

Immunodeficiencies with Autoimmune Consequences

Luigi D. Notarangelo,^{*} Eleonora Gambineri,[†] and
Raffaele Badolato^{*}

^{*}"Angelo Nocivelli" Institute for Molecular Medicine,

Department of Pediatrics, University of Brescia, Brescia, Italy

[†]Department of Pediatrics, University of Florence, Florence, Italy

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Abstract

Far from being mutually exclusive, immunodeficiency and autoimmunity may occur simultaneously. During the last years, analysis of Autoimmune Polyendocrinopathy – Candidiasis – Ectodermal Dystrophy (APECED) and Immunodysregulation – Polyendocrinopathy – Enteropathy – X-linked (IPEX), two rare monogenic forms of immunodeficiency associated with autoimmunity, has led to the identification of Auto Immune Regulator (AIRE) and Forkhead Box P3 (FOXP3), essential transcriptional regulators, involved in central tolerance and peripheral immune homeostasis, respectively. Characterization of the molecular and cellular mechanisms involved in APECED, and recognition that AIRE expression is sustained by effective thymopoiesis, has recently allowed to define that the autoimmunity of Omenn syndrome, a combined immunodeficiency due to defects of V(D)J recombination, also results from defective expression of AIRE. The implications of identification of the basis of autoimmunity in these rare forms of immunodeficiency have important implications for a better understanding of more common autoimmune disorders, and for development of novel therapeutic approaches.

1. Autoimmune Manifestations in Primary Immune Deficiencies: Relevance and Mechanisms

1.1. Introduction

The main function of the immune system is to preserve homeostasis. In vertebrates, this includes the ability to specifically recognize and react against non-self antigens, thus counteracting harm associated with infections and tumors. At the same time, the immune system specifically recognizes self antigens that are tolerated. Failure to accomplish these functions leads to immune deficiency and autoimmunity, respectively. Based on this dichotomy, immune deficiency and autoimmunity have long been considered at opposite sides of the clinical immune response. However, careful analysis of the clinical features associated with congenital immunodeficiencies, and a better understanding of the molecular and cellular mechanisms involved in immunodeficiency and autoimmunity, has shown that, in fact, these conditions are often related. Importantly, the study of autoimmune manifestations associated with primary immune deficiencies (PID) has led to novel insights into the molecular pathophysiology of autoimmune reactions, which now also include exaggerated and harmful inflammatory responses. At the same time, while most autoimmune diseases in humans are of multifactorial origin, the study of rare single-gene disorders that associate immunodeficiency and autoimmunity has shown the clinical relevance of specific events during development and function of immune cells which are essential to the preservation of immune homeostasis.

1.2. Primary Immunodeficiencies Associated with Autoimmunity

Although the clinical hallmark of PID is represented by an increased susceptibility to infections, careful analysis of the clinical features associated with the various forms of PID has shown that many of them are also characterized by autoimmunity and/or aberrant inflammatory responses ([Arkwright *et al.*, 2002](#); [Candotti *et al.*, 2002](#); [Etzioni, 2003](#)). While autoimmune manifestations have been reported in various forms of PID, irrespective of the nature of the immune defect primarily involved in the disease ([Table 1](#)), the molecular and cellular mechanisms responsible for autoimmunity may vary in different forms of PID. In addition, several mechanisms may be involved even within the same form of PID. In particular, the notion that autoimmunity results from a failure of self-tolerance applies only to some of the autoimmune manifestations associated with PID.

For many other forms of PID, autoimmune manifestations do not reflect defects in deletion of autoreactive lymphocytes, or inefficient mechanisms of

Table 1 Autoimmunity and Other Manifestations of Immune Dysregulation in Primary Immune Deficiencies

Main type of immune deficiency	Immunodeficiency disease	Autoimmune manifestations
Humoral immune deficiencies	X-linked agammaglobulinemia	Arthritis, dermatomyositis, AHA, scleroderma
	Common variable immunodeficiency	Thrombocytopenia, AHA, IBD, arthritis, hepatitis, lungs granulomatosis
T cell/combined immune deficiencies	IgA deficiency	Celiac disease, rheumatoid arthritis, SLE, alopecia, thyroiditis, IDDM
	DiGeorge syndrome	ITP, arthritis, erythrodermia, cytopenias
	PNP deficiency	AHA
	ADA deficiency	AHA, IDDM, asthma, eczema
	SCID (other forms)	Skin rash, infiltration of target organs by oligoclonal T cells, AHA
	Omenn syndrome	Erythrodermia, enteropathy, multiple organs lymphoid infiltration
	MHC class I deficiency	Leukocytoclastic vasculitis, arthritis, Wegener-like granulomatosis
	MHC class II deficiency	Sclerosing cholangitis, autoimmune cytopenias
	CD40L or CD40 deficiency	Sclerosing cholangitis, neutropenia
Phagocytic cell defects	Chronic granulomatous disease	IBD, granulomatous lesions of lungs, liver, and urinary tract
Complement deficiency	C1q, C1r, C1s, C2, and C4 deficiency	SLE, nephritis
Immunodeficiency syndromes	APECED	Hypoparathyroidism, Addison disease, primary ovarian failure, IDDM, hepatitis
	IPEX	Enteropathy, IDDM, eczema, autoimmune polyendocrinopathy
	Wiskott-Aldrich syndrome	AHA, vasculitis, IBD, arthritis, renal disease
	X-linked lymphoproliferative disease	EBV-associated hemophagocytic lymphohistiocytosis
	FHL	Virus-associated hemophagocytic lymphohistiocytosis
	ALPS	AHA, thrombocytopenia, neutropenia

AHA: autoimmune haemolytic anemia; IBD: inflammatory bowel disease; SLE: systemic lupus erythematosus; IDDM: insulin-dependent diabetes mellitus; ITP: idiopathic thrombocytopenic purpura; PNP: purine nucleoside phosphorylase; ADA: adenosine deaminase; SCID: severe combined immune deficiency; MHC: major histocompatibility complex; CD40L: CD40 ligand; APECED: autoimmune polyendocrinopathy – candidiasis – ectodermal dystrophy; IPEX: immunodysregulation – polyendocrinopathy – enteropathy – X-linked; EBV: Epstein-Barr virus; FHL: familial hemophagocytic lymphohistiocytosis; ALPS: autoimmune lymphoproliferative syndrome.

peripheral tolerance, but rather are the consequence of inability of the immune system to eliminate pathogens. This often leads to continuous and exaggerated inflammatory responses, which ultimately cause damage of bystander tissues. This mechanism is involved in the hemophagocytic syndromes that follow especially viral infections in patients with defects in T and NK cell-mediated cytotoxicity, as typically observed in X-linked lymphoproliferative disease, familial hemophagocytic lymphohistiocytosis, Chediak-Higashi syndrome, and Griscelli disease (Clark and Griffiths, 2003). Increased inflammatory responses to bacterial microbes are probably involved in the granulomatous lesions associated with chronic granulomatous disease (Barton *et al.*, 1998). Similarly, chronic stimulation by *Cryptosporidium parvum* or *cytomegalovirus* may result in sclerosing cholangitis in patients with hyper-IgM syndrome due to CD40 ligand or CD40 deficiency (Notarangelo and Hayward, 2000), and in infants with Major Histocompatibility Complex class II deficiency (Saleem *et al.*, 2000). Improper handling of pathogens (of viral and bacterial origin) is supposed to be responsible for the autoimmune manifestations of Wiskott-Aldrich syndrome (Schurman and Candotti, 2003). Finally, impaired clearance of immune complexes is likely involved in the Systemic Lupus Erythematosus-like forms associated with defects of early components of the complement classical pathway (Walport *et al.*, 1997).

Vis-à-vis, with this heterogeneity, in this review we will focus our attention on monogenic disorders of central and peripheral tolerance that associate immunodeficiency and autoimmunity, such as Autoimmune Polyendocrinopathy – Candidiasis – Ectodermal Dystrophy (APECED) and Immunodysregulation – Polyendocrinopathy – Enteropathy – X-linked (IPEX), respectively. We will also discuss how characterization of the cellular and molecular bases of these rare disorders has been essential to a better understanding of the pathophysiology of other immunodeficiency disorders with autoimmunity, such as Omenn syndrome. These novel insights have far-reaching implications for the pathophysiology of disorders of immune homeostasis and may lead to novel forms of therapeutic intervention.

2. AIRE, Central Tolerance, and the Pathophysiology of Autoimmune Polyendocrinopathy

2.1. Clinical and Immunological Features of APECED

2.1.1. Clinical Manifestations of APECED

Autoimmune Polyendocrinopathy – Candidiasis – Ectodermal Dystrophy (APECED) is a rare autosomal recessive organ-specific autoimmune disorder that is characterized by a variable combination of (i) chronic mucocutaneous

candidiasis, (ii) polyendocrinopathy and/or hepatitis, and (iii) dystrophy of dental enamel and nails (Ahonen *et al.*, 1990; Halonen *et al.*, 2002). Indeed, the triad of candidiasis, hypoparathyroidism, and adrenal failure constitutes the most typical presentation of the disease. The clinical diagnosis of APECED is established when a subject presents at least two of these major features, but, in the case of a sibling of an APECED patient, one feature is sufficient for suspecting the disease (Ahonen *et al.*, 1990). Although APECED is generally a rare disease, it is more frequently observed in some selected populations, in particular among the Finns (incidence 1:25,000), Iranian Jews (1:9000), and Sardinians (1:14,400) (Aaltonen *et al.*, 1994; Rosatelli *et al.*, 1998; Zlotogora and Shapiro, 1992).

The phenotype of APECED is extremely heterogeneous, varying from one single manifestation, usually candidiasis, up to nine different types of endocrine or dystrophic conditions (Halonen *et al.*, 2002). In the majority of APECED patients, the first symptom to develop is mucocutaneous candidiasis, which is usually resistant to antimicrobial therapy and may recur throughout the patient's life. Hypoparathyroidism is the second symptom to appear, often already in the second year of life. Finally, adrenocortical failure is usually identified after four years of age (Ahonen *et al.*, 1990; Halonen *et al.*, 2002). Definition of a genotype–phenotype correlation in APECED is difficult, since patients, even when from the same family, may present different clinical manifestations in variable sequence. Nevertheless, there are some exceptions to this rule, at least for some populations. In particular, candidiasis, which is observed in the vast majority of APECED patients, is rarely present among the Iranian Jewish patients, suggesting a specific genetic determinant of the APECED phenotype in this population (Zlotogora and Shapiro, 1992).

Hypoparathyroidism is the most frequent endocrinopathy that is identified in APECED. It affects 88% of patients, on average (Ahonen *et al.*, 1990; Halonen *et al.*, 2002). Its incidence, as recently reported, varies greatly among sexes, being more common in females (98%) than in males (71%) (Gylling *et al.*, 2003). Like hypoparathyroidism, also hypogonadism is more frequent in females (60% of APECED patients older than 13 years) than in males (14%), while other endocrinopathies such as adrenocortical failure (observed in 72% of APECED patients), insulin-dependent diabetes mellitus, and hypothyroidism do not seem to be influenced by sex (Table 2).

APECED patients may also display numerous autoimmune manifestations affecting the gastrointestinal tract, including pernicious anemia due to vitamin B12 deficiency (13–15%), and intestinal malabsorption, with onset varying from 4 months to 21 years of age (Ahonen *et al.*, 1990). Steatorrhea and malabsorption are common presenting symptoms of gastrointestinal involvement, and may even mark the clinical onset of the disease (Ahonen *et al.*, 1990).

Table 2 Disease Manifestations in Patients with APECED^a

Disease components	Percentage of patients affected
Candidiasis	88.5
Hypoparathyroidism	84.6
Adrenal failure	72.1
Alopecia	34.6
Ovarian failure	38.5
Vitiligo	23.1
Hepatitis	19.2
Kerathopathy	19.2
Gastrointestinal dysfunction	15.4
Vitamin B12 malabsorption	14.4
Diabetes mellitus	12.5
Hypothyroidism	12.5
Growth hormone deficiency	2.9
Testicular failure	1.2
Oral carcinoma	1.0

^aModified from (Halonen *et al.*, 2002).

Skin and other tissues of ectodermal origin are also frequently involved in APECED: alopecia is particularly common (29–36%) and may appear as early as five years of age, while vitiligo affects 13–26% of the cases, depending on the study. Pitted nails are another cutaneous symptom of the disease; in most cases, they reflect ectodermal dysplasia, because microbial culture of nail specimens are usually negative for fungi, and the lesions may heal spontaneously. However, in some cases, candida infection may play a role in the pathophysiology of the lesions, and specific antifungal therapy is then required to induce complete resolution (Ahonen *et al.*, 1990).

Evaluation of the clinical course of APECED in large cohorts of patients has shown that candidiasis has the highest occurrence in infancy, but its incidence slowly declines in following years. Conversely, hypoparathyroidism and adrenal insufficiency are usually detected during the first decade of life, and gonadal insufficiency is usually not observed before the second decade (Ahonen *et al.*, 1990; Halonen *et al.*, 2002). In addition to these most common APECED manifestations, the other manifestations of the disease, including alopecia, vitiligo, and gastrointestinal dysfunction, may continue to appear also in older patients.

The immune deficiency of APECED is clinically marked by chronic candidiasis. Although this is usually confined to skin and mucosal tissues, a proportion of patients may develop severe lung disease.

Overall, prospective analysis of APECED patients has shown that the recurrence of several endocrine and non-endocrine autoimmune diseases leads to a poor quality of life in the vast majority of patients, without affecting long-term survival. However, in a minority of APECED patients, acute adrenal insufficiency, with or without hypoparathyroidism, and fulminant hepatic failure have been registered as causes of death (Ahonen *et al.*, 1990). Early identification of this genetic disease and strict follow-up of endocrine and of other autoimmune symptoms help to prevent severe complications of the disease.

2.1.2. Autoantibodies

Development of organ-specific autoimmune manifestations is accompanied by the appearance of a variety of autoantibodies directed against enzymes or intracellular proteins (Betterle *et al.*, 2002). Study of large cohorts of APECED patients has shown that the specificity and number of autoantibodies that can be detected is not affected by patients' sex or age (Soderbergh *et al.*, 2004). The majority of APECED subjects present from one to up to eight different types of autoantibodies, while a minority of them (8%) may not show detectable autoantibodies (Table 3). Lack of detectable autoantibodies is more common in patients whose clinical features are restricted to candidiasis and/or hypoparathyroidism, while autoantibodies are usually present in patients with other autoimmune manifestations, suggesting a correlation between antigen specificity and autoimmune reaction against the same organ (Soderbergh *et al.*, 2004).

In fact, most of the patients with adrenal insufficiency present circulating antibodies that recognize several steroidogenic enzymes, including 21-hydroxylase (21-OH), side-chain cleavage enzymes (SCC), and 17 α -hydroxylase (17 α -OH). Two of these autoantibodies (21-OH and SCC) are strongly associated with adrenocortical failure, being detected in 75% and 61% of APECED patients with Addison's disease, while the anti-17 α -OH has a lower predictive power as a risk factor (Soderbergh *et al.*, 2004). However, the autoantibodies that are identified in APECED are not necessarily specific for each of the single clinical manifestations of the disease, but can be variably linked to other endocrinopathies as well. In fact, autoantibodies directed against SCC and/or 17 α -OH (that are often detected in patients with Addison's disease) may also be present in APECED patients with hypogonadism (Soderbergh *et al.*, 2004).

Association has been reported between autoimmune hepatitis and the presence of autoantibodies directed against tryptophan hydroxylase (TPH) (Soderbergh *et al.*, 2004), the cytochromes P450 1A2 (CYP1A2) and 2A6

Table 3 Autoantibodies in Patients with APECED^a

Autoantigen involved	Percentage of patients affected
21-hydroxylase (21-OH)	66
Side-chain cleavage enzyme (SCC)	52
Aromatic L-amino acid decarboxylase (AADC)	51
Tryptophan hydroxylase (TPH)	45
17alpha-hydroxylase (17alpha-OH)	44
Tyrosine hydroxylase (TH)	40
Glutamic acid decarboxylase 65 (GAD65)	38
Cytochrome P450 1A2 (CYP1A2)	8
Protein IA-2 (IA-2)	7
Calcium-sensing receptor (CaSR)	0

^aModified from (Soderbergh *et al.*, 2004).

(CYP2A6), and aromatic L-amino acid decarboxylase (AADC) (Clemente *et al.*, 1997, 1998; Husebye *et al.*, 1997; Perniola *et al.*, 2000). However, recent studies suggest lack of correlation of anti-CYP2A6 antibody with the disease and a low sensitivity of anti-CYP1A2 antibodies, suggesting that anti-TPH antibodies are a more sensitive marker for autoimmune hepatitis (Obermayer-Straub *et al.*, 2001). TPH and GAD65 autoantibodies also have an independent correlation with intestinal dysfunction; in particular, each of them has been detected in 75% of APECED patients with this manifestation (Ekwall *et al.*, 1998; Soderbergh *et al.*, 2004).

In patients with cutaneous autoimmune manifestations of APECED, such as alopecia or vitiligo, association with serum autoantibodies directed against the transcription factor SOX10 (Hedstrand *et al.*, 2000), or against the enzyme tyrosine hydroxylase (Hedstrand *et al.*, 2001), respectively, has been demonstrated, and might account for the pathophysiology of these conditions.

