occurred on day 10 post-BMT and 100% donor chimerism was documented. Initial post-transplant complications were minor, consisting of neutropenic fever and mild haemorrhagic cystitis. The patient experienced significant decrease in stool frequency and volume beginning approximately three weeks after grafting and showed initial weight gain. Hyperalimentation was discontinued before discharge to his home state. Follow up endoscopy showed no significant abnormalities. Three months after transplant, donor engraftment had declined to approximately 50%, where it remained until the time of death. The patient was readmitted for the last time five months post-transplant with recurrent diarrhoea, fevers, and progressive marrow failure eventually leading to a diagnosis of adenovirus infection. He developed Gram negative pneumonia and died quietly on day +194 post-BMT of respiratory insufficiency.

Sequencing of genomic DNA showed a missense mutation in the winged helix domain of the predicted scurfin protein (table 1).

Case 2

This patient, currently 5 years old, is the only child born after an uncomplicated pregnancy. He weighed 4252 g at birth. He developed otitis media during the first month of life, followed by diarrhoea, and presented in diabetic ketoacidosis at 6 weeks of age. Anti-islet cell antibodies were documented. While in hospital, the patient developed interstitial pneumonitis. Lung biopsy and endoscopic GI biopsies showed CMV. In infancy, the CT scan of the pancreas was within normal limits. After resolution of the CMV infection with antiviral therapy, diarrhoea persisted. GI biopsies showed villous atrophy and chronic inflammation. Steroid therapy was initiated for a presumptive diagnosis of autoimmune enteritis. Episodic diarrhoea has continued throughout the patient's life, but he has been growing on oral intake alone. The family reported no similarly affected relatives.

Around 1 year of age lymphadenopathy, hepatosplenomagaly, and eczema appeared. Owing to massive lymphadenopathy and symptoms of obstructive apnoea the patient underwent tonsillectomy, adenoidectomy, lymph node biopsy, and bone marrow biopsy at 20 months of age. These showed Epstein-Barr virus (EBV) infection. The patient subsequently recovered spontaneously and currently maintains protective convalescent anti-EBV viral capsid antigen and anti-Epstein-Barr nuclear antigen titres. Hypothyroidism was diagnosed and treated at 2 years of age. At 2½ years the patient developed severe, relapsing, autoimmune haemolytic anaemia, which was initially managed with corticosteroids. Owing to complications of severe hypertension and cardiomegaly, monthly rituximab (anti-CD20 antibody) therapy was initiated 15 months ago. This has permitted substantial weaning of the steroids without recrudescence of haemolysis. With reduction in steroid therapy, systemic eczema and massive lymphadenopathy have returned. The patient has experienced one brief episode of immune neutropenia, and has suffered with chronic sinus and ear infections requiring frequent antibiotics, surgery, and, more recently, empirical IV immunoglobulin G (IgG). In the past, he has shown adequate responses to all immunisations.

DNA sequencing showed no *FOXP3* coding region mutations, but a previously reported population variant in intron 9[1] was observed.

Case 3

The clinical diagnosis of IPEX was made at 9 years of age after a review of the clinical history and an immunological evaluation. The patient presented with enteritis and T1DM at 2 months of age. His older brother had died at 15 months of age of infectious complications secondary to immunosuppression following a similar clinical presentation. The patient had life long problems with diabetes and enteropathy requiring chronic immunosuppression with steroids, CSA, and tacrolimus. In addition, he developed debilitating polyarticular arthritis that was treated with rofecoxib, methotrexate, and infliximab, and suffered chronic idiopathic thrombocytopenic purpura during the last two and a half years of his life, treated with steroids, high dose IV IgG, and rituximab. Hepatomegaly and mild hepatitis with focal hepatosteatosis were present on liver biopsy. In addition to chronic failure to thrive, the patient showed evidence of progressive renal insufficiency and hypertension, believed to be secondary to the chronic dependence on CSA and tacrolimus. At 9 years, height and weight were further than 3 standard deviations below the mean for age.

Because of his deteriorating course, the patient's parents consented to treatment with a matched unrelated donor transplant. As in case 1, the pre-transplant conditioning consisted of IV cyclophosphamide, total body irradiation, and rabbit antithymocyte globulin. The marrow was partially T cell depleted. The patient showed prompt engraftment and 100%

donor peripheral cells initially. The transplant was complicated by haemorrhagic cystitis and a number of infections, including reactivation of cytomegalovirus (CMV), first noted on the day of transplant. Despite these problems, the patient's stool output normalised for a while and he tolerated full enteral feedings. One month post-transplant, colon biopsies showed only "slight chronic architectural distortion, most likely a residue of the chronic inflammatory bowel disease antecedent to the BMT". Arthritis resolved despite a decline in peripheral donor chimerism to 70%. Approximately three months post-grafting the patient was readmitted to the intensive care unit with respiratory distress thought to result from infection and fluid overload, and he died on post-BMT day 94. At necropsy, an EBV related lymphoproliferative disorder involving the lung and all other major organs was observed. Chronic sclerosing pancreatitis with absent pancreatic islets, CSA glomerulopathy, and testicular atrophy were also noted.

His *FOXP3* DNA sequence incorporated an in frame, three nucleotide deletion expected to cause the deletion of a single scurfin amino acid residue in a predicted coiled coil domain upstream of the winged helix domain. A previously described silent variation at serine 181 was also detected.

BACKGROUND

Incidence of IPEX

Based on clinical experience and the limited number of published reports, IPEX is probably extremely rare. No estimates of incidence have been proposed. However, new mutations or cases without a family history or with variant presentation are likely to have been under-reported, so the true incidence may be higher than is currently perceived. Males appear to be affected in the expected Mendelian proportions in family reports, but no affected females have been reported. Obligate carrier females are healthy.

Animal model

The scurfy mouse (*sf*) is a natural mutant resembling IPEX. Scurfy mice show X linked recessive inheritance of scaly skin, runting, progressive anaemia, thrombocytopenia, leucocytosis and lymphadenopathy, hypogonadism in males, apparent infection, diarrhoea, gastrointestinal bleeding, cachexia, and death by 3-4 weeks of age.[2 3] Several elegant immunology studies have shown that CD4+ T cells mediate scurfy disease.[4 5] Scurfy T cells are hyper-responsive to activation via the T cell receptor (TCR), have a reduced requirement for co-stimulation via CD-28, and are resistant to CSA.[6] Initiation of illness appears to require antigen stimulation via the TCR.[7] High levels of circulating cytokines are present in scurfy mice, especially in the skin.[8] In particular, raised interleukin (IL)-2, IL-4, IL-5, IL-6, IL-10, interferon-γ, and tumour necrosis factor-α have been documented.[5 8]

The gene mutated in scurfy was recently obtained by positional cloning and named *Foxp3*. Located near the centromere of the mouse X chromosome,[3 9] *Foxp3* encodes a 429 residue protein, scurfin.[10] This novel protein has at the carboxy-terminus a conserved domain belonging to the forkhead class of winged helix transcription factors and a zinc finger motif in the middle third of the sequence. These features suggest it has DNA binding properties and may function in the regulation of gene transcription. The scurfy mutation is a 2 bp insertion that results in the loss of the carboxy-terminal half of the protein including the winged helix domain.

The functions of normal scurfin and the molecular mechanisms by which mutated versions cause disease are not wholly known. Recent molecular analysis of human scurfin function indicates that it binds DNA elements in the IL-2 promoter and granulocyte macrophage colony stimulating factor enhancer near nuclear factor of activated T cells (NFAT) sites and is capable of repressing transcription of these genes and reducing IL-2 expression in an activated CD4+ T cell line. These functions, as well as nuclear localisation, do not occur in the absence of the forkhead domain.[11] However, scurfy mice can be rescued with a single injection of normal T cells (Wildin *et al*, submitted), suggesting that *Foxp3* deficiency results primarily in the lack of functional regulatory T cells, rather than in the failure to repress inflammatory cytokine expression.

GENETICS OF IPEX

Genetic mapping in a few large families isolated the human IPEX locus to Xp11.3-q13.3.[12–14] The clinical similarities between IPEX and the scurfy mouse and the synteny of their respective map locations recently led us and others to seek mutations in the human orthologue (*JM2, FOXP3*) of the mouse gene in several unrelated IPEX patients.[12 15–17] Coding region mutations consistent with a disruption of function were found in 10 of 11 families (table 1). The eleventh family has recently been shown to have a mutation in the 3′ untranslated region of *FOXP3* (see below). In addition, two of the patients reported here have mutations predicted to alter the amino acid sequence of the scurfin protein. Thus, more than 90% of IPEX families tested have mutations in *FOXP3*.

Table 1 lists the known variations in IPEX probands. None of the disease associated mutations has been seen in a screen of >200 ethnically diverse people.[16]

Several of the IPEX mutations occur in the winged helix domain of scurfin in ways that have been predicted to alter a putative DNA binding activity of this motif.[16] Others truncate the protein leaving it without a winged helix domain or extend the carboxy-terminus.[15] The former may prevent nuclear transport and/or eliminate putative transcriptional repression,[11] while the latter probably interferes with folding or positioning of the winged helix. Thus, loss or functional alteration of the winged helix domain can cause IPEX in the recessive, hemizygous state. Other mutations, including those in cases 1 and 3, suggest that other scurfin domains are also essential for preventing autoimmunity.