Although more rare, hypopituitarism and growth arrest have also been observed in APECED patients. In such cases, circulating autoantibodies directed against hypothalamic-hypophyseal structures have been occasionally detected, suggesting that these may be additional potential targets of autoimmune destruction (Arvanitakis and Knouss, 1973; Cocco *et al.*, 2005; Franzese *et al.*, 1999; Ward *et al.*, 1999).

Identification of the autoantigen(s) responsible for the development of hypoparathyroidism has been especially elusive. Descriptions of autoantibodies against parathyroid gland and epithelia have been controversial (Irvine and Scarth, 1969). Even the demonstration of antibodies against the extracellular domain of the Ca²⁺-sensing receptor (Li *et al.*, 1996) has not been confirmed. Nevertheless, immunofluorescence and western blot analysis has

shown that the serum from 37% of APECED patients contains antibodies that bind to the parathyroid gland tissue, but identification of the specific autoantigen(s) is still missing (Gylling *et al.*, 2003).

Finally, the presence of autoantibodies does not necessarily indicate a destructive autoimmune process leading to clinically active disease. In fact, reactivity against pancreatic islet cell antigens, including the major autoantigen glutamic acid decarboxylase 65 (GAD65) of insulin-dependent diabetes mellitus, is detected in a large proportion of APECED patients (37–43%), but development of diabetes is observed only in a minority of these patients, suggesting that GAD65 antibodies have a low predictive value for development of diabetes in APECED (Gylling *et al.*, 2000; Soderbergh *et al.*, 2004). In contrast, autoantibodies against IA-2 tyrosine phosphatase-like protein (IA-2) or insulin (IAA) have a strong association with IDDM, but a low sensitivity (Gylling *et al.*, 2000; Soderbergh *et al.*, 2004).

2.2. Molecular Genetics of APECED

The gene responsible for APECED, known as *AIRE* (Auto Immune Regulator), was identified by positional cloning in 1997 on chromosome 21q22.3 (Nagamine *et al.*, 1997; The Finnish–German APECED Consortium, 1997). The gene extends for approximately 13 kb, and is composed of 14 exons. Up to now, more than 40 APECED-causing mutations and at least 6 polymorphisms have been identified in the coding and flanking regions of *AIRE*. These mutations are spread throughout the gene, but a few of them account for the majority of APECED patients identified among Caucasians (Bjorses *et al.*, 2000a; Heino *et al.*, 2001). Transition of the C nucleotide of a CpG dinucleotide leading to premature stop at codon 257 (R257X) is found in 83% of Finnish APECED chromosomes, but it has also been identified in other APECED patients of continental European nationality and/or ancestry (Bjorses *et al.*, 2000a; Heino *et al.*, 1999b, 2001; Scott *et al.*, 1998; Wang *et al.*, 1998). Another frequently occurring mutation is a deletion of 13 nucleotides (1094–1106del) that creates a premature termination at position 371 and a consequent loss of the last 73 amino acid residues. This mutation accounts for 70% of British and 53% of North American APECED alleles (Heino *et al.*, 1999b; Pearce *et al.*, 1998; Wang *et al.*, 1998). Together, the R257X and the 1094–1106del mutations account for more than 70% of North American APECED patients (Wang *et al.*, 1998).

There have been numerous attempts to define whether such high frequency of mutant alleles is the consequence of a founder effect or may reflect recurrent mutations. The R257X mutation involves a CpG dinucleotide, a well-known mutational hot spot, whereas the 1094–1106del may possibly

arise from formation of hairpin structures because of imperfect inverted repeats near the mutation site (Scott *et al.*, 1998). Therefore, these observations are suggestive of recurrent mutations being caused by mutational hot spots. To further support this hypothesis, the 1094–1106del mutation has been shown to be carried on different haplotypes of various APECED patients, as defined by analysis of microsatellites that flank the *AIRE* gene (Scott *et al.*, 1998; Wang *et al.*, 1998). On the other hand, it is still possible that divergence of current haplotypes from a common ancestral haplotype carrying the 1094–1106 deletion might be the consequence of recombinational events which occurred in the recent past. It is interesting that almost all the Sardinian APECED patients are homozygous for a nonsense mutation resulting from transition of a C residue of a CpG dinucleotide that creates a premature stop in exon 3 at arginine 139 (R139X) (Rosatelli *et al.*, 1998). This mutation is mostly associated with one single haplotype that is rarely found in healthy individuals from the same region, suggesting that this is a “private” mutation that probably occurred between 500 and 600 years ago (Meloni *et al.*, 2002; Rosatelli *et al.*, 1998). Another *AIRE* mutation with limited geographical distribution has been identified in Iranian Jewish APECED patients, who are known to have an elevated incidence of the disease. Almost all affected patients from this region carry a single homozygous nucleotide substitution in exon 2 (G374A), which leads to a tyrosine to cysteine substitution at position 85 (Y85C). Heterozygosity for the same mutant allele was detected in 1 of 35 unrelated Iranian Jewish subjects (Bjorses *et al.*, 2000a). In contrast to other individual populations affected by APECED, Iranian Jewish subjects present a lower incidence of mucocutaneous candidiasis and of other symptoms related to ectodermal dystrophy, suggesting that this mutation is associated with a milder clinical phenotype (Bjorses *et al.*, 2000a).

2.3. Cellular Expression of the AIRE Protein

AIRE encodes a protein of 545 amino acids with a molecular weight of 58 kDa that mainly localizes in the cell nucleus, but it is also detectable at lower levels in the cytosol (Nagamine *et al.*, 1997; The Finnish-German APECED Consortium, 1997). The AIRE protein is characterized by conserved motifs that are typical of a transcriptional regulator. It is composed of a highly conserved 100 amino acid domain at the N-terminus, known as the homogeneously staining region (HSR), a nuclear localization signal (NLS) between amino acids 113 and 133, a putative DNA-binding domain named “SAND,” two zinc fingers of plant homeodomain (PHD)-type motifs that are structural components of several nuclear proteins, and four nuclear receptor-binding LXXLL motifs (Fig. 1).

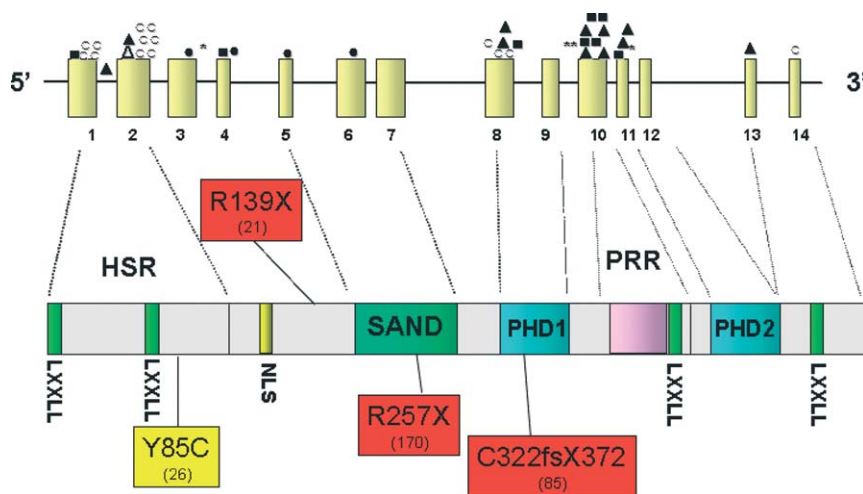


Figure 1 Top: Schematic representation of the AIRE gene, and illustration of the mutations identified in patients with APECED. Open circles identify missense mutations; closed circles, nonsense mutations; open triangles, in-frame deletions; closed triangles, out-of-frame deletions; closed squares, out-of-frame insertions; and asterisks, splice-site mutations, respectively. Bottom: Schematic representation of the AIRE protein, with the main functional domains. Position of the most common mutations identified in APECED patients (Y85C, R139X, R257X, and C322fsX372) is shown.

In keeping with these findings, transfection of a tagged-AIRE construct in cell lines, including COS-1 and U937 cells, shows that most of the protein is detectable as speckled structures in the nucleus (Fig. 2), and are reminiscent of the nuclear dots that are observed in promyelocytic leukaemia cells (Bjorses *et al.*, 2000a; Halonen *et al.*, 2004; Heino *et al.*, 1999a; Pitkanen *et al.*, 2001; Rinderle *et al.*, 1999). However, the AIRE protein is also detected in the cytoplasm, where it is organized in filamentous structures resembling intermediate filaments (Fig. 2), and co-localizes with vimentin (Bjorses *et al.*, 2000a; Halonen *et al.*, 2004; Heino *et al.*, 1999a; Pitkanen *et al.*, 2001; Rinderle *et al.*, 1999).

Several APECED-causing mutations that are permissive for protein expression have been shown to interfere with the subcellular localization of the protein. In particular, expression of the major Finnish mutation (R257X) leads to production of a 28 kDa protein that lacks the two PHD fingers and is detectable as filamentous and/or granular structures in the cytoplasm, but is undetectable in the nucleus (Bjorses *et al.*, 2000a). In addition, analysis of the C1313del mutant, which disrupts the second PHD finger, shows that the

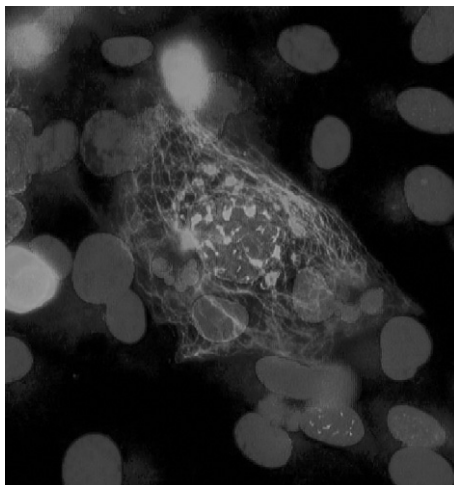


Figure 2 Illustration of AIRE expression in AIRE-transfected COS-1 cells. Staining with anti-AIRE antibody shows delicate cytoplasmic filaments and nuclear dots.

protein is retained in the cytoplasm as well (Bjorses *et al.*, 2000a). These observations suggest that both PHD fingers and the nuclear localization signal are required for the correct subcellular distribution of AIRE and its interaction with other nuclear proteins. Other *AIRE* mutations that are predicted to result in the synthesis of a prematurely truncated polypeptide lacking either one of the PHD finger domains also lead to prevalent expression in the cytoplasm (Bjorses *et al.*, 2000a).

It is noticeable that many missense mutations in the *AIRE* gene are located in exons 2 and 8, which encode for the HSR and PHD1 finger domains, respectively. This suggests that these domains are critically required for essential functions of the protein. The HSR domain is also present in the Sp100 and Sp140 proteins, and has been reported to be necessary for homodimerization of the protein (Pitkanen *et al.*, 2000b; Sternsdorf *et al.*, 1999). The common Iranian Jewish mutation (Y85C) is located in the four-helix bundle of the HSR domain (Halonen *et al.*, 2004; Pitkanen *et al.*, 2000a). Transfection of this mutant and of other constructs with mutations in exons 1 and 2 leads to homogenous expression of AIRE protein in the cytoplasm and in the nucleus, but neither the nuclear dots, nor the cytoplasmic filaments can be observed (Bjorses *et al.*, 2000a; Halonen *et al.*, 2004). Indeed, study of homomultimerization of AIRE by a two-hybrid system shows that the same missense mutations affecting the HSR domain severely reduce the homomultimerization ability of AIRE, and prevent ability of the mutant to interact with the wild-type

protein (Halonen *et al.*, 2004; Meloni *et al.*, 2005). Homodimerization of the protein is required for normal transcriptional activating property of AIRE, because inhibition of dimerization prevents transactivating activity of AIRE, as assessed by use of AIRE constructs carrying a copy of the gene fused to a heterologous binding domain in combination with the appropriate reporter gene (Bjorses *et al.*, 2000b; Halonen *et al.*, 2004; Pitkanen *et al.*, 2000a).

Characterization of the nuclear proteins and/or of DNA sequences that interact with AIRE is still a matter of debate. It has been shown that the common transcriptional coactivator CREB-binding protein (CBP) interacts with AIRE *in vitro*, suggesting that CBP, and possibly other nuclear factors, might act as cofactors for increasing binding of AIRE to target genes (Pitkanen *et al.*, 2000a). Subsequent studies by Kumar and coworkers (2001) have identified a DNA motif with specific binding activity for AIRE multimeric protein that might constitute a potential target for the three putative DNA binding domains (PHD fingers and SAND) of AIRE, but this observation has not been confirmed by other investigators.

Heteronuclear nuclear magnetic spectroscopy, which has been used to determine the solution structure of the first PHD finger, has demonstrated that the AIRE-PHD1 domain has a canonical PHD finger fold, including two coordinated zinc ions, which resemble RING finger domains with potential ubiquitylation activity (Bottomley *et al.*, 2005). In addition to this structural evidence, Uchida and coworkers (2004) have shown that the first PHD finger domain of AIRE mediates ubiquitylation and has intrinsic E3 ligase activity. Moreover, APECED-causing missense mutations located in the first PHD finger domain result in abrogation of the E3 ligase activity, while mutations in the second PHD finger only affect transactivating activity of the AIRE protein. It is likely that ubiquitylation activity of AIRE is important to regulate the steady-state level of transcription factors that are present in the nucleus and/or in the cytoplasm, but the identity of these proteins is still unknown.

2.4. Immunobiology of AIRE

2.4.1. Distribution and Regulation of AIRE Expression

In humans, AIRE expression is essentially restricted to lymphoid tissues, including thymus, lymph node, spleen, and fetal liver (Nagamine *et al.*, 1997). This contrasts with what is observed in mice, in which Aire is widely expressed in numerous non-hematopoietic organs, including adrenal glands, pancreas, intestine, liver, and kidney, suggesting a broader function of this regulatory factor in mouse (Halonen *et al.*, 2001). In both species, however, the highest levels of AIRE are detected in thymic epithelial cells, where it

concentrates in the nucleus to form the same dot-like structures that are observed after AIRE expression *in vitro* (Heino *et al.*, 1999a, 2000; Zuklys *et al.*, 2000). Comparison of AIRE staining pattern in the thymus to cytokeratin staining of epithelial cells demonstrates that only a fraction of thymic epithelial cells express AIRE mRNA and protein (Heino *et al.*, 1999a). In particular, AIRE is more expressed in a subset of thymic medullary epithelial cells that strongly express HLA class II antigens and the costimulatory molecules CD80, CD86, and CD40. This suggests that levels of AIRE protein are tightly regulated during maturation of medullary epithelial cells (Heino *et al.*, 1999a). Isolation of medullary epithelial cells from murine thymi on the basis of the level of CD80 expression has shown a positive correlation between the intensity of Aire expression and CD80 levels, supporting the model that regulation of costimulatory molecules increases in parallel with Aire mRNA levels in thymic epithelial cells (Derbinski *et al.*, 2005).

Analysis of AIRE expression in the thymic medulla shows that a minor proportion of AIRE-expressing cells presents a dendritic morphology and lacks epithelial antigens. In fact, these cells express the cell surface markers typical of myeloid cells, such as CD11c and CD83 (Heino *et al.*, 1999a). Moreover, monocytes and monocyte-derived dendritic cells generated in culture with IL-4, GM-CSF, and TNF- α also express AIRE, although at lower levels than thymic epithelial cells (Kogawa *et al.*, 2002). Therefore, it is likely that AIRE expression by DCs may account for the low levels of AIRE expression that are observed in many lymphoid organs, including lymph nodes and spleen.

Study of Aire expression during thymus ontogeny in mice has revealed that CD3 ϵ transgenic mice, which present a complete arrest at the early stage of thymocyte differentiation (CD44⁺ CD25⁺) before appearance of CD4⁺ CD8⁺ double positive (DP) T cells, completely fail to express Aire (Zuklys *et al.*, 2000). In contrast, RAG^{null} mutant mice, which present a block of thymocyte differentiation at the phase of CD4⁺/CD8⁺ DP T cells, display detectable transcripts of Aire mRNA (Zuklys *et al.*, 2000).

On the basis of these observations it seems that AIRE expression in the thymus is strictly influenced by thymocyte differentiation and requires a precise spatial and temporal interaction between these two cell populations of thymus. In RelB^{null} thymi, the cortico-medullary structure of the organ is completely subverted and there is a lack of Aire expression, even in the presence of normal mature epithelial cells (Heino *et al.*, 1999a; Zuklys *et al.*, 2000). Investigation of other animal models has shown that lymphotoxin- α (LT- α) might be important for the interaction between thymocytes and epithelial cells and for Aire expression, because LT- α gene-targeted mice are defective in Aire expression, but it is unclear whether the lymphotoxin

beta receptor is required for this pathway (Boehm *et al.*, 2003; Chin *et al.*, 2003).

2.4.2. Animal Models of APECED

Genetic disruption of the *Aire* gene by homologous recombination has provided a unique opportunity to investigate the function of Aire and to develop an animal model for APECED. Although mucocutaneous candidiasis and polyendocrinopathy have never been observed in the *Aire*^{null} mice, these animals present other features of the human disease, including multiorgan lymphocyte infiltration and circulating autoantibodies (Anderson *et al.*, 2002; Ramsey *et al.*, 2002). The autoantibodies are directed against several organs, such as salivary glands, liver, exocrine pancreas, and their presence correlates with the lymphocyte infiltration in the same tissues (Anderson *et al.*, 2002; Kuroda *et al.*, 2005; Ramsey *et al.*, 2002).

Despite the humoral and cellular autoimmune manifestations, the *Aire*^{null} mice present normal immunoglobulin levels, normal B and T subpopulations, and normal cytokine secretion in response to mitogens (Anderson *et al.*, 2002; Kuroda *et al.*, 2005; Ramsey *et al.*, 2002). In addition, circulating lymphocytes from *Aire*^{null} mice do not present any obvious defect in lymphocyte apoptosis, and in the expression of activation markers (CD25 and CD69), suggesting that Aire is not essential for functions of peripheral blood lymphocytes.

Both the expression studies of AIRE and the role of thymus in central tolerance suggest that the most crucial function of AIRE is related to regulation of thymocyte differentiation and selection. Although thymi from *Aire*^{null} mice are of normal size and show a normal architecture of the organ with a preserved cortico-medullary demarcation, epithelial cells from *Aire*^{null} mice present a striking reduction in the number of mRNA transcripts in comparison to cells of wild-type animals, as assessed by microarray analysis (Anderson *et al.*, 2002). Analysis of the genes that are downregulated in *Aire*^{null} mice showed that the vast majority of them are ectopically expressed transcripts of tissue-specific antigens, including preproinsulin, cythochrome P450 1A2, and many others. This observation prompted the authors to suggest that *Aire* expression by medullary epithelial cells is required to promote antigen presentation by medullary epithelial cells and subsequent negative selection of autoreactive T cells that are generated during TCR rearrangement (Anderson *et al.*, 2002) (Fig. 3).

Matching this observation, Liston and coworkers have demonstrated that *Aire* mutation causes a severe defect in the deletion of organ-specific autoreactive T cell clones giving further support to the role of AIRE in the regulation of central tolerance (Liston *et al.*, 2003).

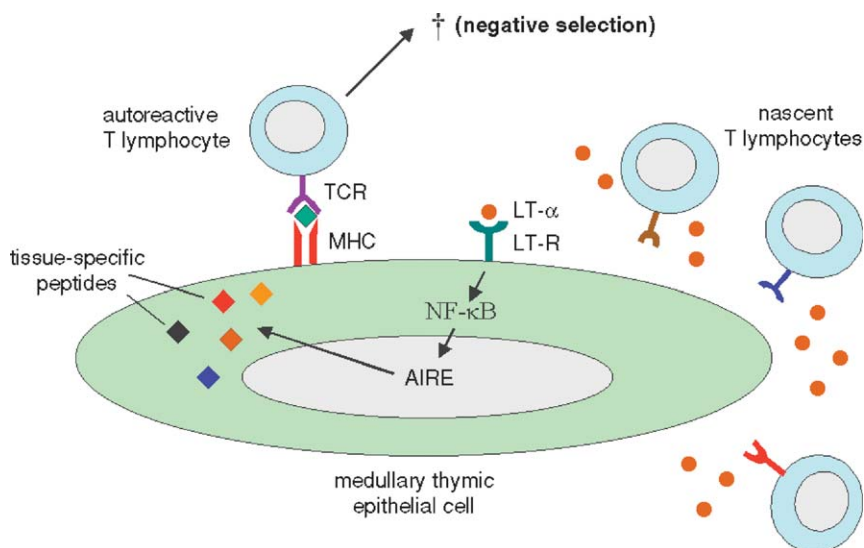


Figure 3 Schematic representation of the role of AIRE in central tolerance. Active thymopoiesis sustains AIRE expression by medullary thymic epithelial cells through a lymphotoxin- α (LT- α)-dependent mechanism that involves activation of the transcription factor NF- κ B. By poorly characterized mechanisms, AIRE induces expression of tissue-specific antigens, which are presented to nascent T cells in the context of major histocompatibility complex (MHC) antigens. Autoreactive T cells, whose T cell receptor (TCR) is specific for the ectopically expressed tissue-specific antigens, are deleted in the thymus.

These observations propose AIRE as an exclusive regulator of lymphocyte selection in the thymus. However, several observations argue in favor of additional factors involved in this phenomenon. In particular, a significant proportion of APECED patients carry mutations of the *AIRE* gene on one allele only, suggesting that other genetic loci influence central tolerance (Buzi *et al.*, 2003; Heino *et al.*, 2001; Wang *et al.*, 1998). There is evidence that the effect of Aire on thymic expression of promiscuous genes is strictly subjected to a gene-dosage effect. Loss of a single copy of *Aire* results in proportional reduction of insulin gene expression in thymic medulla, and increase in the number of autoreactive T cell clones that escape from thymic deletion (Liston *et al.*, 2004). In addition, recent studies have directly suggested that *AIRE* is not the only gene that induces promiscuous gene expression in the thymus. In fact, mature CD80^{bright} medullary epithelial cells express a broader array of promiscuous genes than those known to be regulated by Aire (Derbinski *et al.*, 2005). Furthermore, Derbinski and coworkers have shown that maturation of medullary epithelial results in increased CD80 levels on the cell surface and in

parallel upregulation of more than 300 mRNA transcripts, most of which are not affected by AIRE (Derbinski *et al.*, 2005). Promiscuous genes that are expressed by medullary thymic epithelium present an uneven distribution in the mouse genome and tend to cluster on chromosomes. This has suggested that genomic clustering may be a mechanism by which coordinated regulation of genes expressed during thymocyte selection is achieved (Derbinski *et al.*, 2005; Johnnidis *et al.*, 2005). It is also possible that other “AIRE-like” factors regulate promiscuous gene expression in medullary epithelial cells and participate with AIRE in the mechanism of central tolerance.

In any case, characterization of the critical role played by AIRE in the control of central tolerance has opened the way to a better understanding of other autoimmune disorders, including some associated with PID characterized by defective thymopoiesis.

3. Defective AIRE Expression Explains the Pathophysiology of Autoimmunity in Omenn Syndrome

3.1. Omenn Syndrome: Clinical and Laboratory Features

Omenn syndrome (OS) is a rare and severe form of combined immunodeficiency, characterized by early-onset diffuse erythrodermia, lymphadenopathy, and hepato-splenomegaly (Fig. 4A) (Omenn, 1965). Protracted diarrhea, generalized oedema, failure to thrive, and alopecia are also common findings in OS (Aleman *et al.*, 2001).

Typical laboratory features of OS include hypoprotidemia and hypogammaglobulinemia with increased serum IgE, defective antibody production, and marked eosinophilia. While the total lymphocyte count may vary from low to increased, analysis of lymphocyte subpopulations reveals a variable number of T cells, with absent or very few circulating B lymphocytes. The circulating T lymphocytes from OS patients are autologous (i.e., they do not reflect transuterine passage and expansion of maternally derived T cells in an immunocompromised fetus), and show a highly restricted T cell receptor (TCR) repertoire (Rieux-Laucat *et al.*, 1998; Signorini *et al.*, 1999; Villa *et al.*, 1998). In most cases, a selective expansion of CD4⁺ or—less frequently—of CD8⁺ cells is observed. Although the vast majority of OS patients show circulating T cells carrying the $\alpha\beta$ form of the TCR, accumulation of TCRGD cells has also been described (Brugnoni *et al.*, 1997; de Saint Basile *et al.*, 1991). Occasionally, OS patients present a predominance of TCRAB⁺ CD4[−] CD8[−] cells (Wirt *et al.*, 1989).

In addition, T lymphocytes from OS patients carry activation markers (DR, CD30, CD25), are poorly responsive to mitogens, and tend to infiltrate target

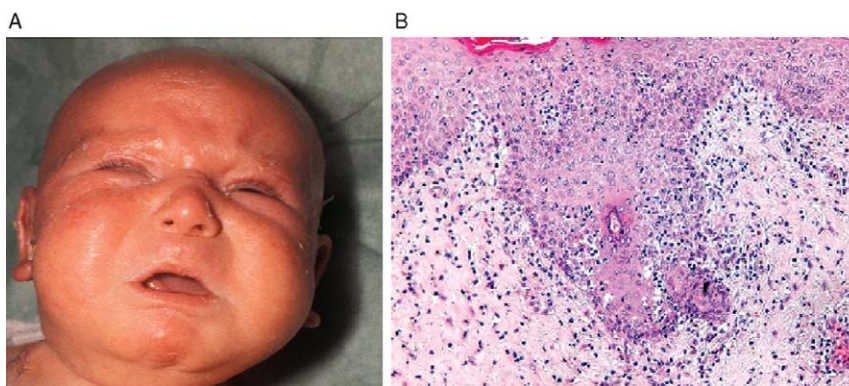


Figure 4 Panel A: Typical features of Omenn syndrome, with diffuse erythrodermia and oedema. Panel B: Skin section for an infant with Omenn syndrome, showing abundant lymphocytic infiltrate in the superficial dermis and in the epidermis, associated with keratinocytes damage and several apoptotic bodies and abundant presence of eosinophils. (Courtesy: Prof. F. Facchetti)

organs (skin, gut, liver, and spleen) (Fig. 4B), thus contributing to the clinical manifestations of the disease (Brugnoni *et al.*, 1997; Rieux-Laucat *et al.*, 1998; Signorini *et al.*, 1999; Villa *et al.*, 1998). The pathological findings observed in patients with OS are very similar to what is observed in some infants with severe combined immune deficiency (SCID) who develop graft-versus-host-like reactions following maternal T cell engraftment (Denianke *et al.*, 2001). Analysis of the profile of cytokine secretion indicates that OS T lymphocytes are skewed towards a T-helper 2 (Th2) phenotype (Chilosi *et al.*, 1996; Schandené *et al.*, 1993), thus accounting for the eosinophilia and the increased serum IgE levels.

Architecture of lymphoid tissues is drastically altered in OS. Lymph nodes show absence of primary follicles and germinal centers, and an expansion of interdigitating reticulum cells in the paracortex (Facchetti *et al.*, 1998; Martin *et al.*, 1995). Lymphoid depletion is also apparent in the thymus, with lack of cortico-medullary demarcation, and a reduced number of CD3⁺ cells (Signorini *et al.*, 1999).

In the absence of hematopoietic stem cell transplantation (HSCT), the disease is rapidly and uniformly fatal within the first few years of life. In a review of OS patients reported in the literature (1965–1999), 69% of the untransplanted infants with OS died within the first year of life (Aleman *et al.*, 2001). Mortality is most often due to overwhelming infections and to metabolic disturbances associated with the severe autoimmune manifestations of the disease. Immune suppression with steroids and/or cyclosporin A has

been shown to be effective in ameliorating the clinical conditions, while waiting for HSCT, which represents at present the only way to provide a definitive cure. We have recently reported an excellent long-term survival (81.8%) in OS infants treated by HSCT, even with the use of matched unrelated donors or of partially-matched related donors (Mazzolari *et al.*, 2005b).

3.2. Molecular Pathophysiology of Omenn Syndrome

The description of a family with OS in one sibling, and SCID with complete absence of T and B lymphocytes (T^BSCID) in another sibling, suggested that OS may be a “leaky” form of T^BSCID (de Saint Basile *et al.*, 1991). In addition, the demonstration of a restricted TCR repertoire in OS, associated with a severe block in B cell differentiation, was indicative of a putative defect in V(D)J recombination (Rieux-Laucat *et al.*, 1998).

This process is initiated by the lymphoid-specific recombinase activating gene (RAG) proteins, which specifically recognize the recombination signal sequences (RSS) that flank the Variable (V), Diversity (D), and Joining (J) gene segments. The RAG proteins introduce a nick in the DNA double-strand between a coding V, (D), or J element and the heptamer of its flanking RSS. The resulting free 3'-OH attacks the phosphodiester bond on the opposite strand, resulting in formation of covalently sealed hairpins at the coding ends. The recently identified Artemis protein (Moshous *et al.*, 2001) is responsible for opening these coding end hairpins, and requires DNA-dependent protein kinase catalytic subunit (DNA-PKcs) for its activity (Ma *et al.*, 2002). The process of DNA repair in V(D)J recombination is then completed by other proteins, including Ku70, Ku80, XRCC4, and DNA ligase IV.

A few years ago, we showed that in most cases OS is due to hypomorphic mutations in the *RAG1* or *RAG2* (Villa *et al.*, 1998). While null mutations in these genes are associated with T^BSCID (Schwarz *et al.*, 1996), hypomorphic mutations in the same genes, which impair, but do not completely abolish, the V(D)J recombination process, may lead to the OS phenotype. Several molecular mechanisms may account for defective (but not abolished) V(D)J recombination associated with hypomorphic mutations in the *RAG* genes detected in patients with OS. In particular, some missense mutations in the RAG1 non-amer binding domain (NBD) impair RSS recognition and cleavage (Villa *et al.*, 1998). Another RAG1 common mutation (R561H) observed in patients with OS affects a region of the protein that mediates interaction with RAG2, whereas the Y912 substitution in the catalytic region is highly inefficient in DNA nicking (Santagata *et al.*, 1999). Another common mutation in patients with OS is represented by a dinucleotide deletion (del nt 368–369) in the

RAG1 gene. This mutation results in usage of a downstream translation initiation site; the resulting N-terminal deleted protein shows improper subcellular localization (Santagata *et al.*, 2000). Among the *RAG2* gene mutations associated with OS, most are located on one side of a predicted six-bladed β -propeller in the active core of the molecule (Callebaut *et al.*, 1998; Gomez *et al.*, 2000); in particular, the mutations associated with OS occur at the borders of the β -propeller β -strands, or within the loops that connect the various β -strands, in regions that are more tolerant to substitutions. Also, these mutants have been shown to retain partial recombinational activity when tested *in vitro* (Gomez *et al.*, 2000).

While these findings of hypomorphic *RAG* mutations in patients with OS have been confirmed by several other groups, the impact of the gene defect onto clinical phenotype has been challenged. In particular, it has been recognized that the same missense mutations in either *RAG1* or *RAG2* genes may lead to different phenotypes, ranging from T-B-SCID to OS, and including atypical cases with some but not all features of OS (Corneo *et al.*, 2001; Villa *et al.*, 2001). These data indicate that the clinical and immunological phenotype of OS is possibly contributed by the following mechanisms, which are not mutually exclusive: (1) defective V(D)J recombination activity, due to *RAG* gene defects; (2) the existence of yet undefined modifier genes that may control expression of the *RAG* genes, or efficiency of the V(D)J recombination process; (3) epigenetic factors, which may affect the function of the *RAG* proteins, or may favor expansion of selected T cell clonotypes.

As mentioned above, one of the typical features of OS is represented by the presence of a detectable number of circulating T lymphocytes, with virtual absence of B cells. While the observation that serum IgE levels are often increased in OS suggests that some B cell maturation occurs (possibly in the bone marrow), these data indicate that the *RAG* gene mutations differently affect the process of V(D)J recombination and cell differentiation in the T vs. B cell lineages. In particular, by studying TCR and immunoglobulin genes rearrangement, Noordzij and coworkers have shown that N-terminal truncated *RAG1* protein can direct TCR but not immunoglobulin gene rearrangements (Noordzij *et al.*, 2000b). The same authors have also found that in most patients with *RAG* defects, the B cell differentiation is arrested at the transition from cytoplasmic Igu⁻ pre-B-I cells to cytoplasmic Igu⁺ pre-B-II cells (Noordzij *et al.*, 2000a). The hypothesis that *RAG* defects exert a stronger constraint onto immunoglobulin than TCR genes rearrangements is also supported by the observation that cross-lineage TCR rearrangements are frequently detected in precursor B cell acute lymphoblastic leukaemia, whereas immunoglobulin gene rearrangements are rare in T cell acute lymphoblastic leukaemia (Szczepanski *et al.*, 1999a,b).

The notion that OS is indeed a syndrome, whose clinical phenotype reflects impaired, but not completely abolished thymopoiesis, has received support through the description of several OS patients in which no defects could be identified in the *RAG1* or *RAG2* genes (Aleman *et al.*, 2001; Gennery *et al.*, 2005; Wada *et al.*, 2000). More recently, one patient was described, in which OS was due to *ARTEMIS* gene mutations (Ege *et al.*, 2005). Interestingly, similar to that observed for null mutations in the *RAG1* and *RAG2* genes, severe mutations in *ARTEMIS* are also associated with a T-B-SCID phenotype (Moshous *et al.*, 2001). Altogether, these data reinforce the notion that hypomorphic mutations in genes involved in V(D)J recombination may allow for residual T cell development, and thus possibly result in OS phenotype.

3.3. Unraveling the Molecular Basis of Immune-Mediated Damage in Omenn Syndrome: Implications for Other Forms of T Cell Immunodeficiency with Autoimmunity

3.3.1. Autoimmunity in Omenn Syndrome is Sustained by Impaired Expression of AIRE and of Tissue-Specific Transcripts in the Thymus

Tissue infiltration by oligoclonal T cells is a hallmark of OS and leads to detrimental clinical consequences that mimic that observed in graft-versus-host disease (erythrodermia, hepato-splenomegaly, abnormalities of gut mucosa and chronic diarrhea, alopecia). During the last years, the mechanisms accounting for the expansion of selected T cell clonotypes in OS have been studied in detail. The notion that the diversity of TCR repertoire among tissue-infiltrating lymphocytes is different in various tissues suggests peripheral expansion of selected clonotypes in response to tissue-restricted (auto)antigens (Signorini *et al.*, 1999). Importantly, preferential usage of the same TCRBV chain has been found in different patients with OS. In particular, oligoclonal expansion of TCRBV14 has been documented in several patients with severe erythrodermia (Pirovano *et al.*, 2003; Rieux-Laucat *et al.*, 1998; Signorini *et al.*, 1999; Villa *et al.*, 1998), suggesting that the same (auto)antigen may elicit peripheral expansion of selected clonotypes in different patients. However, the nature of the antigens that drive this peripheral expansion remains largely unknown.