Two patients with clinical disease consistent with IPEX have not yielded mutations in the coding region of *FOXP3*[15 16] (case 2). The absence of a *FOXP3* coding mutation despite good mapping data to this region in the large family reported by Powell *et al*[18] suggests that regulatory or conditional mutations may occur outside the *FOXP3* coding regions.[16] Indeed, Bennett *et al*[19] recently identified a mutation in the first canonical polyadenylation signal following the final coding exon of *FOXP3* in this family. This change is associated with a reduction in *FOXP3* mRNA expression, presumably because of non-specific degradation of the resulting aberrant RNA species. A non-coding mutation in our case 2 has not been excluded.

Females who are obligate carriers for IPEX associated mutations lack the skewed pattern of X inactivation that is seen in the peripheral lymphocytes of some X linked immunodeficiency syndromes (R S Wildin, unpublished data).

CLINICAL REVIEW

Clinical features

Table 2 summarises reported cases with clinical and/or molecular diagnosis of IPEX and patients the authors believe resemble IPEX. In general, cases were selected when the combination of both early T1DM and severe enteropathy existed in a male or among males in a family where X linked recessive inheritance was possible. Many of these cases have not previously been linked or associated as a single disorder. At least seven additional families meeting these criteria have been summarised in two series of protracted diarrhoea of infancy and are not reproduced here.[20 21]

Table 2 Summary of IPEX or IPEX-like cases

Reported families*	Case No	Gestation (wk)	Birth weight (g)	Age at presentation	Age at death	Diabetes mellitus**	Failure to thrive	Diarrhoea or ileus	Eczema or atopy	Thrombocytopenia	Haemolytic anaemia***	Hypothyroidism	Lymphadenopathy
1970 Meyer[31]†	1	Term	2860	36 d	4.5 mth	+	+	+					
	2	Term	2550	3 d	32 d	+	+						
1977 Dodge[23]†	1	39	2130	Birth	40 h	+							
1982 Powell[18]†	1	Term	3300	7 mth	10 mth	+	+	+	+	–	+		
	2			9 mth	30 y	+	+	+	+		(+)		
	3			Early	Alive @ 10 y			+	+		(+)		
	4			Birth	4 y	+		+					
	5			Birth	3 mth	+		+					
	6			3 mth	25 mth		+	+					
	7				2 y				+				
	8			4 mth	6 mth			+					
1982 Ellis[27]	1		3100	6 wk	21.5 mth	–	+	+	+[f]	+	+	–	
	2			2 wk	11 wk	+	+	+	+[f]	–	+	+	
1982 Walker-Smith[35]	1			1.5 y	Alive	–	+	+					
	2			3 wk	1 y	+		+					
1982 Savage[41]	1			4 mth	16 mth	+	+	+				+	
1982 Hattevig[25]	1	Term	3080	8 d		+	+	+[g]					
1990 Seidman[40]	1			4 mth		+	+	+			+		
1991 Jonas[32]	1	37	1820	<3 d	Infancy	+	+	+					
1991 Jonas[32]	2	36	2120	1 d	6 mth	+	+	+					
1993 Satake[33]	1	Term	2980	Birth	Alive @ 18 mth	(+)	+	+			+	+	
	2		3000	Birth	10 mth	(+)	+	+				+	
	3		3750	Birth	6 mth	–	+	+			+		
1994 Zeller[26]	1		SGA	1 d	Mths	+		+	+				
1995 Roberts[30]†	1	42	3240	2 wk	10 mth	+	+	+	+				+
1996 Finel[37]	1	38	2420	1 d	2 y	+	+	+		+	+		
1996 DiRocco[24]	III.4			Birth	3 mth	–		+	+	+			
	III.1				6 mth	+		+					
	III.3			1 mth	7 mth	+		+					
	III.7			45 d	12 mth	–		+					
	III.8				2 mth			+					
	III.9				4 mth	+							
	III.11				Alive @ 7 y			+[g]					
1996 Peake[29]	1	42	3240	6 wk	10 mth	+	+	+	+		(+)		+
	2	41	3750	4 wk	19 mth	+		+	+	–			+
	3			17 d	10 wk	+	+	+					
	4				Early		+	+					
1998 Kobayashi[28]†	2			6 mth	3 y	+		+				+	
2000 Ferguson[14]†	1	Term	3629	1 mth	Alive @ 8 y		+	+	+				
	2	36–38	2438	2 mth	10 mth		+	+[i]	+			+	
	3	Term	3657	2 wk	2 y	+	+	+	+				
	4	Term	3430	3 wk	12 wk	+						+	
2000 Cilio[36]	1	34	1600	Birth	26 d	+		+	+				
2001 Levy-Lahad[22]†	1	32	2000	Birth	19 d	–	+	+[i]	+	+	+	+	
	2	37	1350	Birth	5 wk	+	+	+[i]		+		–	
	3	36	2090	Birth	5 mth	+	+	+			(+)	–	
2001 Baud[38]	1				4.5 mth	+		+	+	+			
	2	Term	Normal	2 wk	34 mth	+	+	+	+	+	+	–	
This report	1†			3 mth	14 y	+	+	+					
This report	2			<1 mth	Alive	+		+	+		+	+	+
This report	3†			2 mth	10 y	+	+	+		+			
2001Chatila[12]	1–5			3 wk–3 mth		5/5	5/5	5/5	4/5		(3/5)		