While the resemblance with clinical manifestations of graft-versus-host disease suggests autoantigen-driven T cell proliferation, the possibility that exogenous antigens may initiate the process should also be considered. In particular, it has been recently reported that among three patients belonging to the same family with typical T-B-SCID due to *RAG2* defect, one infant developed typical features of OS following a parainfluenza 3 virus infection

(Dalal *et al.*, 2005). It remains unclear whether the expansion of oligoclonal T cells in this patient reflected a direct response to the virus, or whether it represented a proliferative response to autoantigens, possibly sustained through a mechanism of molecular mimicry with virus-specific antigens. In any case, this observation illustrates the interaction of genetic (*RAG* defect) and environmental (parainfluenza 3 virus) factors in determining the clinical and immunological phenotype of OS. In particular, it demonstrates that antigens may elicit clonal proliferation of T lymphocytes also in patients with OS.

In view of defective V(D)J recombination, oligoclonal representation of T cells in OS may reflect disturbed and restricted thymopoiesis, in addition to (auto)antigen-driven peripheral expansion. To address this issue, we have analyzed TCR repertoire and the sequence of productively rearranged TCR gene segments in OS thymocytes, and compared these data with similar data obtained on circulating and tissue-infiltrating T cells from the same patients (Signorini *et al.*, 1999). We have been able to show that while thymus from control infants shows a fully polyclonal TCR representation, predominance of few TCR clonotypes is present in the thymus from OS infants. The pattern and sequence of productively rearranged TCR gene segments is different in the thymus vs. other tissues, indicating that thymic oligoclonality does not simply reflect recirculation of expanded clonotypes, but rather is due to inability to generate a polyclonal repertoire. In addition, we have also shown that *RAG* gene defects in OS do not interfere with the process of N and P nucleotide insertions, and that they may cause only a marginal reduction in CDR3 length (Signorini *et al.*, 1999).

The critical role for residual V(D)J recombination activity in allowing production and expansion of selected TCR clonotypes has been recently confirmed by Wada and coworkers (2005). These authors have studied a patient with T-B-SCID due to *RAG1* deficiency in which appearance of somatic second-site mutations, capable of restoring the *RAG1* reading frame, resulted in development of autologous, activated T cells with a highly restricted TCR repertoire, associated with clinical features of OS.

Altogether, these data indicate that the autoimmune manifestations of OS are sustained by (auto)antigen-specific T cell clonotypes, whose production reflects both an intrathymic restriction of TCR repertoire and a peripheral expansion. In spite of this, the mechanisms that account for the accumulation of such clonotypes have long remained obscure. In particular, it was not known whether the autoimmune manifestations of OS are due to altered central or peripheral tolerance. The studies performed on the role of AIRE in central tolerance and on the molecular pathophysiology of APECED have been essential to a better understanding of autoimmunity in OS.

In particular, the notion that AIRE expression in the thymus is strictly influenced by thymocyte differentiation (through secretion of LT- α) and requires a precise spatial and temporal interaction between nascent thymocytes and medullary thymic epithelial cells, has led to the hypothesis that impairment of T cell development (as observed in OS) may cause reduced AIRE expression and poor negative selection of newly generated autoreactive T cells in the thymus. To address this possibility, we have investigated AIRE expression in the thymus derived from controls, patients with OS, and patients with T-B-SCID due to *RAG* gene defects (Cavadini *et al.*, 2005). As shown in Fig. 5, we have confirmed that in humans AIRE expression in the thymus is

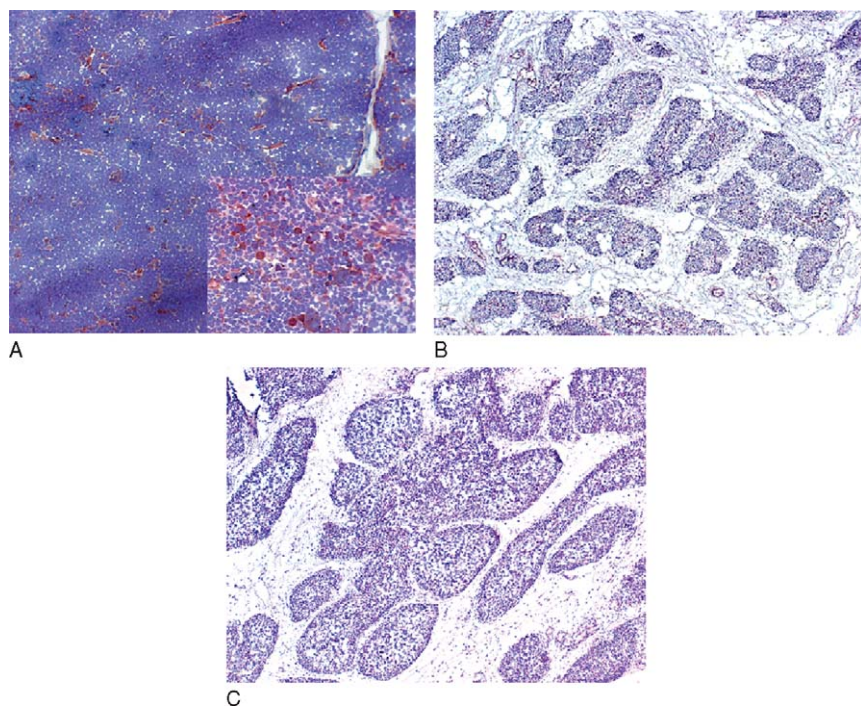


Figure 5 Expression of AIRE in the thymus from a control subject (panel A), infant with Omenn syndrome (panel B), and a patient with T-B-SCID (panel C). Positive staining can be clearly appreciated in the normal thymus, in particular in the medulla, where epithelial cells show cytoplasmic and nuclear expression of AIRE (inset in panel A). Thymi from the Omenn syndrome patient and the infant with T-B-SCID show grossly abnormal architecture, with severe depletion of lymphoid cells, and lack of corticomedullary demarcation. In these thymi, expression of AIRE is absent or negligible.

largely restricted to medullary thymic epithelial cells and to few myeloid dendritic cells. However, AIRE expression is markedly reduced in the thymus derived from OS or from T-B-SCID infants. Based on what was observed in *Aire*^{-/-} mice, we hypothesized and indeed demonstrated that low AIRE expression in the thymus from OS and SCID infants results in markedly reduced expression of tissue-specific transcripts, such as insulin, cytochrome P450 1a2, and fatty acid binding protein (Fig. 6) (Cavadini *et al.*, 2005).

3.3.2. Implications for Other Forms of T Cell Immunodeficiency with Autoimmunity

The data on reduced expression of AIRE and of tissue-specific transcripts in the thymus from patients with OS and with T-B-SCID have important implications for human immunopathology. They offer the first demonstration that, in humans as well as mice, AIRE expression in the thymus is tightly controlled by the efficiency of T cell development. However, they also indicate that regulation of AIRE expression by thymopoiesis occurs differently in mice and humans. In fact, while *RAG* mutations in humans are severely detrimental to AIRE expression, this is not the case in *Rag*^{null} mice (Zuklys *et al.*, 2000).

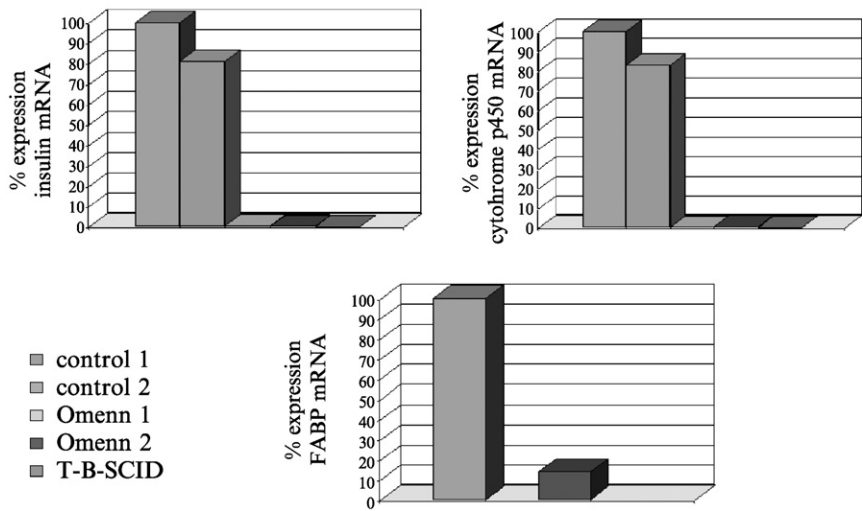


Figure 6 Impaired AIRE expression in the thymus from patients with Omenn syndrome or with T-B-SCID results in defective expression of tissue-specific transcripts. Semi-quantitative evaluation (as measured by RT-PCR) of the levels of transcripts for insulin, cytochrome p450, and fatty acid binding protein (FABP) in thymic extracts from two controls, two infants with Omenn syndrome, and one T-B-SCID infant is shown. Modified from (Cavadini *et al.*, 2005).

Furthermore, our data strongly support the notion that in OS the process of negative selection is impaired, as the result of inadequate expression of tissue-specific self-antigens by medullary thymic epithelial cells. The few T cell clonotypes that may develop in infants with OS, in spite of defective V(D)J recombination, are likely to contain a proportion of autoreactive T cells, which upon maturation and thymic egression, may expand in peripheral tissues, upon contact with the specific autoantigen. The different clinical phenotype (OS vs. T-B-SCID) that may be observed in patients with RAG defects is justified by the residual V(D)J recombination activity detected in patients with OS, that allows for partial T cell development, which is, however, insufficient to sustain AIRE and ectopic transcripts expression, and thus causes impaired deletion of autoreactive T cells. In contrast, the complete block in the process of V(D)J recombination observed in patients with T-B-SCID prevents generation of autoreactive T cells, in spite of an equally defective expression of AIRE and of tissue-specific transcripts.

While these data strongly support the hypothesis that impairment of central tolerance plays a major role in the pathophysiology of OS, additional mechanisms might also be involved. In particular, a dysfunction of regulatory CD4⁺ T cells that express FOXP3 and are of thymic origin might also be postulated. However, we have found that FOXP3 is normally expressed by circulating CD4⁺ T cells derived from a series of patients with OS (Badolato and Notarangelo, unpublished observations).

Importantly, recognition that AIRE expression is reduced in the thymus from patients with OS or with RAG-deficient T-B-SCID suggests that a similar phenomenon may occur in other forms of PID with ineffective thymopoiesis, and contribute to possibly autoimmune manifestations. In particular, autoimmunity may be observed in patients with DiGeorge syndrome, a developmental defect of the thymus, heart, and parathyroid glands, and is more common among patients with pronounced deficiency of T cell development. Some of these patients with so-called “complete” DiGeorge syndrome may develop clinical manifestations that may mimic OS, with generalized erythrodermia and lymphadenopathy (Sullivan, 2003). Also in such cases, reduced thymic output (as detected by a low number of T cell receptor excision circles) was associated with oligoclonal representation of circulating T cells (Markert *et al.*, 2004; Pirovano *et al.*, 2003).

Immune dysregulation, including autoimmune haemolytic anemia, thrombocytopenia, insulin-dependent diabetes mellitus, asthma, and skin rash, has also been reported in patients with combined immune deficiency due to adenosine deaminase deficiency (Notarangelo *et al.*, 1992). Furthermore, autoimmune manifestations are particularly common (up to a third of all cases) among infants with purine nucleoside phosphorylase deficiency (Hershfield, 2004).

Finally, dense lymphocytic infiltration in several target organs (gut, lung, bone, and soft tissues) has been reported in patients with mutations of the *IL2RA* gene, encoding for interleukin-2 receptor α chain (Sharfe *et al.*, 1997). Similar disturbances have been occasionally observed in patients with combined immune deficiency due to defects of the common γ chain (γ_c), of JAK3, and of the interleukin-7 receptor α chain gene (*IL7RA*), who showed evidence of lymphoproliferative disease, or developed oligoclonal and activated T lymphocytes (Frucht *et al.*, 2001; Notarangelo and Giliani, unpublished observation). Although immune dysregulation in these cases may reflect abnormal function of CD4⁺ CD25⁺ regulatory T cells or perturbed peripheral immune homeostasis, the observation of thymic abnormalities (with impaired expression of CD1 on cortical thymocytes) in *IL2RA* deficiency and of profound defect of thymic output in γ_c and JAK3 deficiency suggests that inefficient presentation of self-antigens to autoreactive T cells may contribute to the immunological and clinical phenotype.

4. CD4⁺ CD25⁺ Regulatory T Cells and the Pathophysiology of IPEX (Immunodysregulation – Polyendocrinopathy – Enteropathy – X-linked)

4.1. IPEX Syndrome: Clinical and Laboratory Features

4.1.1. Introduction

IPEX (OMIM: 304930) is a rare X-linked recessive disorder of immune regulation resulting in overwhelming and systemic autoimmunity. The three most common clinical features are early onset insulin-dependent diabetes mellitus, severe watery diarrhea, and dermatitis (Fig. 7A) that typically correlate with elevated titers of serum immunoglobulin E (IgE). Other clinical manifestations that have been described less consistently include thyroiditis, hemolytic anemia, thrombocytopenia, and nephropathy. The disease is rare, but retrospective data on clinical cases of early autoimmune enteritis associated with Type I diabetes, or of neonatal diabetes of unknown origin, suggest that the actual frequency of the disease may be underestimated (Marquis *et al.*, 2002). In the majority of cases, affected males develop symptoms early in infancy and most die prematurely of either metabolic alterations due to intractable diarrhea or severe infections, which occur in some patients prior to the initiation of immune suppressive treatment.

The animal model of IPEX, called *scurfy* mouse, is a natural mutant, described more than 40 years ago. *Scurfy* mice develop a severe lymphoproliferative disorder resulting from an inability of the immune system to properly regulate CD4⁺CD8⁻ T cell activity. In these mice, autoaggressive phenomena and multiorgan infiltration, leading to death in a few weeks, are prominent features.

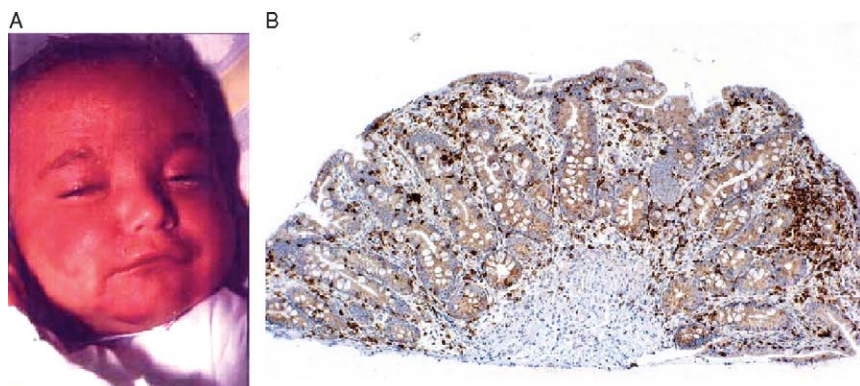


Figure 7 Panel A: Severe psoriasiform erythrodermia in an infant with immunedysregulation – polyendocrinopathy – enteropathy – X-linked (IPEX) syndrome. Panel B: Small-bowel biopsy from an infant with IPEX, showing characteristic villous atrophy and lymphocytic infiltrates. Infiltrating lymphocytes are T cells, as indicated by staining with anti-CD3 antibody. (Courtesy: Prof. F. Facchetti.)

IPEX and *scurfy* are caused by mutations of Forkhead Box P3 (*FOXP3*) gene, located on the X chromosome and encoding for a DNA-binding protein. Previously, adoptive transfer experiments and $CD4^+CD8^-$ T cell subset depletion studies had indicated that Scurfy and IPEX can be the result of immune dysregulation. More recent findings have revealed the crucial role of *FOXP3* for $CD4^+CD25^+$ regulatory T cells (T_{reg}) development and function, and consequently provided the most direct evidence of an alteration of the immune homeostasis in IPEX and its murine orthologue model.

4.1.2. Historical Aspects

An early description of a syndrome similar to IPEX was reported by Meyer and coworkers (1970), who describe two brothers with absent islet cells, neonatal onset type I diabetes, enteropathy, and failure to thrive. Both brothers were affected by several severe infections (including fungal infections, thigh abscess, and discharge from ears) and died early in life. The mother had 10 brothers who had died of unknown causes in infancy, and a healthy sister, suggesting X-linked inheritance. The first detailed report of the complete IPEX phenotype was published by Powell and coworkers (1982), who described an X-linked syndrome in a large kindred in which 19 affected males in five generations experienced similar illnesses with variable onset and severity. In these patients, the disease was mostly characterized by intractable diarrhea, eczema, and endocrine abnormalities associated with various combinations of hemolytic anemia, thrombocytopenia, autoimmune hepatitis, collagen-vascular

disease, and infections. Satake and coworkers (1993) described a Japanese family in which two brothers and their maternal uncle suffered from autoimmune enteropathy with hemolytic anemia and polyendocrinopathy. Two of the boys died from severe diarrhea accompanied by total or subtotal intestinal villous atrophy. The third affected male showed the same symptoms and had circulating antibodies against enterocytes, but his condition improved when treated with immune suppressive drugs. Peake and coworkers (1996) reported the cases of four related males who presented with neonatal diabetes mellitus, intractable diarrhea, and exfoliative dermatitis with extremely high IgE serum levels. These patients died early in life, mainly of severe infections. Additional families were reported by Roberts and Searle (1995) and Di Rocco and Marta (1996). Also in these series, most of the affected males developed autoimmune phenomena within the first six months of life and died within the first year of life.

Although isolated cases with the characteristic phenotype of the IPEX syndrome have been documented over many years, they had not been related to a single disorder nor linked to the *scurfy* mouse syndrome. One of the first suggestions that the human and mouse diseases might represent orthologue models came from linkage analysis which located the responsible locus of each disease to the syntenic pericentromeric region of the X chromosome (Xp11.23 to Xq21.1) (Ferguson *et al.*, 2000; Means *et al.*, 2000). Since the gene encoding the Wiskott-Aldrich syndrome (WAS) protein also lies within this region, it was thought that IPEX could represent a variant of WAS. However, sequence of the WAS protein (WASP) gene was found to be normal in IPEX patients and carrier females (Bennett *et al.*, 2000; Ferguson *et al.*, 2000).

Subsequently, combining genetic and physical mapping with large-scale sequence analysis, Brunkow and coworkers (2000) identified the gene defective in *scurfy* mice, *foxp3*. The consequent association of the human IPEX syndrome with mutations in *FOXP3*, the human orthologue of the mouse gene, proved that these two syndromes are in fact homologous and distinct from Wiskott-Aldrich syndrome (Bennett *et al.*, 2001b; Chatila *et al.*, 2000; Wildin *et al.*, 2001).

4.1.3. Clinical Features of IPEX

In recent years, several IPEX cases have been described (Gambineri *et al.*, 2003; Ochs *et al.*, 2005; Wildin *et al.*, 2002). As more patients were documented, three basic features of symptoms were recognized (Table 4). Enteropathy was found to be present in every case of IPEX. The enteropathy consists of a severe refractory and life-threatening diarrhea causing failure to thrive. The onset typically occurs

Table 4 Clinical Features of IPEX Syndrome

<i>Basic Clinical and Laboratory Features</i>	
Enteropathy	Watery diarrhea (rarely bloody) with villous atrophy Inflammatory bowel disease
Dermatitis	Eczema Erythroderma Psoriasiform dermatitis Alopecia
Endocrinopathy	Type I Diabetes Thyroid abnormalities
Elevated IgE	Usually present
<i>Other Clinical and Laboratory Features</i>	
Hematologic	Coombs (+) hemolytic anemia Autoimmune thrombocytopenia Autoimmune neutropenia
Renal	Nephrosis or nephritis
Hepatic	Autoimmune hepatitis
Lymphadenopathy	Rare
Arthritis / Vasculitis	Rare
Cytokines production	Low IFN- γ
Autoantibodies (Ab)	May be present: AIE-75 (Ab against gut and kidney specific antigen) Anti-nuclear antibodies Antibodies against different organs (thyroid, pancreatic islets, erythrocytes, platelets, smooth muscle)

in early infancy with watery, and sometimes bloody, diarrhea. Characteristic severe villous atrophy and mucosal erosion with lymphocytic infiltrates of the submucosa or lamina propria are observed in small bowel biopsies (Fig. 7B), often leading to an incorrect diagnosis of inflammatory bowel disease. Other common features of the IPEX phenotype, present in nearly all cases, are skin disorders, primarily eczema, but also erythroderma and psoriasiform dermatitis. Alopecia and nail abnormalities have been described in one patient (Nieves *et al.*, 2004). Endocrinopathy is also frequently observed in most but not all patients. The most common endocrine manifestation is early-onset type I diabetes, frequently characterized by a difficult control of glycemia. Thyroid abnormalities include abnormal serum triiodothyronine and/or thyroxine levels, possibly associated with clinical evidence of hypo- or hyperthyroidism. Lymphocytic infiltrates, observed also 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 of abnormal organogenesis.

In addition to these recurrent features, other autoimmune manifestations may occur. Hematologic disorders such as Coombs's positive hemolytic anemia, thrombocytopenia, and neutropenia are frequent. Less common manifestations include tubular nephropathy, hepatitis, arthritis, and/or lymphadenopathy, with or without hepato-splenomegaly (Table 4).

An increased risk of recurrent infections has been observed (Ferguson *et al.*, 2000; Jonas *et al.*, 1991; Kobayashi *et al.*, 1999; Levy-Lahad and Wildin, 2001; Peake *et al.*, 1996; Powell *et al.*, 1982; Roberts and Searle, 1995). It is still unclear whether the increased susceptibility to infections in IPEX patients reflects a primary defect in immune regulation or whether it is the consequence of a barrier defect. Severe enteropathy and eczema may in fact facilitate bacterial entry via the gut and skin. Finally, immunosuppressive therapy used to treat the disorder may also increase the risk of infections. The severity of infections typically varies from moderate infections of the upper respiratory or gastrointestinal tract, to severe and invasive infections such as sepsis, meningitis, and osteomyelitis. The most common infectious organisms are *Enterococcus* and *Staphylococcus* species, *Cytomegalovirus*, and *Candida* (Ochs *et al.*, 2005).

Interestingly, heterozygous females appear to be healthy even though X-inactivation occurs randomly. The X chromosome inactivation analysis performed on one carrier female has demonstrated that both the normal and the mutated *FOXP3* alleles are equally expressed in circulating T lymphocytes (Tommasini *et al.*, 2002), thereby masking the disease by a compensatory mechanism. However, such analysis has been performed on total CD4⁺ cells. No data are available on the pattern of X chromosome inactivation in purified T_{reg} cells.

4.1.4. Laboratory Findings

There is not a significant commonality in the immune aberrations of IPEX patients, and the laboratory findings are not a remarkable aspect of the syndrome. Elevated IgE levels seem to be an important feature, and have been reported in the majority of patients; persistent or periodic eosinophilia has also been commonly described (Wildin *et al.*, 2002). In contrast, IgG, M, and A levels are generally normal, although they may decrease over time due to a progressive loss of protein through the bowel caused by the enteropathy. Most patients generate a variety of autoantibodies against different organs including thyroid, pancreatic islets, erythrocytes, platelets, smooth muscle, and intestine. Autoantibodies to a novel 75 kDa gut and kidney-specific antigen, AIE-75, have been described in IPEX patients (Kobayashi *et al.*, 1998, 1999). Antinuclear antibodies are either absent or present at low titer

(Baud *et al.*, 2001; Powell *et al.*, 1982). The total lymphocyte count and T/B lymphocyte subsets are generally within the normal range (Baud *et al.*, 2001; Di Rocco and Marta, 1996; Ferguson *et al.*, 2000; Jonas *et al.*, 1991; Peake *et al.*, 1996). In one case, reported by Satake and coworkers (1993), CD4⁺ lymphocytes were elevated at birth, and predominantly naïve; activation markers (HLA-DR⁺ and CD25⁺) increased over time, in association with clinical deterioration. The *in vitro* lymphocyte proliferation to mitogens and specific antigens is generally normal in most of the patients. Only one patient with increased responses to mitogens and enhanced IL-2 production has been reported (Shigeoka *et al.*, 1991). In a single family, one affected male had low T cell responses to mitogens, a second showed a 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 (Ferguson *et al.*, 2000), thus revealing a significant variability in the immune defect of IPEX patients even within the same family. Two reports have described increased response of peripheral mononuclear cells to *in vitro* activation, associated with production of high levels of Th2 cytokines and decreased production of the Th1 cytokine interferon- γ (IFN- γ) (Chatila *et al.*, 2000; Nieves *et al.*, 2004; Powell *et al.*, 1982). Patients with IPEX have normal neutrophil function and normal complement levels. However, caution must be used when interpreting data regarding the immunological responses of IPEX patients since most are already on immunosuppressive treatment at the time of immunological evaluation.

4.1.5. Treatment

In order to target T cell activation, the use of immunosuppressive agents such as cyclosporin A and FK506, either alone or in combination with steroids, constitute the best available choice for treatment of IPEX patients (Ferguson *et al.*, 2000; Kobayashi *et al.*, 1995, 2001). Most patients respond at least temporarily to immunosuppressive treatment with steroids and/or cyclosporin A, which may attenuate or delay the clinical course. However, these drugs often fail to sustain long-term remission of symptoms. Use of rapamycin has been recently advocated as a possibly better alternative (Battaglia *et al.*, 2005). In any case, chronic immunosuppression often leads to drug toxicity and increases the chance of severe or opportunistic infections. Use of rituximab (anti-CD20 monoclonal antibody) has been reported in patients where autoantibody-mediated symptoms are predominant.

Supportive therapy with total parenteral nutrition, insulin, and blood transfusion is frequently required. Intravenous immunoglobulins are used in patients with evidence of primary or secondary antibody deficiency.

Up to now, HSCT is the only effective cure for the disease. At least four cases of IPEX patients who received HSCY from an HLA identical sibling have been reported in the literature. One boy received HSCT at four months of age from his HLA-identical sister, who was not a carrier (Baud *et al.*, 2001). Remission began during the conditioning phase that consisted of anti-T-lymphocyte globulin, busulfan, and cyclophosphamide, and continued after the transplant. Enteropathy, type I diabetes, and eczema were all resolved. The patient remained in a complete remission of symptoms for 29 months, despite the fact that only 20–30% of the T cells were of donor origin. Subsequently he developed a rapidly progressive haemophagocytic syndrome and died suddenly when he was nearly three years old. Two other affected males, reported by Wildin and coworkers (2002), received HSCT as a final choice after developing life-threatening complications of long-term immunosuppressant medications. Although both died from infectious complications of their transplants, their symptoms showed marked improvement.

Recently, we performed transplantation of allogenic bone marrow from an HLA identical family member in a one-year-old boy with IPEX who failed therapy with steroids and cyclosporin A (Mazzolari *et al.*, 2005a). The patient achieved a complete clinical and immunological remission of his diseases after transplantation and at 20 months after HSCT is still asymptomatic. Engraftment studies showed the presence of a stable, mixed host/donor chimerism throughout remission indicating that, despite previous unsuccessful attempts, partial chimerism is sufficient to obtain a complete rescue of IPEX, as also shown in a transplantation model in the *scurfy* mouse. This experience confirms that HSCT is so far the only effective and definitive cure for this otherwise fatal disease.

4.2. The Mouse Model for IPEX

“*Scurfy*” is an X-linked recessive mouse mutant which was first described in 1949 and that occurred spontaneously in a partially inbred strain of mice at the Oak Ridge National Laboratory (Russell, 1959). IPEX shares many phenotypic features with the *scurfy* mice. Affected mice tend to be smaller and appear sick early after birth. They present with X-linked recessive inheritance of scaly skin, small-thickened ears, marked runting secondary to diarrhea and malabsorption, progressive anemia, thrombocytopenia, leucocytosis, lymphadenopathy, and hepatosplenomegaly. Scurfy mice typically die by three to four 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 as well as into lymph nodes and spleen, which have abnormal cellular architecture. Previous studies in *scurfy* mice have demonstrated that the clinical phenotype

is the result of immune dysregulation (Godfrey *et al.*, 1991; Lyon *et al.*, 1990). The lymphoproliferative disease is mediated by $CD4^+CD8^-$ T lymphocytes constitutively activated in affected males, as suggested by spontaneous expression of activation markers (CD69, CD25, CD80, and CD86) and by increased levels of cytokine production. Although *scurfy* T cells are hyperresponsive to T cell receptor (TCR) ligation, they still require costimulation through CD28, even if at decreased intensity (Blair *et al.*, 1994; Clark *et al.*, 1999; Kanangat *et al.*, 1996). Interestingly, T cell activation in *scurfy* mice is not downregulated by inhibitors of tyrosine kinases, such as the immune-suppressant cyclosporine A (Clark *et al.*, 1999). Both depletion and adoptive transfer experiments have indicated that $CD4^+$ T cells are primarily responsible for the disease phenotype in mice. Moreover, *Foxp3* overexpression studies carried out on different transgenic mice resulted in reduction of lymphoid organ size as well as of $CD4^+$ T lymphocyte number. $CD4^+$ T cells from these transgenic mice also display decreased proliferative responses and markedly diminished IL-2 production following *in vitro* activation, indicating the crucial role played by *Foxp3* in regulating their ability to respond to TCR-mediated signals (Kasprowicz *et al.*, 2003; Khattri *et al.*, 2001). Recent works in murine models highlighted that *Foxp3* is preferentially expressed in the $CD4^+CD25^+$ T_{reg} cells and that these cells are absent in the *scurfy* mouse, strongly suggesting that the lack of T_{reg} cells is most likely responsible for the defect observed in downregulation of T cell activation (Fontenot *et al.*, 2003; Hori *et al.*, 2003; Khattri *et al.*, 2003).

4.3. Molecular and Cellular Pathophysiology of IPEX

4.3.1. Molecular Aspects of IPEX and Scurfy

Brunkow and coworkers (2001) identified the gene defective in *scurfy* mice. The protein (scurfin) encoded by this gene, designated *Foxp3* (*FOXP3* in humans), is a member of the forkhead/winged-helix family of transcriptional regulators and is highly conserved in humans. Scurfin has several interesting structural features including a proline-rich domain at the N-terminus, a zinc finger, and a leucine zipper, conserved structural motifs involved in protein-protein interactions located within the central portion, and a forkhead DNA-binding domain at the C-terminus embracing a putative nuclear localization signal at the C-terminal portion (Clark *et al.*, 1993; Kaestner, Knochel, and Martinez, 2000; Qian and Costa, 1995). In most members of the FOX family of transcription factors, the forkhead domain is located near the amino terminus, but in *Foxp3* the forkhead domain is uniquely positioned at the C terminus (amino acids residues 337–420) (Brunkow *et al.*, 2001). Proteins bearing

forkhead DNA-binding motifs consist of a large family of related molecules that play diverse roles in enhancing or suppressing transcription from specific binding sites. Several members of this protein family are involved in cell lineage commitment and developmental differentiation. Two other members of Foxp subfamily, Foxp1 and Foxp2, have been shown to act as transcriptional repressors in the lung (Shu *et al.*, 2001). At present there is very little understanding of the function of Foxp3 at the molecular level. Foxp3 binds DNA, localizes to the nucleus, and can act as a transcriptional repressor. In fact, as demonstrated by Khattri and coworkers (2001), the overexpression of scurf in mice leads to a significant repression of the immune function, suggesting a possible role as a repressor of transcription in T cells. Moreover, identification of consensus forkhead binding domains nearby to Nuclear-Factor-of-Activated-T cells (NFAT) transcription factor binding sites in the promoter of several cytokine genes, suggests a possible competition between NFAT and Foxp3 for the control of the expression of genes involved in T cell activation (Schubert *et al.*, 2001). Based on these and other findings, it has also been proposed that Foxp3 is induced in different cell types as a general mechanism of negative immune regulation, through repression of multiple cytokines production. A recent study indicates how Foxp3 interacts with NF- κ B and NFAT protein, and as a consequence, inhibits IL-2, IL-4, and IFN- γ production by CD4⁺ effector T cells (Bettelli *et al.*, 2005). However, so far there is no clear evidence of Foxp3 target genes, and the mechanisms by which it controls transcription of target genes have not been completely characterized.

The human *FOXP3* gene is located at Xp11.23 and consists of 11 translated exons that encode a protein of 431 amino acids 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 shows that FOXP3 is predominantly expressed in CD4⁺CD25⁺ T_{reg} lymphocytes. In mice, *Foxp3* is expressed at low levels in CD4⁺CD25⁻ cells but not in CD8⁺ cells (Bennett *et al.*, 2001b; Hori *et al.*, 2003); human CD8⁺ cells can express FOXP3, although typically at lower levels (Cosmi *et al.*, 2003; Walker *et al.*, 2003).

Identification of the genes responsible for IPEX and for the *scurfy* phenotype has allowed mutation analysis in the patients and in the murine model of the disease.

The spontaneous *scurfy* mutation is the result of two base-pair insertion leading to a frameshift and loss of the entire forkhead domain. Based on this finding, affected members from different families who suffer from a disease resembling IPEX were studied for mutation of the *FOXP3* gene.