*Cases from same family if reference is not repeated. †Also noted other maternally related affected males in family history. **(+) glucose intolerance. [f]Follicular dermatitis. [g]Gluten responsive. [i]Ileus or recurrent vomiting rather than diarrhoea. ***(+) anaemia, aetiology unspecified. Note: Case 1 of Peake *et al*,[29] is the same patient as reported by Roberts and Searle[30] (J Searle, personal communication), and further reports on case 1 of Satake *et al*[33] are found in references 17, 28, 34, 42, and 43.

Table 3 Initial presenting features

Feature	No of patients	References
Diarrhoea	19	14, 18, 25, 27–29, 31, 32, 35, 40, 41, this report
T1DM	14	14, 22, 23, 25, 26, 29, 31, 32, 35, 37, this report
Eczema or atopic dermatitis	9	14, 18, 22, 24, 29–31, 38
Poor feeding, ileus	3	22, 29
Anaemia or thrombocytopenia	3	22, 24, 33
Hypothyroidism	2	22, 33
Lymphadenopathy	2	29, 30
Respiratory distress	1	23
Bruising	1	22

Table 4 Causes of death (where stated)

Cause	No of patients	References
Diarrhoea or malnutrition	8	18, 24, 26, 29, 32, 33, 38
Sepsis	6	14, 18, 27–29, 32
T1DM or complication	4	18, 23, 24, 31
Haemorrhage	4	18, 22, 33
Pneumonitis, respiratory failure	4	14, 22, 31, 37
Acute reaction to immunisation, spleen extract, or special formula	3	18, 29
Renal disease	3	24, 27
Peritonitis	2	22
Infectious complications of BMT	2	This report
Lymphoproliferative, haemophagocytic syndrome following BMT	1	38
Congestive heart failure	1	14
Jaundice	1	35

The initial presenting signs, where stated, are listed in table 3. Onset of signs and symptoms usually occurred in the perinatal or infancy period. Later onset was occasionally described, particularly in the family reported by Powell *et al*,[18] where one member first presented in adulthood. The family history of our case 1 suggests that the maternal grandfather may also have been affected. Thus, a diagnosis of IPEX should not be ruled out on the basis of age.

The most common clinical features were T1DM and enteropathy manifesting as secretory diarrhoea or ileus. In diabetic patients, euglycaemia was often elusive despite careful insulin therapy. T1DM is probably the result of inflammatory destruction of the islet cells rather than islet cell agenesis, as previously suggested.[22 23] Diarrhoea was sometimes present before feeding, but generally appeared or worsened after the initial feeding and often failed to abate with enteric rest. Institution of a gluten free diet rarely altered the course of enteropathy, although two patients responded well,[24 25] and two gained mild or transient benefit[18] (case 1).

Other clinical features appeared less frequently or were omitted from clinical descriptions. These included eczema (or exfoliative or atopic dermatitis), thrombocytopenia, Coombs positive anaemia, and lymphadenopathy. Several patients were hypothyroid. The initial presentation sometimes included metabolic acidosis, leading to evaluation for inherited metabolic disorders. Polyarticular arthritis, asthma, and ulcerative colitis occurred in a non-diabetic survivor in the family of Powell *et al*,[18] glomerulonephropathy was diagnosed in three cases,[18 26 27] and interstitial nephritis independent of medication in three.[27 28] Arthritis also occurred in our case 3 and ulcerative colitis in case 1. Hypotonia and muscle atrophy was only noted in a single case.[22]

Sepsis, especially catheter related sepsis, and other serious infections including peritonitis, pneumonitis, and septic arthritis, complicated treatment in several cases[14 18 22 27–32] (case 1). The most common organisms were *Enterococcus* and *Staphylococcus* species. Sepsis occasionally occurs in the absence of immunosuppressive drugs.[22 28] These increased infections may relate to a primary defect in immune regulation, as postulated by Powell *et al*,[18] or arise from autoimmune neutropenia or the use of immunosuppressive drugs. In addition, severe enteropathy and eczema could facilitate bacterial entry via the gut and skin, and malnutrition may decrease the effectiveness of the immune system and contribute to severe infection.