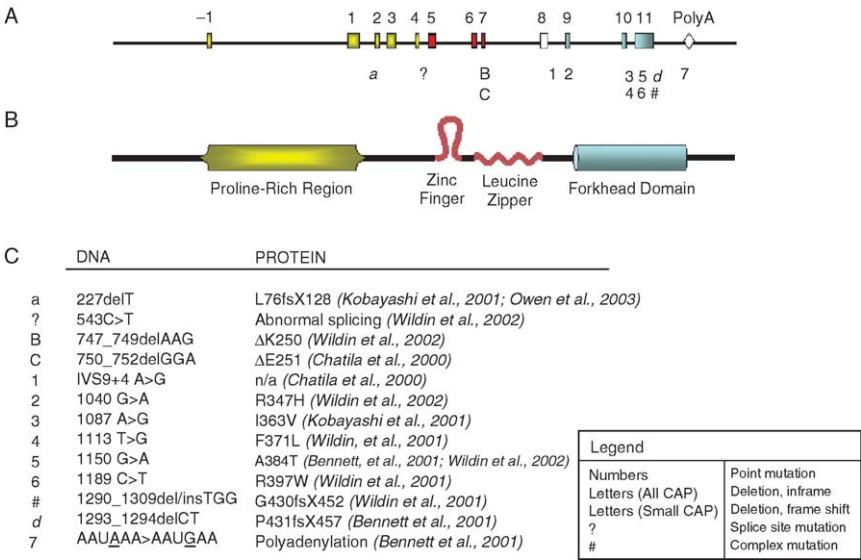


Figure 8 Schematic representation of the *FOXP3* gene, the encoded proteins, and of the mutations identified in patients with IPEX. Panel A: Representation of the *FOXP3* gene. Exons are identified by solid boxes. Locations of the mutations identified in IPEX patients are also shown, and the correspondence between symbols and type of mutations is illustrated in the legend. Panel B: Linear organization of the functional domains of the FOXP3 protein. Panel C: Description of the mutations identified in IPEX patients. For each mutation, the genomic abnormality, and the corresponding effect on protein structure and sequence, are shown, along with appropriate quotations from the literature.

Mutations within the *FOXP3* gene reported to date in different families are shown in Fig. 8. The majority of these are missense mutations within the C-terminal forkhead DNA binding domain, but there are also mutations affecting the leucine zipper and the N-terminal proline-rich domain, demonstrating their importance for FOXP3 function (*Bennett et al., 2001b; Chatila et al., 2000; Gambineri et al., 2003; Kobayashi et al., 2001; Wildin et al., 2001*). In particular, the other mutations reported outside of the forkhead domain include one deletion in exon 2 at nucleotide 227 (*Kobayashi et al., 2001; Owen et al., 2003*), causing frame-shift and early termination; two different deletions of 3-bp each involving nucleotides 747–749 and 750–752 respectively, within exon 7, presumably altering the function of the leucine-zipper domain (*Chatila et al., 2000; Wildin et al., 2002*); an A to G transition at position +4 of intron 8 that causes exon 9 skipping, resulting in frame-shift and early termination (*Chatila et al., 2000*); and two deletions that remove the stop codon and create

products with C-terminal extensions (Bennett *et al.*, 2001b; Wildin *et al.*, 2001). Phenotypically, every patient with one of these *FOXP3* mutations has classic IPEX and the symptomatic differences are unremarkable. Two other mutations, one within an intron/exon splice junction and one in the first polyadenylation signal of the gene, tend to lead to a milder, late-onset form of the disease (Bennett *et al.*, 2001a; Gambineri *et al.*, 2003).

4.3.2. $CD4^+CD25^+$ Regulatory T Cells

Naturally arising $CD4^+CD25^+$ regulatory T cells are a $CD4^+$ T cell subpopulation (5–10% of peripheral $CD4^+$ T cells) that is highly specialized for suppressive function. These cells play a key role not only in maintenance of self-tolerance, but also in the control of aberrant or excessive immune responses to invading or commensal microbes and to innocuous environmental substances (Levings and Roncarolo, 2005). Deficiency or dysfunction of these cells can be a cause of autoimmune or other inflammatory diseases in animals and humans. $CD4^+CD25^+T_{reg}$ cells are produced by normal thymus as a functionally distinct and mature T cell subpopulation and are not induced *de novo* from naïve T cells after antigen exposure in the periphery. $CD4^+CD25^+T_{reg}$ cells constitutively express the CD25 molecule (IL-2 receptor α -chain), and are anergic. However, upon activation, they suppress proliferation and IL-2 production by memory and naïve $CD4^+$ T cells, through a cell-mediated and cytokine-independent mechanism (Sakaguchi, 2004; Sakaguchi *et al.*, 2001). Recent reports indicated that T_{reg} cell-mediated suppression can be overridden by large amounts of IL-2 (Shevach, 2002). It should be noted that the concentration of IL-2 present in the environment is a critical determinant of T_{reg} cell function. In fact, IL-2 is also required for $CD4^+CD25^+ T_{reg}$ cell suppressive activity, since this function can be abrogated in the presence of IL-2 neutralizing antibody (de la Rosa *et al.*, 2004; Thornton *et al.*, 2004). Altogether, these data suggest the possibility of a regulatory network where the function of T_{reg} cells is conditional on IL-2 production by non-regulatory T cells.

Moreover, specific TCR stimulation is necessary for T_{reg} function as shown by studies on TCR-transgenic mice, which develop organ-specific autoimmunity. In addition, T_{reg} cells isolated from organ-draining lymph nodes are more efficient in mediating protection against organ-specific autoimmunity as compared to T_{reg} cells found elsewhere (Fontenot and Rudensky, 2005; Green *et al.*, 2002). Costimulatory signals mediated by CD28 engagement of CD80 and CD86 are also important in the generation of T_{reg} cells in the thymus, and presumably play a role in survival of T_{reg} cells in the periphery. In fact,

CD4⁺CD25⁺ T_{reg} cells are reduced in the absence of CD28–CD80 or CD28–CD86 interaction (Tai *et al.*, 2005).

Recent works in murine models have revealed the specific role of *Foxp3* in development and function of CD4⁺CD25⁺ T_{reg} cells. It has been demonstrated that *Foxp3* is preferentially expressed in CD4⁺CD25⁺ T_{reg} cells and that these cells are absent in the *scurfy* mouse, thus strongly suggesting that the absence of T_{reg} cells is responsible for the lack of downregulation of effector lymphocytes in the *scurfy* mouse. Moreover, murine CD4⁺CD25[−] cells, if activated *in vitro*, do not express *Foxp3* and do not acquire suppressor activity, despite the expression of CD25 on their surface (Fontenot *et al.*, 2003; Hori *et al.*, 2003; Khattri *et al.*, 2003). In contrast, human CD4⁺CD25[−] cells can be activated *in vitro* to express FOXP3 and to suppress T cell proliferation (Walker *et al.*, 2003). The importance of CD4⁺CD25⁺ T_{reg} cells for the maintenance of immune homeostasis has been previously demonstrated by depletion of this subpopulation from healthy mice. These animals spontaneously develop various T cell-mediated autoimmune diseases, such as thyroiditis, gastritis, and type I diabetes. Reconstitution of the depleted mice with CD4⁺CD25⁺ T cells promptly suppresses the development of multi-organ autoimmunity (Sakaguchi *et al.*, 2001). Furthermore, retroviral transduction or transgenic expression studies of *Foxp3* in murine CD4⁺CD25[−] T lymphocytes phenotypically and functionally converts them to T_{reg} cells that are able to suppress proliferation of other T cells *in vitro* as well as to prevent the development of autoimmune diseases *in vivo* (Fontenot *et al.*, 2003; Hori *et al.*, 2003; Khattri *et al.*, 2003). Based on these findings, FOXP3 seems to play a crucial role in the maintenance of the immune homeostasis, by controlling the development and function of CD4⁺CD25⁺ T_{reg} cells. Thus, the *scurfy* mouse and the human disease IPEX constitute a significant model not only to better understand the developmental process and the mechanism of suppression mediated by CD4⁺CD25⁺ T_{reg} cells, but also to elucidate how the function of these cells is modulated.

5. Concluding Remarks

Far from being at the opposite ends of immunopathology, immune deficiency and autoimmunity may actually go hand-in-hand, both being examples of immune dysregulation. Careful analysis of autoimmune manifestations associated with PID has shown that they are heterogeneous in nature, ranging from organ-specific autoimmunity to systemic manifestations. Furthermore, it has been appreciated that the mechanisms involved in autoimmunity associated with PID are also variable, and may be related to defective central or

peripheral tolerance, inability to clear pathogens and extinguish inflammatory reactions, or inadequate removal of immune complexes.

In spite of their rarity, PID continues to represent an extraordinary model to dissect human immunopathology. In particular, the study of two unique forms of PID with associated autoimmunity (APECED and IPEX) has revealed the crucial role played by transcriptional regulators in central and peripheral tolerance. These achievements have not only led to a better understanding of the molecular and cellular mechanisms underlying APECED and IPEX, but have allowed more accurate investigation of the basis of autoimmunity associated with other forms of PID as well. Thus, recognition that active thymopoiesis is essential for normal expression of AIRE and of tissue-specific antigens in the thymus (both critical for induction of central tolerance), has led to the identification that failure in this mechanism is responsible for autoimmunity of Omenn syndrome, a combined immune deficiency with very severe immune dysregulation. It is likely that the same mechanism also applies to other forms of defective T cell development associated with autoimmunity, and complete DiGeorge syndrome in particular.

On the other hand, detailed studies of patients with IPEX and of the murine model (the *scurfy* mouse) have revealed the critical role played by FOXP3, a transcriptional repressor of immune response, required for the generation of CD4⁺CD25⁺ regulatory T cells and peripheral immune homeostasis. Important studies on the murine model indicate that if *Foxp3* is mutated, T_{reg} cells cannot develop, thus causing autoimmune lymphoproliferative phenomena. Thus, IPEX/scurfy provides an ideal model to study immune dysregulation and the role played by regulatory T cells in controlling autoimmune and lymphoproliferative disease development. Further investigation of the structure, function, and regulation of the *FOXP3* gene product will allow a better understanding of its role in generating regulatory T cells and will provide new insights in comprehending the mechanism of immune tolerance. Already at this stage, however, it is possible to envisage the use of T_{reg} cells not only in the treatment of autoimmune diseases, but even to control alloreactivity (Hauben *et al.*, 2005).

Finally, characterization of the molecular and cellular defects that are responsible for these rare monogenic disorders of immunodeficiency associated with autoimmunity has important implications also for a better understanding of the more common autoimmune disorders, since they indicate two crucial steps (central tolerance and peripheral immune homeostasis), whose abnormalities may contribute to the multifactorial nature of most autoimmune diseases.

Acknowledgments

This work was partially supported by Ministero dell'Istruzione dell'Università e della Ricerca (MIUR)-COFIN 2004, European Union (EUROPOLICY-PID grant, SP23-CT-2005-006411), and MIUR-Fondo per gli Investimenti della Ricerca di Base (FIRB) to L.D.N., by Fondazione Berlucci to LDN, PF2003 from Min-Salute to LDN and RB, Telethon Italia Grant No. GGP0485 to RB and to EG.

References

- Aaltonen, J., Bjorses, P., Sandkuijl, L., Perheentupa, J., and Peltonen, L. (1994). An autosomal locus causing autoimmune disease: Autoimmune polyglandular disease type I assigned to chromosome 21. *Nat. Genet.* **8**, 83–87.
- Ahonen, P., Myllarniemi, S., Sipila, I., and Perheentupa, J. (1990). Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N. Engl. J. Med.* **322**, 1829–1836.
- Aleman, K., Noordzij, J. G., de Groot, R., *et al.* (2001). Reviewing Omenn syndrome. *Eur. J. Pediatr.* **160**, 718–725.
- Anderson, M. S., Venzani, E. S., Klein, L., Chen, Z., Berzins, S. P., Turley, S. J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science* **298**, 1395–1401.
- Arkwright, P. D., Abinun, M., and Cant, A. J. (2002). Autoimmunity in human primary immunodeficiency diseases. *Blood* **99**, 2694–2702.
- Arvanitakis, C., and Knouss, R. F. (1973). Selective hypopituitarism. Impaired cell-mediated immunity and chronic mucocutaneous candidiasis. *JAMA: The Journal of the American Medical Association* **225**, 1492–1495.
- Barton, L. L., Moussa, S. L., Villar, R. G., and Hulett, R. L. (1998). Gastrointestinal complications of chronic granulomatous disease: Case report and literature review. *Clin. Pediatr.* **37**, 231–236.
- Battaglia, M., Stabilini, A., and Roncarolo, M. G. (2005). Rapamycin selectively expands CD4⁺ CD25⁺ FoxP3⁺ regulatory T cells. *Blood* **105**, 4743–4748.
- Baud, O., Goulet, O., Canioni, D., Le Deist, F., Radford, I., Rieu, D., Dupuis-Girod, S., Cerf-Bensussan, N., Cavazzana-Calvo, M., Brousse, N., Fischer, A., and Casanova, J. L. (2001). Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. *N. Engl. J. Med.* **344**, 1758–1762.
- Bennett, C. L., Brunkow, M. E., Ramsdell, F., O'Brian, K. C., Zhu, Q., Fuleihan, R. L., Shigeoka, A. O., Ochs, H. D., and Chance, P. F. (2001a). A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA→AAUGAA) leads to the IPEX syndrome. *Immunogenetics* **53**, 435–439.
- Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., Kelly, T. E., Saulsbury, F. T., Chance, P. F., and Ochs, H. D. (2001b). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* **27**, 20–21.
- Bennett, C. L., Yoshioka, R., Kiyosawa, H., Barker, D. F., Fain, P. R., Shigeoka, A. O., and Chance, P. F. (2000). X-Linked syndrome of polyendocrinopathy, immune dysfunction, and diarrhoea maps to Xp11.23-Xq13.3. *Am. J. Hum. Genet.* **66**, 461–468.
- Bettelli, E., Dastrange, M., and Oukka, M. (2005). Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. *Proc. Natl. Acad. Sci. USA* **102**, 5138–5143.

- Betterle, C., Dal Pra, C., Mantero, F., and Zanchetta, R. (2002). Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: Autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr. Rev.* **23**, 327–364.
- Bjorses, P., Halonen, M., Palvimo, J. J., Kolmer, M., Aaltonen, J., Ellonen, P., Perheentupa, J., Ulmanen, I., and Peltonen, L. (2000a). Mutations in the AIRE gene: Effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am. J. Hum. Genet.* **66**, 378–392.
- Bjorses, P., Halonen, M., Palvimo, J. J., Kolmer, M., Aaltonen, J., Ellonen, P., Perheentupa, J., Ulmanen, I., and Peltonen, L. (2000b). Mutations in the AIRE gene: Effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am. J. Hum. Genet.* **66**, 378–392.
- Blair, P. J., Bultman, S. J., Haas, J. C., Rouse, B. T., Wilkinson, J. E., and Godfrey, V. L. (1994). CD4 +CD8- T cells are the effector cells in disease pathogenesis in the scurfy (sf) mouse. *J. Immunol.* **153**, 3764–3774.
- Boehm, T., Scheu, S., Pfeffer, K., and Bleul, C. C. (2003). Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTbetaR. *J. Exp. Med.* **198**, 757–769.
- Bottomley, M. J., Stier, G., Pennacchini, D., Legube, G., Simon, B., Akhtar, A., Sattler, M., and Musco, G. (2005). NMR structure of the first PHD finger of autoimmune regulator protein (AIRE1). Insights into autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) disease. *J. Biol. Chem.* **280**, 11505–11512.
- Brugnoni, D., Airò, P., Facchetti, F., Blanzuoli, L., Ugazio, A. G., Cattaneo, R., and Notarangelo, L. D. (1997). *In vitro* cell death of activated lymphocytes in Omenn's syndrome. *Eur. J. Immunol.* **27**, 2765–2773.
- Brunkow, M. E., Jeffery, E. W., Hjerrild, K. A., Paepers, B., Clark, L. B., Yasayko, S. A., Wilkinson, J. E., Galas, D., Ziegler, S. F., and Ramsdell, F. (2001). Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* **27**, 68–73.
- Buzi, F., Badolato, R., Mazza, C., Giliani, S., Notarangelo, L. D., Radetti, G., Plebani, A., and Notarangelo, L. D. (2003). Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Syndrome: Time to review diagnostic criteria? *J. Clin. Endocrinol. Metab.* **88**, 3146–3148.
- Callebaut, I., and Mornon, J. P. (1998). The VDJ recombination activating protein rag2 consists of a six-bladed propeller and PHD finger-like domain, as revealed by sequence analysis. *Cell. Mol. Life* **54**, 880–891.
- Candotti, F., Notarangelo, L., Visconti, R., and O'Shea, J. (2002). Molecular aspects of primary immunodeficiencies: Lessons from cytokine and other signaling pathways. *J. Clin. Invest.* **109**, 1261–1269.
- Cavadini, P., Vermi, W., Facchetti, F., Fontana, S., Nagafuchi, S., Mazzolari, E., Sediva, A., Marrella, V., Villa, A., Fischer, A., Notarangelo, L. D., and Badolato, R. (2005). AIRE deficiency in thymus of 2 patients with Omenn syndrome. *J. Clin. Invest.* **115**, 728–732.
- Chatila, T. A., Blaeser, F., Ho, N., Lederman, H. M., Voulgaropoulos, C., Helms, C., and Bowcock, A. M. (2000). JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J. Clin. Invest.* **106**, R75–R81.
- Chilosi, M., Facchetti, F., Notarangelo, L. D., Romagnani, S., Del Prete, G., Almerigogna, F., De Carli, F., and Pizzolo, G. (1996). CD30 cell expression and abnormal soluble CD30 serum accumulation in Omenn's syndrome: Evidence for a T helper-2 mediated condition. *Eur. J. Immunol.* **26**, 329–334.