Growth retardation, which may begin prenatally, and cachexia are striking features of the syndrome. This may be the result of T1DM and enteropathy, but given its persistence despite adequate parenteral nutrition and exogenous insulin, it more likely represents inanition related to chronic illness and cytokine excess.

The causes of death for reported cases are listed in table 4. Haemorrhage, sepsis, intractable diarrhoea, and diabetic complications are the most frequent. Death from reactions related to acute stimulation of the immune system were present in the family reported by Powell *et al*,[18] which exhibits an attenuated phenotype and a non-coding mutation. Of all reported patients, only six are clearly stated to have survived[14 18 24 33–35] (case 2) and more than half were treated long term with immunosuppressants.

Laboratory findings

Laboratory abnormalities consistent with T1DM, severe enteropathy, hypothyroidism, and cytopenias are common. Apart from these, clinical laboratory testing has shown few consistent diagnostic findings and shed only a little light on the underlying pathophysiology.

Karyotypes were normal in six patients.[22 24 30 32 33 36] Urinary acids were normal in one,[24] but raised glycine and serine were noted in another,[29] and non-selective amino aciduria was found in still another.[30] Hypocalcaemia with normal or raised parathyroid hormone was present in two cases,[18 22] suggesting some parathyroid hormone resistance.

Serum cortisol, somatostatin, gastrin releasing peptide, calcitonin, substance P, neurotensin, adrenalin, noradrenaline, cholecystokinin, pepsinogen I, and urine 5-hydroxyindoleacetic acid were normal in one or two patients.[32] Mild increases of gastrin, vasoactive intestinal peptide, pancreatic peptide, and urinary vanillylmandelic and homovanillic acids were variably found.[32] Biochemical evidence of hepatic parenchymal disease and cholestasis was common[14 22 24 29 32 35 37 38] (case 3).

T lymphocyte subsets and $CD4^+/CD8^+$ ratios were normal in most,[14 24 29 32 37 38] although slight increases in $CD4^+$ cells were reported by Satake *et al*.[33] T cell stimulations with phytohaemagglutinin, streptokinase-dornase, poke weed mitogen, concanavalin-A, OKT3, and Candida were slightly low or normal,[18 29 33 38] although Shigeoka *et al*[39] reported increased proliferation with phytohaemagglutinin, along with increased IL-2 production from CD-4 positive and total T cells in a member of the family reported by Powell *et al*.[18] This patient showed mildly increased T cell apoptosis, increased numbers of CD4+ T cells at birth, and increases in T cell activation markers associated with episodes of clinical deterioration (Bakke and Wildin, submitted). In one family, one boy had markedly diminished T cell responses, while another had hypogammaglobulinaemia and no response to immunisation with diphtheria and tetanus toxoid.[14] A third affected boy had normal antibody responses to protein immunogens, but defective response to Pneumovax™. Post-immunisation titres of antibodies to tetanus toxoid and poliovirus were normal in one boy.[38] Lymphocyte mitogen stimulation resulted in exaggerated expression of IL-4, IL-5, IL-10, and IL-13, and reduced expression of interferon-γ.[38]

CD16 positive natural killer cells were high in one case,[29] and CD20 positive cells and NKH-1 positive cells were raised in

another.[33] Class II-DR expressing T cells were raised.[33 39] Neutrophil chemotaxis, nitroblue tetrazolium reduction, and myeloperoxidase activity were also normal.[14 18 29] Complement was slightly low or normal.[14 29 30 37] IgG, IgM, and IgA levels were generally normal, but IgE was often raised, sometimes dramatically.[12 14 24 27–30 33 36–38] Persistent or periodic eosinophilia was frequent.[14 18 24 29 30 36] Allergo-absorbent and skin prick tests for immediate hypersensitivity were consistent with heightened allergic response.[12 28] Neutropenia was present in at least two cases[40] (case 2). A detailed summary of immune function testing in the three patients described here is being prepared for publication elsewhere. No unusual findings in lymphocyte subsets, mitogen responses, or immunoglobulin levels were identified.