- Chin, R. K., Lo, J. C., Kim, O., Blink, S. E., Christiansen, P. A., Peterson, P., Wang, Y., Ware, C., and Fu, Y. X. (2003). Lymphotoxin pathway directs thymic Aire expression. *Nat. Immunol.* **4**, 1121–1127.
- Clark, K. L., Halay, E. D., Lai, E., and Burley, S. K. (1993). Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature* **364**, 412–420.
- Clark, L. B., Appleby, M. W., Brunkow, M. E., Wilkinson, J. E., Ziegler, S. F., and Ramsdell, F. (1999). Cellular and molecular characterization of the scurfy mouse mutant. *J. Immunol.* **162**, 2546–2554.
- Clark, R., and Griffiths, G. M. (2003). Lytic granules, secretory lysosomes, and disease. *Curr. Opin. Immunol.* 516–521.
- Clemente, M. G., Meloni, A., Obermayer-Straub, P., Frau, F., Manns, M. P., and De Virgiliis, S. (1998). Two cytochromes P450 are major hepatocellular autoantigens in autoimmune polyglandular syndrome type 1. *Gastroenterology* **114**, 324–328.
- Clemente, M. G., Obermayer-Straub, P., Meloni, A., Strassburg, C. P., Arangino, V., Tukey, R. H., De Virgiliis, S., and Manns, M. P. (1997). Cytochrome P450 1A2 is a hepatic autoantigen in autoimmune polyglandular syndrome type 1. *J. Clin. Endocrinol. Metab.* **82**, 1353–1361.
- Cocco, C., Meloni, A., Boi, F., Pinna, G., Possenti, R., Mariotti, S., and Ferri, G. L. (2005). Median eminence dopaminergic nerve terminals: A novel target in autoimmune polyendocrine syndrome². *J. Clin. Endocrinol. Metab.* **90**, 4108–4111.
- Corneo, B., Moshous, D., Gungor, T., Wulfraat, N., Philippet, F. L., Le Deist, A., Fischer, A., and de Villartay, J. P. (2001). Identical mutations in RAG1 or RAG2 genes leading to defective V(D)J recombinase activity can cause either T-B-severe combined immune deficiency or Omenn syndrome. *Blood* **97**, 2772–2776.
- Cosmi, L., Liotta, F., Lazzeri, E., Francalanci, M., Angeli, R., Mazzinghi, B., Santarlasci, V., Manetti, R., Vanini, V., Romagnani, P., Maggi, E., Romagnani, S., and Annunziato, F. (2003). Human CD8⁺CD25⁺ thymocytes share phenotypic and functional features with CD4⁺CD25⁺ regulatory thymocytes. *Blood* **102**, 4107–4114.
- Dalal, I., Tabori, U., Bielorai, B., Golan, H., Rosenthal, E., Amariglio, N., Rechavi, G., and Toren, A. (2005). Evolution of T-B-SCID into Omenn syndrome phenotype following parainfluenza 3 virus infection. *Clin. Immunol.* **115**, 70–73.
- de la Rosa, M., Rutz, S., Dorninger, H., and Scheffold, A. (2004). Interleukin-2 is essential for CD4⁺CD25⁺ regulatory T cell function. *Eur. J. Immunol.* **34**, 2480–2488.
- Denianke, K. S., Frieden, I. J., Cowan, M. J., Williams, M. L., and McCalmont, T. H. (2001). Cutaneous manifestations of maternal engraftment in patients with severe combined immunodeficiency: A clinicopathological study. *Bone Marrow Transplant.* **28**, 227–233.
- Derbinski, J., Gabler, J., Brors, B., Tierling, S., Jonnakuty, S., Hergenhausen, M., Peltonen, L., Walter, J., and Kyewski, B. (2005). Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels. *J. Exp. Med.* **202**, 33–45.
- de Saint Basile, G., Le Deist, F., de Villartay, J. P., Cerf-Bensussan, N., Journet, O., Brousse, N., Griscelli, C., and Fischer, A. (1991). Restricted heterogeneity of T lymphocytes in combined immunodeficiency with hypereosinophilia (Omenn's syndrome). *J. Clin. Invest.* **87**, 1352–1359.
- Di Rocco, M., and Marta, R. (1996). X linked immune dysregulation, neonatal insulin dependent diabetes, and intractable diarrhoea. *Arch. Dis. Child Fetal Neonatal Ed.* **75**, F144.
- Ege, M., Ma, Y., Manfras, B., Kalwak, K., Lu, H., Lieber, M. R., Schwarz, K., and Pannicke, U. (2005). Omenn syndrome due to ARTEMIS mutations. *Blood* **105**, 4179–4186.
- Ekwall, O., Hedstrand, H., Grimelius, L., Haavik, J., Perheentupa, J., Gustafsson, J., Husebye, E., Kampe, O., and Rorsman, F. (1998). Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* **352**, 279–283.

- Etzioni, A. (2003). Immune deficiency and autoimmunity. *Autoimmunity Reviews* **2**, 364–369.
- Facchetti, F., Blanzuoli, L., Ungari, M., Alebardi, O., and Vermi, W. (1998). Lymph node pathology in primary combined immunodeficiency diseases. *Springer Semin. Immunopathol.* **19**, 459–478.
- Ferguson, P. J., Blanton, S. H., Saulsbury, F. T., McDuffie, M. J., Lemahieu, V., Gastier, J. M., Francke, U., Borowitz, S. M., Sutphen, J. L., and Kelly, T. E. (2000). Manifestations and linkage analysis in X-linked autoimmunity-immunodeficiency syndrome. *Am. J. Med. Genet.* **90**, 390–397.
- Fontenot, J. D., Gavin, M. A., and Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat. Immunol.* **4**, 330–336.
- Fontenot, J. D., and Rudensky, A. Y. (2005). A well adapted regulatory contrivance: Regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat. Immunol.* **6**, 331–337.
- Franzese, A., Valerio, G., Di Maio, S., Iannucci, M. P., Bloise, A., and Tenore, A. (1999). Growth hormone insufficiency in a girl with the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J. Endocrinol. Invest.* **22**, 66–69.
- Frucht, D. M., Gadina, M., Jagadees, G. J., Aksentijevic, I., Takata, K., Blessing, J., Nelson, J., Muul, L. M., Perham, T. G. M., Morgan, G., Gerritsne, E. J. A., Schumacher, R. F., Mella, P., Veys, P., Fleisher, T. A., Kaminsky, E. R., Notarangelo, L. D., O'Shea, J. J., and Candotti, F. (2001). Unexpected and variable phenotypes in a family with JAK3 deficiency. *Genes Immun.* **2**, 422–432.
- Gambineri, E., Torgerson, T. R., and Ochs, H. D. (2003). 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. *Curr. Opin. Rheumatol.* **15**, 430–435.
- Gennery, A. R., Hodges, E., Williams, A. P., Harris, S., Villa, A., Angus, B., Cant, A. J., and Smith, J. L. (2005). Omenn's syndrome occurring in patients without mutations in recombinase activating genes. *Clin. Immunol.* **116**, 246–256.
- Godfrey, V. L., Wilkinson, J. E., and Russell, L. B. (1991). X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. *Am. J. Pathol.* **138**, 1379–1387.
- Gomez, C. A., Ptaszek, L. M., Villa, A., Bozzi, F., Sobacchi, C., Brooks, E. G., Notarangelo, L. D., Spanopoulou, E., Pan, Z. Q., Vezzoni, P., Cortes, P., and Santagata, S. (2000). Mutations in conserved regions of the predicted RAG2 kelch repeats block initiation of V(D)J recombination and result in primary immunodeficiencies. *Mol. Cell. Biol.* **20**, 5653–5664.
- Green, E. A., Choi, Y., and Flavell, R. A. (2002). Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: Highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity* **16**, 183–191.
- Gylling, M., Kaariainen, E., Vaisanen, R., Kerosuo, L., Solin, M. L., Halme, L., Saari, S., Halonen, M., Kampe, O., Perheentupa, J., and Miettinen, A. (2003). The hypoparathyroidism of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy: Protective effect of male sex. *J. Clin. Endocrinol. Metab.* **88**, 4602–4608.
- Gylling, M., Tuomi, T., Bjorses, P., Kontiainen, S., Partanen, J., Christie, M. R., Knip, M., Perheentupa, J., and Miettinen, A. (2000). ss-cell autoantibodies, human leukocyte antigen II alleles, and type 1 diabetes in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J. Clin. Endocrinol. Metab.* **85**, 4434–4440.
- Halonen, M., Eskelin, P., Myhre, A. G., Perheentupa, J., Husebye, E. S., Kampe, O., Rorsman, F., Peltonen, L., Ulmanen, I., and Partanen, J. (2002). AIRE mutations and human leukocyte antigen genotypes as determinants of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy phenotype. *J. Clin. Endocrinol. Metab.* **87**, 2568–2574.

- Halonen, M., Kangas, H., Ruppell, T., Ilmarinen, T., Ollila, J., Kolmer, M., Vihinen, M., Palvimo, J., Saarela, J., Ulmanen, I., and Eskelin, P. (2004). APECED-causing mutations in AIRE reveal the functional domains of the protein. *Hum. Mutat.* **23**, 245–257.
- Halonen, M., Peltto-Huikko, M., Eskelin, P., Peltonen, L., Ulmanen, I., and Kolmer, M. (2001). Subcellular location and expression pattern of autoimmune regulator (Aire), the mouse orthologue for human gene defective in autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). *J. Histochem. Cytochem.* **49**, 197–208.
- Hauben, E., Bacchetta, R., and Roncarolo, M. G. (2005). Utilizing regulatory T cells to control alloreactivity. *Cytotherapy* **7**, 158–165.
- Hedstrand, H., Ekwall, O., Haavik, J., Landgren, E., Betterle, C., Perheentupa, J., Gustafsson, J., Husebye, E., Rorsman, F., and Kampe, O. (2000). Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem. Biophys. Res. Commun.* **267**, 456–461.
- Hedstrand, H., Ekwall, O., Olsson, M. J., Landgren, E., Kemp, E. H., Weetman, A. P., Perheentupa, J., Husebye, E., Gustafsson, J., Betterle, C., Kampe, O., and Rorsman, F. (2001). The transcription factors SOX9 and SOX10 are vitiligo autoantigens in autoimmune polyendocrine syndrome type I. *J. Biol. Chem.* **276**, 35390–35395.
- Heino, M., Peterson, P., Kudoh, J., Nagamine, K., Lagerstedt, A., Ovod, V., Ranki, A., Rantala, I., Nieminen, M., Tuukkanen, J., Scott, H. S., Antonarakis, S. E., Shimizu, N., and Krohn, K. (1999a). Autoimmune regulator is expressed in the cells regulating immune tolerance in thymus medulla. *Biochem. Biophys. Res. Commun.* **257**, 821–825.
- Heino, M., Peterson, P., Kudoh, J., Shimizu, N., Antonarakis, S. E., Scott, H. S., and Krohn, K. (2001). APECED mutations in the autoimmune regulator (AIRE) gene. *Hum. Mutat.* **18**, 205–211.
- Heino, M., Peterson, P., Sillanpaa, N., Guerin, S., Wu, L., Anderson, G., Scott, H. S., Antonarakis, S. E., Kudoh, J., Shimizu, N., Jenkinson, E. J., Naquet, P., and Krohn, K. J. (2000). RNA and protein expression of the murine autoimmune regulator gene (Aire) in normal, RelB-deficient and in NOD mouse. *Eur. J. Immunol.* **30**, 1884–1893.
- Heino, M., Scott, H. S., Chen, Q., Peterson, P., Maebpaa, U., Papasavvas, M. P., Mittaz, L., Barras, C., Rossier, C., Chrousos, G. P., Stratakis, C. A., Nagamine, K., Kudoh, J., Shimizu, N., Maclaren, N., Antonarakis, S. E., and Krohn, K. (1999b). Mutation analyses of North American APS-1 patients. *Hum. Mutat.* **13**, 69–74.
- Hershfield, M. S. (2004). Combined immune deficiencies due to purine enzyme defects. In “Immunologic disorders in infants and children” (E. R. Stiehm, H. D. Ochs, and J. A. Winkelstein, Eds.), 5th Ed. pp. 480–504. W.B. Saunders, Philadelphia.
- Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061.
- Husebye, E. S., Gebre-Medhin, G., Tuomi, T., Perheentupa, J., Landin-Olsson, M., Gustafsson, J., Rorsman, F., and Kampe, O. (1997). Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* **82**, 147–150.
- Irvine, W. J., and Scarth, L. (1969). Antibody to the oxyphil cells of the human parathyroid in idiopathic hypoparathyroidism. *Clin. Exp. Immunol.* **4**, 505–510.
- Johnnidis, J. B., Venanzi, E. S., Taxman, D. J., Ting, J. P., Benoist, C. O., and Mathis, D. J. (2005). Chromosomal clustering of genes controlled by the aire transcription factor. *Proc. Natl. Acad. Sci. USA* **102**, 7233–7238.
- Jonas, M. M., Bell, M. D., Eidson, M. S., Koutouby, R., and Hensley, G. T. (1991). Congenital diabetes mellitus and fatal secretory diarrhea in two infants. *J. Pediatr. Gastroenterol. Nutr.* **13**, 415–425.

- Kaestner, K. H., Knochel, W., and Martinez, D. E. (2000). Unified nomenclature for the winged helix/forkhead transcription factors. *Genes Dev.* **14**, 142–146.
- Kanangat, S., Blair, P., Reddy, R., Daheshia, M., Godfrey, V., Rouse, B. T., and Wilkinson, E. (1996). Disease in the scurfy (sf) mouse is associated with overexpression of cytokine genes. *Eur. J. Immunol.* **26**, 161–165.
- Kasprowicz, D. J., Smallwood, P. S., Tyznik, A. J., and Ziegler, S. F. (2003). Scurfin (FoxP3) controls T-dependent immune responses *in vivo* through regulation of CD4⁺ T cell effector function. *J. Immunol.* **171**, 1216–1223.
- Khatti, R., Cox, T., Yasayko, S. A., and Ramsdell, F. (2003). An essential role for Scurfin in CD4⁺CD25⁺ T regulatory cells. *Nat. Immunol.* **4**, 337–342.
- Khatti, R., Kasprowicz, D., Cox, T., Mortrud, M., Appleby, M. W., Brunkow, M. E., Ziegler, S. F., and Ramsdell, F. (2001). The amount of scurf protein determines peripheral T cell number and responsiveness. *J. Immunol.* **167**, 6312–6320.
- Kobayashi, I., Imamura, K., Kubota, M., Ishikawa, S., Yamada, M., Tonoki, H., Okano, M., Storch, W. B., Moriuchi, T., Sakiyama, Y., and Kobayashi, K. (1999). Identification of an autoimmune enteropathy-related 75-kilodalton antigen. *Gastroenterology* **117**, 823–830.
- Kobayashi, I., Imamura, K., Yamada, M., Okano, M., Yara, A., Ikema, S., and Ishikawa, N. (1998). A 75-kD autoantigen recognized by sera from patients with X-linked autoimmune enteropathy associated with nephropathy. *Clin. Exp. Immunol.* **111**, 527–531.
- Kobayashi, I., Kawamura, N., and Okano, M. (2001). A long-term survivor with the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *N. Engl. J. Med.* **345**, 999–1000.
- Kobayashi, I., Nakanishi, M., Okano, M., Sakiyama, Y., and Matsumoto, S. (1995). Combination therapy with tacrolimus and betamethasone for a patient with X-linked auto-immune enteropathy. *Eur. J. Pediatr.* **154**, 594–595.
- Kobayashi, I., Shiari, R., Yamada, M., Kawamura, N., Okano, M., Yara, A., Iguchi, A., Ishikawa, N., Ariga, T., Sakiyama, Y., Ochs, H. D., and Kobayashi, K. (2001). Novel mutations of FOXP3 in two Japanese patients with immune dysregulation, polyendocrinopathy, enteropathy, X linked syndrome (IPEX). *J. Med. Genet.* **38**, 874–876.
- Kogawa, K., Nagafuchi, S., Katsuta, H., Kudoh, J., Tamiya, S., Sakai, Y., Shimizu, N., and Harada, M. (2002). Expression of AIRE gene in peripheral monocyte/dendritic cell lineage. *Immunol. Lett.* **80**, 195–198.
- Kumar, P. G., Laloraya, M., Wang, C. Y., Ruan, Q. G., Davoodi-Semiromi, A., Kao, K. J., and She, J. X. (2001). The autoimmune regulator (AIRE) is a DNA-binding protein. *J. Biol. Chem.* **276**, 41357–41364.
- Kuroda, N., Mitani, T., Takeda, N., Ishimaru, N., Arakaki, R., Hayashi, Y., Bando, Y., Izumi, K., Takahashi, T., Nomura, T., Sakaguchi, S., Ueno, T., Takahama, Y., Uchida, D., Sun, S., Kajiura, F., Mouri, Y., Han, H., Matsushima, A., Yamada, G., and Matsumoto, M. (2005). Development of autoimmunity against transcriptionally unrepressed target antigen in the thymus of Aire-deficient mice. *J. Immunol.* **174**, 1862–1870.
- Levings, M. K., and Roncarolo, M. G. (2005). Phenotypic and functional differences between human CD4⁺CD25⁺ and type 1 regulatory T cells. *Curr. Top. Microbiol. Immunol.* **293**, 303–326.
- Levy-Lahad, E., and Wildin, R. S. (2001). Neonatal diabetes mellitus, enteropathy, thrombocytopenia, and endocrinopathy: Further evidence for an X-linked lethal syndrome. *J. Pediatr.* **138**, 577–580.
- Li, Y., Song, Y.-H., Rais, N., Connor, E., Schatz, D., Muir, A., and Maclaren, N. (1996). Auto-antibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *J. Clin. Invest.* **97**, 910–914.