Autoantibodies have been identified in many patients but are absent in others.[26 27 32 37] Positive reports include antibodies against pancreatic islets[14 29 30 32 35 37 38 41] (case 2), insulin,[36 37] glutamic acid decarboxylase (GAD),[36 38] thyroid (anti-microsomal and anti-thyroglobulin),[14 18 24 33 40 41] smooth muscle,[18 33 35 37] blood group B,[18] intestinal epithelium (enterocytes[24 37] rat and human small intestine and colon,[35] human jejunum,[33 40] human duodenum, jejunum, and colon,[41] and rabbit colon and small intestine and human rectum[27] (case 1), and reticulin and/or gliadin[14 35]). Kobayashi *et al*[28 42] found circulating autoantibodies to a novel 75 kDa gut and kidney specific antigen, AIE-75, in the family first reported by Satake *et al*,[33] but AIE-75 specific antibodies were not detected by Baud *et al*.[38] Thyroid function remained normal in some patients despite the presence of antithyroid antibodies. In one case, anti-insulin antibodies developed only after exogenous insulin therapy.[37] Antinuclear antibody has been either absent or present at low titre.[14 18 27 37 38] Late appearance of antibodies to proximal renal tubules, keratinocytes, pancreatic ascini and canals, and adrenals has also been reported.[37] Cilio *et al*[36] reported a diabetes susceptibility *HLA* allele inherited from the father (DQB1*0201, DR3) in a severely affected infant.

Pathology

The pathological findings in IPEX are summarised in table 5. By far the most striking and consistent feature is the absence of normal small bowel mucosa and the presence of inflammatory cells in the lamina propria and/or submucosa. Involvement of the large intestine is not uncommon. Inflammatory infiltrates are observed in multiple organs. Foci of inflammation are often seen in the pancreas and islet cells have been reduced or absent in most cases with T1DM. Cholestasis and hepatic fatty change are also common, though the cause in many cases may be chronic parenteral nutrition rather than the underlying autoimmune disorder. Pathology in lymphoid organs is variable, and evidence of bleeding or haemorrhage is not unusual. Histology of the skin shows immune cell infiltration with other changes that are characterised as psoriaform hyperplasia or eczematoid. In one case, IgG and complement component C3 deposition were associated with bullae.[14 29] Renal pathology may include tubulointerstitial nephritis, focal tubular atrophy, membranous glomerulopathy, and irregular granular immune deposits in glomeruli and tubular basement membranes.[27 28]

Table 5 Pathology of examined cases[14 18 22–25 27–33 35–38 40 41]

Biopsy or necropsy findings	No of patients	
	Abnormal	Normal
Small intestine		
Villous atrophy or mucosal erosion	25	
Lamina propria expansion	11	
Inflammatory infiltrate	19	
Plasma cells	6	
Crypts, absent or decreased	5	4
Paneth or argentaffin cells, absent or decreased	4	
Abnormal nuclear polarity	2	
Immature/foregut appearance	4	
Large intestine		3
Mucosal erosion or oedema	5	
Goblet cells, decreased	1	
Crypt abscesses	3	
Pancreas		
Exocrine deficiency or immature lobules	3	4
Acini, atrophy, fibrotic, dilated, or cystic	7	2
Ducts, increased or dilated	3	
Islets of Langerhans, decreased or absent	8	2
Inflammation	13	
Liver		
Fatty change	6	
Cholestasis	5	
Cholangitis/pericholangitis	4	2
Thyroid, infiltrates	3	1
Thymus, atrophy	3	
Lymph nodes, follicular/reactive hyperplasia	4	2
Lung		
Consolidation	4	
Haemorrhage	1	
Inflammation	5	
Kidney		
Interstitial fibrosis and inflammation	3	
Immune glomerulopathy	1	

Treatment

Many interventions have been attempted (table 6). Most are ineffective, but two approaches show promise: immunosuppression and BMT. Supportive measures, such as parenteral nutrition and red blood cell and platelet transfusions, are often necessary.

Immunosuppression

Chronic immunosuppression has proven partially effective in some patients, but ineffective in others. Regimens that include tacrolimus have shown significant effect, but its prolonged use is limited by its direct toxicity. Furthermore, even those with prolonged survival continue to have significant clinical disease. CSA seemed to benefit some members of the kindred reported by Powell *et al*,[18] where one member still survives on chronic CSA (Buist and Wildin, unpublished data). CSA alone may have prolonged life in a few other cases but generally did not prevent a fatal outcome. Following eventual failure of oral CSA in case 1 of Satake *et al*,[33] Kobayashi *et al*[34 43] reported improvement with oral tacrolimus plus dexamethasone. This patient survives, but suffers from renal tubular disease, osteoporosis, a steroid induced cataract, and growth failure.[28 43] Case 1 of Ferguson *et al*[14] had a similar response to tacrolimus, and his skin responded to a topical combination of CSA, prednisone, and dapsone. The patients reported here survived for many years with chronic immunosuppressive medications, including CSA, tacrolimus, methotrexate, corticosteroids, infliximab, and rituximab, but also developed progressive toxicities. These included severe hypertension, renal insufficiency, cardiac hypertrophy, and sepsis related to indwelling catheters. Psychiatric complications including clinical depression and conduct disturbance required medical intervention in the two older patients.

T cells from scurfy mice are resistant to CSA suppression,[10] suggesting that it and other agents inhibiting TCR signalling by the same mechanism may not be good choices for modulating the abnormally high reactivity of the mutant T cells. The limited success of CSA and tacrolimus in humans is consistent with this idea. Experience with newer, more potent immunosuppressive medications is lacking.

Chronic immunosuppression also increases the chance of severe or opportunistic infections. It is possible that the disordered immune function of IPEX patients contributes to the development of infections in the context of immunosuppressive medications.

Table 6 Attempted treatments

Intervention	References
Nutritional	
Gluten restriction	18, 24, 25, 29, 30, 35, 38, 41, this report
Parenteral nutrition	14, 18, 22, 24–30, 32, 33, 35, 38, 40, 41, this report
Pancreatic enzymes	29, 37
Somatostatin analogue	32, 40
Peptide formula	28, this report
Colostrum	40
Immunological	
Corticosteroids	14, 18, 22, 24, 32, 33, 35, 37, 38, 40, 41, this report
Cyclosporin A	14, 22, 24, 33, 37, 40, this report
Tacrolimus	14, 34, 38, this report
Topical dapsone and CSA	14
IV immunglobulin	14, 22, 33, 40, this report
Anti-RIL2 and anti-lymphocyte serum	37
Rituximab	This report
Rofecoxib, methotrexate, infliximab	This report
Cellular	
Bone marrow transplant	38, this report
Other	
Fresh frozen plasma	29, 30
Cromoglycate	35
Cyclophosphamide (without BMT)	35
Azothioprine	35, 41
5-aminosalicylic acid and 6-mercaptopurine	40

Bone marrow transplantation

HLA identical sib BMT has been attempted in at least three cases. One boy received HLA identical bone marrow at 4 months of age from his sister, who was not a carrier.[38] Remission began during the conditioning phase and continued after the transplant. Enteropathy, T1DM, and eczema all resolved. He remained disease free for 29 months, then developed a lymphoproliferative, haemophagocytic syndrome and died suddenly. Engraftment studies showed host/donor chimerism throughout remission with a donor contribution of 3-30% among peripheral blood cell subsets.

Two of the affected boys reported here received BMT as a final resort after developing life threatening complications of long term immunosuppression medications. Although both died from infectious complications of their transplants, their enteropathy, arthritis, immune thrombocytopenic purpura, and eczema showed marked improvement. In addition, a decreased insulin requirement was intermittently observed in both cases despite a long history of insulin dependence.

These mixed results suggest that BMT may, with further experience, become an effective treatment, especially if performed early in the course of clinical manifestations. However, this option should be used cautiously and selectively until long term survival potential with BMT is proven. The discovery of the underlying genetic defect in IPEX permits us to perform molecular diagnosis in newborn boys at risk and to contemplate presymptomatic BMT. It is possible that this approach would improve the long term outcome.

Other treatments

Peake *et al*[29] reported some improvement of diarrhoea with fresh frozen plasma and pancreatic enzymes. Seidman *et al*[40] reported marked but transient improvement in enteropathy with human colostrum given at 18 months of age.

DISCUSSION

Diagnosis and differential diagnosis

The diagnosis of IPEX rests on the clinical presentation, the family history, and the elimination of other diagnoses with similar presentation. IPEX displays clinical overlap with a number of genetic disorders. Table 7 proposes features that may help clinicians differentiate them from IPEX. Non-genetic disorders, especially pre- or postnatal viral infections, may present in a similar fashion. These should be considered and appropriately managed until ruled out.

Clinical variation among and within families

Families reported by Powell *et al*,[18] Satake *et al*,[33] and perhaps Ferguson *et al*[14] differ from the majority of reports in that the disease is sometimes compatible with survival, and appears to show some response to immunosuppression. Members of the kindred reported by Powell *et al*[18] had later ages of onset and showed episodic, rather than persistent diarrhoea. Many of the deaths were associated with first immunisation, viral infection, or other exogenous immune stimulating events. Those surviving past infancy manifested arthritis, glomerulonephritis, ulcerative colitis, hypertension, recurrent infections, sarcoidosis, and peripheral nerve sensory neuropathy.[18] Similarly, the brothers reported by Satake *et al*[33] lacked overt T1DM, although two had glucose intolerance and the diarrhoea responded to strong immunosuppression in one.[33] The current cases also showed a clinical response to chronic immunosuppression and a longer lifespan. It is not yet possible to identify a reliable genotype-phenotype relationship for the observed variations, but the mutation affecting mRNA stability found in the family reported by Powell *et al*[18] might explain the variable, sometimes attenuated phenotype.

The patient reported by Hattevig *et al*[25] had neonatal T1DM and recurrent diarrhoea; the latter eventually responded to withdrawal of dietary gluten and the patient survived. He is included here because of the combination of neonatal illnesses, the similarity of gut pathology, and a pedigree that is complicated, but consistent with an X linked recessive contribution. One member of the family reported by DiRocco and Marta[24] also did well on a gluten free diet. In several other cases, gluten could be excluded as a contributing agent. Of interest, symptomatic and asymptomatic coeliac disease may occur at increased frequency among children with the common form of juvenile T1DM.[44]

In contrast, the remaining families and individual cases had early, even prenatal onset, were almost uniformly fatal, and immunosuppression, when used, did not prevent death. It may prove clinically useful to distinguish disease variants. However, it remains to be seen whether the differences are the result of chance, alternate genetic mechanisms, variably

Table 7 Syndromes with overlapping features

Disorder (OMIM)	Inheritance	Common features	Distinguishing features
Transient neonatal diabetes (601410)	Usually sporadic	Neonatal onset insulin dependent diabetes	Transient, isolated DM without autoimmune features. Uniparental disomy or paternal duplication at 6q22-q23
Pancreatic hypoplasia (260370, 60001) or agenesis (260370)	AR, AD for 60001	Neonatal onset insulin dependent diabetes	May have exocrine pancreatic insufficiency. No autoimmune features.
Islet beta cell developmental defect (600089, 606176)	AR	Neonatal onset insulin dependent diabetes	No autoimmune features. Mutant glucokinase gene in some.
Wiskott-Aldrich syndrome (301000)	XR	Autoimmunity, including eczema, thrombocytopenia, and occasional enteropathy, early death	Later age of onset, chronic immunodeficiency, abnormalities of platelet size, low CD8+ T cell count, skewed X inactivation in lymphocytes from female carriers. Mutant WAS gene.
Autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy (APECED) (240300)	AR	Malabsorbtion, diarrhoea, insulin dependent diabetes or hypothyroidism occurs infrequently	Later age of onset, candidiasis, and ectodermal dystrophy rather than eczema. Mutant *AIRE* gene
Schmidt syndrome/polyglandular autoimmune syndrome type II (269200)	Complex	Autoimmune thyroiditis, T1DM, anaemia	Later age of onset, Addison disease, gonadal atrophy; pernicious anemia
Omenn syndrome (603554)	AR	Erythroderma with T cell infiltration and thickening, lymphadenopathy, protracted diarrhoea, failure to thrive, eosinophilia and raised IgE	Reduced or absent B cells, poor T cell proliferative responses. No T1DM. Mutant *RAG1* or *RAG2* genes.
Intractable diarrhoea with persistent villous atrophy/X linked autoimmune enteropathy	XR	Severe enteropathy with flattening or loss of mucosa and immune cell infiltration, eczema	T1DM less frequent. Allelism with IPEX has not been excluded.

severe mutations of *FOXP3* or its regulatory regions, or modifying genes such as HLA. Environmental influences and variable management may also influence outcomes.

In addition to clinical variation among families, presentation and expression varies considerably within families. Enteropathy may be present in the absence of T1DM[22 24 29] and vice versa. Indeed, several reports of X linked autoimmune enteropathy (AIE) strongly resemble IPEX with or without T1DM (Goulet's types Ia and Ib, respectively[20]), but allelism has not yet been shown. The age of onset and the prevalence of atopic dermatitis, thrombocytopenia, and haemolytic anaemia vary within families as well.

Immunology and pathophysiology

The current cases add substantially to the limited knowledge of the immune milieu in IPEX patients. Remarkably few abnormalities are found, and none supports the presence of an underlying deficiency of immunoprotective cells or molecules. Instead, the pathological evidence suggests a hyperactive immune or immune dysregulated state that results in destruction or compromise of various essential organs, as well as secondary effects such as cytokine mediated cachexia. This concept is supported by the available data from the scurfy mouse, where abnormal T cell reactivity appears central to the development of autoimmunity and cytokine excess.

In this context, the therapeutic efficacy of partial chimerism for normal bone marrow is of particular interest. This observation suggests that the abnormally reactive T cells can be made quiescent by the addition of normal haematopoietic cells. Patel[45] alluded to a similar effect in scurfy mice. The phenomenon is presumably also responsible for the absence of disease in female heterozygotes. These females are probably mosaic by Lyonisation for cells expressing only the normal *FOXP3* allele and cells expressing only the IPEX associated allele, yet the latter fail to cause disease. One of us has recently shown a therapeutic effect of normal T cells in the scurfy mouse, suggesting that scurfy T cells are susceptible to regulation by regulatory T cells, but are not themselves capable of providing T regulation (R S Wildin, submitted).

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