- Liston, A., Gray, D. H. D., Lesage, S., Fletcher, A. L., Wilson, J., Webster, K. E., Scott, H. S., Boyd, R. L., Peltonen, L., and Goodnow, C. C. (2004). Gene dosage-limiting role of *aire* in thymic expression, clonal deletion, and organ-specific autoimmunity. *J. Exp. Med.* **200**, 1015–1026.
- Liston, A., Lesage, S., Wilson, J., Peltonen, L., and Goodnow, C. C. (2003). Aire regulates negative selection of organ-specific T cells. *Nat. Immunol.* **4**, 350–354.
- Lyon, M. F., Peters, J., Glenister, P. H., Ball, S., and Wright, E. (1990). The scurfy mouse mutant has previously unrecognized hematological abnormalities and resembles Wiskott-Aldrich syndrome. *Proc. Natl. Acad. Sci. USA* **87**, 2433–2437.
- Ma, Y., Pannicke, U., Schwarz, K., and Lieber, M. R. (2002). Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in non-homologous end-joining and V(D)J recombination. *Cell* **108**, 781–794.
- Markert, M. L., Alexieff, M. J., Li, J., Sarzotti, M., Ozaki, D. A., Devlin, B. H., Sempowski, G. D., Rhein, M. E., Szabolcs, P., Hale, L. P., Buckley, R. H., Coyne, K. E., Rice, H. E., Mahaffey, S. M., and Skinner, M. A. (2004). Complete DiGeorge syndrome: Development of rash, lymphadenopathy, and oligoclonal T cells in 5 cases. *J. Allergy Clin. Immunol.* **113**, 734–741.
- Marquis, E., Robert, J. J., Bouvattier, C., Bellanne-Chantelot, C., Junien, C., and Diatloff-Zito, C. (2002). Major difference in aetiology and phenotypic abnormalities between transient and permanent neonatal diabetes. *J. Med. Genet.* **39**, 370–374.
- Martin, J. V., Willoughby, P. B., Giusti, V., Price, G., and Cerezo, L. (1995). The lymph node pathology of Omenn's syndrome. *Am. J. Surg. Pathol.* **19**, 1082–1087.
- Mazzolari, E., Forino, C., Fontana, M., D'Ippolito, C., Lanfranchi, A., Gambineri, E., Ochs, H., Badolato, R., and Notarangelo, L. D. (2005a). A new case of IPEX receiving bone marrow transplantation. *Bone Marrow Transplant.* **35**, 1033–1034.
- Mazzolari, E., Moshous, D., Forino, C., DeMartis, D., Offer, C., Lanfranchi, A., Giliani, S., Imberti, L., Pasic, S., Ugazio, A. G., Porta, F., and Notarangelo, L. D. (2005b). Hematopoietic stem cell transplantation in Omenn syndrome: A single-center experience. *Bone Marrow Transplant.* **36**, 107–114.
- Means, G. D., Toy, D. Y., Baum, P. R., and Derry, J. M. (2000). A transcript map of a 2-Mb BAC contig in the proximal portion of the mouse X chromosome and regional mapping of the scurfy mutation. *Genomics* **65**, 213–223.
- Meloni, A., Fiorillo, E., Corda, D., Perniola, R., Cao, A., and Rosatelli, M. C. (2005). Two novel mutations of the AIRE protein affecting its homodimerization properties. *Hum. Mutat.* **25**, 319.
- Meloni, A., Perniola, R., Faa, V., Corvaglia, E., Cao, A., and Rosatelli, M. C. (2002). Delineation of the molecular defects in the AIRE gene in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients from Southern Italy. *J. Clin. Endocrinol. Metab.* **87**, 841–846.
- Moshous, D., Callebaut, I., de Chasseval, R., Corneo, B., Cavazzana-Calvo, M., Le Deist, F., Tezcan, I., Sanal, O., Bertrand, Y., Philippe, N., Fischer, A., and de Villartay, J. P. (2001). Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* **105**, 177–186.
- Nagamine, K., Peterson, P., Scott, H. S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K. J., Lalioti, M. D., Mullis, P. E., Antonarakis, S. E., Kawasaki, K., Asakawa, S., Ito, F., and Shimizu, N. (1997). Positional cloning of the APECED gene. *Nat. Genet.* **17**, 393–398.
- Nieves, D. S., Phipps, R. P., Pollock, S. J., Ochs, H. D., Zhu, Q., Scott, G. A., Ryan, C. K., Kobayashi, I., Rossi, T. M., and Goldsmith, L. A. (2004). Dermatologic and immunologic

- findings in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *Arch. Dermatol.* **140**, 466–472.
- Noordzij, J. G., de Bruin-Versteeg, S., Verkaik, N. S., Vossen, J. M. J. J., de Groot, R., Bernatowska, E., Langerak, A. W., van Gent, D. C., and van Dongen, J. J. M. (2000a). The immunophenotypic and immunogenotypic B cell differentiation arrest in bone marrow of RAG-deficient SCID corresponds to residual recombination activities of mutated RAG proteins. *Blood* **100**, 2145–2152.
- Noordzij, J. G., Verkaik, N. S., Hartwig, N. G., de Groot, R., van Gent, D. C., and van Dongen, J. J. M. (2000b). N-terminal truncated RAG1 proteins can direct T cell receptor but not immunoglobulin gene rearrangements. *Blood* **96**, 203–209.
- Notarangelo, L. D., Stoppoloni, G., Toraldo, R., Mazzolari, E., Coletta, A., Airò, P., Bordignon, C., and Ugazio, A. G. (1992). Insulin-dependent diabetes mellitus and severe atopic dermatitis in a child with adenosine deaminase deficiency. *Eur. J. Pediatr.* **151**, 811–814.
- Notarangelo, L. D., and Hayward, A. R. (2000). X-linked immunodeficiency with hyper-IgM (XHIM). *Clin. Exp. Immunol.* **120**, 399–405.
- Obermayer-Straub, P., Perheentupa, J., Braun, S., Kayser, A., Barut, A., Loges, S., Harms, A., Dalekos, G., Strassburg, C. P., and Manns, M. P. (2001). Hepatic autoantigens in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Gastroenterology* **121**, 668–677.
- Ochs, H. D., Ziegler, S. F., and Torgerson, T. R. (2005). FOXP3 acts as a rheostat of the immune response. *Immunol. Rev.* **203**, 156–164.
- Omenn, G. S. (1965). Familial reticuloendotheliosis with eosinophilia. *N. Engl. J. Med.* **273**, 427–432.
- Owen, C. J., Jennings, C. E., Imrie, H., Lachaux, A., Bridges, N. A., Cheetham, T. D., and Pearce, S. H. (2003). Mutational analysis of the FOXP3 gene and evidence for genetic heterogeneity in the immunodysregulation, polyendocrinopathy, enteropathy syndrome. *J. Clin. Endocrinol. Metab.* **88**, 6034–6039.
- Peake, J. E., McCrossin, R. B., Byrne, G., and Shepherd, R. (1996). X-linked immune dysregulation, neonatal insulin dependent diabetes, and intractable diarrhoea. *Arch. Dis. Child Fetal Neonatal Ed.* **74**, F195–F199.
- Pearce, S. H., Cheetham, T., Imrie, H., Vaidya, B., Barnes, N. D., Bilous, R. W., Carr, D., Meeran, K., Shaw, N. J., Smith, C. S., Toft, A. D., Williams, G., and Kendall-Taylor, P. (1998). A common and recurrent 13-bp deletion in the autoimmune regulator gene in British kindreds with autoimmune polyendocrinopathy type 1. *Am. J. Hum. Genet.* **63**, 1675–1684.
- Perniola, R., Falorni, A., Clemente, M. G., Forini, F., Accogli, E., and Lobreglio, G. (2000). Organ-specific and non-organ-specific autoantibodies in children and young adults with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *Eur. J. Endocrinol.* **143**, 497–503.
- Pirovano, S., Mazzolari, E., Pasic, S., Alberini, A., Notarangelo, L. D., and Imberbti, L. (2003). Impaired thymic output and restricted T cell repertoire in two infants with immunodeficiency and early-onset generalized dermatitis. *Immunol. Lett.* **86**, 93–97.
- Pitkanen, J., Doucas, V., Sternsdorf, T., Nakajima, T., Aratani, S., Jensen, K., Will, H., Vahamurto, P., Ollila, J., Vihinen, M., Scott, H. S., Antonarakis, S. E., Kudoh, J., Shimizu, N., Krohn, K., and Peterson, P. (2000a). The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common coactivator CREB-binding protein. *J. Biol. Chem.* **275**, 16802–16809.
- Pitkanen, J., Vahamurto, P., Krohn, K., and Peterson, P. (2001). Subcellular localization of the autoimmune regulator protein. Characterization of nuclear targeting and transcriptional activation domain. *J. Biol. Chem.* **276**, 19597–19602.

- Pitkanen, J., Doucas, V., Sternsdorf, T., Nakajima, T., Aratani, S., Jensen, K., Will, H., Vahamurto, P., Ollila, J., Vihinen, M., Scott, H. S., Antonarakis, S. E., Kudoh, J., Shimizu, N., Krohn, K., and Peterson, P. (2000b). The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common coactivator CREB-binding protein. *J. Biol. Chem.* **275**, 16802–16809.
- Powell, B. R., Buist, N. R., and Stenzel, P. (1982). An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J. Pediatr.* **100**, 731–737.
- Qian, X., and Costa, R. H. (1995). Analysis of hepatocyte nuclear factor-3 beta protein domains required for transcriptional activation and nuclear targeting. *Nucleic Acids Res.* **23**, 1184–1191.
- Ramsey, C., Winqvist, O., Puhakka, L., Halonen, M., Moro, A., Kampe, O., Eskelin, P., Peltto-Huikko, M., and Peltonen, L. (2002). Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum. Mol. Genet.* **11**, 397–409.
- Rieux-Laucat, F., Bahadoran, P., Brousse, N., Selz, F., Fischer, A., Le Deist, F., and de Villartay, J. P. (1998). Highly restricted human T cell repertoire in peripheral blood and tissue-infiltrating lymphocytes in Omenn's syndrome. *J. Clin. Invest.* **102**, 312–321.
- Rinderle, C., Christensen, H. M., Schweiger, S., Lehrach, H., and Yaspo, M. L. (1999). AIRE encodes a nuclear protein co-localizing with cytoskeletal filaments: Altered subcellular distribution of mutants lacking the PHD zinc fingers. *Hum. Mol. Genet.* **8**, 277–290.
- Roberts, J., and Searle, J. (1995). Neonatal diabetes mellitus associated with severe diarrhea, hyperimmunoglobulin E syndrome, and absence of islets of Langerhans. *Pediatr. Pathol. Lab. Med.* **15**, 477–483.
- Rosatelli, M. C., Meloni, A., Devoto, M., Cao, A., Scott, H. S., Peterson, P., Heino, M., Krohn, K. J., Nagamine, K., Kudoh, J., Shimizu, N., and Antonarakis, S. E. (1998). A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *Hum. Genet.* **103**, 428–434.
- Russell, W. L., Russell, L. B., and Gower, J. S. (1959). Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex chromosome constitution is female. *Proc. Natl. Acad. Sci. USA* **45**, 554–560.
- Sakaguchi, S. (2004). Naturally arising CD4⁺ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* **22**, 531–562.
- Sakaguchi, S., Sakaguchi, N., Shimizu, J., Yamazaki, S., Sakihama, T., Itoh, M., Kuniyasu, Y., Nomura, T., Toda, M., and Takahashi, T. (2001). Immunologic tolerance maintained by CD25⁺ CD4⁺ regulatory T cells: Their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev.* **182**, 18–32.
- Saleem, M. A., Arkwright, P. D., Davies, E. G., Cant, A. J., and Veys, P. (2000). Clinical course of patients with major histocompatibility complex class II deficiency. *Arch. Dis. Child* **83**, 356–359.
- Santagata, S., Besmer, E., Villa, A., Bozzi, F., Allingham, J. S., Sobacchi, C., Haniford, D. B., Vezzoni, P., Nussenzweig, M. C., Pan, Z. Q., and Cortes, P. (1999). The RAG1/RAG2 complex constitutes a 3' flap endonuclease: Implications for junctional diversità in V(D)J and transpositional recombination. *Mol. Cell* **4**, 1–20.
- Santagata, S., Gomez, C. A., Sobacchi, C., Bozzi, F., Abinun, M., Pasic, S., Cortes, P., Vezzoni, P., and Villa, A. (2000). N-terminal RAG1 frameshift mutations in Omenn's syndrome: Internal methionine usage leads to partial V(D)J recombination activity and reveals a fundamental role *in vivo* for the N-terminal domains. *Proc. Natl. Acad. Sci. USA* **19**, 14572–14577.
- Satake, N., Nakanishi, M., Okano, M., Tomizawa, K., Ishizaka, A., Kojima, K., Onodera, M., Ariga, T., Satake, A., Sakiyama, Y., *et al.* (1993). A Japanese family of X-linked auto-immune enteropathy with haemolytic anaemia and polyendocrinopathy. *Eur. J. Pediatr.* **152**, 313–315.
- Schandéné, L., Ferster, A., Mascart-Lemone, F., Crusiaux, A., Gérard, C., Marchant, A., Lybin, M., Velu, T., Sariban, E., and Goldman, M. (1993). T helper 2-like cells and therapeutic effects of

- interferon- γ in combined immunodeficiency with hypereosinophilia (Omenn's syndrome). *Eur. J. Immunol.* **23**, 56–60.
- Schubert, L. A., Jeffery, E., Zhang, Y., Ramsdell, F., and Ziegler, S. F. (2001). Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J. Biol. Chem.* **276**, 37672–37679.
- Schurman, S. H., and Candotti, F. (2003). Autoimmunity in Wiskott-Aldrich syndrome. *Curr. Opin. Rheumatol.* **15**, 446–453.
- Schwarz, K., Gauss, G. H., Ludwig, L., Pannicke, U., Li, Z., Lindner, D., Friedrich, W., Seger, R. A., Hansen-Hagge, T. E., Desiderio, S., and Lieber, M. R. (1996). RAG mutations in human B cell-negative SCID. *Science* **274**, 97–99.
- Scott, H. S., Heino, M., Peterson, P., Mittaz, L., Lalioti, M. D., Betterle, C., Cohen, A., Seri, M., Lerone, M., Romeo, G., Collin, P., Salo, M., Metcalfe, R., Weetman, A., Papasavvas, M. P., Rossier, C., Nagamine, K., Kudoh, J., Shimizu, N., Krohn, K. J., and Antonarakis, S. E. (1998). Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. *Mol. Endocrinol.* **12**, 1112–1119.
- Sharfe, N., Dadi, H. K., Shahar, M., and Roifman, C. M. (1997). Human immune disorder arising from mutation of the α chain of the interleukin-2 receptor. *Proc. Natl. Acad. Sci. USA* **94**, 3168–3171.
- Shevach, E. M. (2002). CD4⁺ CD25⁺ suppressor T cells: More questions than answers. *Nat. Rev. Immunol.* **2**, 389–400.
- Shigeoka, A. O., Araneo, B., Carey, J., and Rallison, M. (1991). An X-linked T cell activation syndrome and response to Cyclosporin A in one affected infant. *Pediatr. Res.* **29**, 163A.
- Shu, W., Yang, H., Zhang, L., Lu, M. M., and Morrissey, E. E. (2001). Characterization of a new subfamily of winged-helix/forkhead (Fox) genes that are expressed in the lung and act as transcriptional repressors. *J. Biol. Chem.* **276**, 27488–27497.
- Signorini, S., Imberti, L., Pirovano, S., Villa, A., Facchetti, F., Ungari, M., Bozzi, F., Albertini, A., Ugazio, A. G., Vezzoni, P., and Notarangelo, L. D. (1999). Intrathymic restriction and peripheral expansion of the T cell repertoire in Omenn syndrome. *Blood* **94**, 3468–3478.
- Soderbergh, A., Myhre, A. G., Ekwall, O., Gebre-Medhin, G., Hedstrand, H., Landgren, E., Miettinen, A., Eskelin, P., Halonen, M., Tuomi, T., Gustafsson, J., Husebye, E. S., Perheentupa, J., Gylling, M., Manns, M. P., Rorsman, F., Kampe, O., and Nilsson, T. (2004). Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab.* **89**, 557–562.
- Sternsdorf, T., Jensen, K., Reich, B., and Will, H. (1999). The nuclear dot protein sp100, characterization of domains necessary for dimerization, subcellular localization, and modification by small ubiquitin-like modifiers. *J. Biol. Chem.* **274**, 12555–12566.
- Szczepanski, T., Beishuizen, A., Pongers-Willemsse, M. J., Hahlen, K., van Wering, E. R., Wijkhuijs, A. G., Tibbe, G. J., de Bruijn, M. A., and van Dongen, J. J. (1999a). Cross-lineage T cell receptor gene rearrangements occur in more than ninety percent of childhood precursor-B acute lymphoblastic leukemias: Alternative PCR targets for detection of minimal residual disease. *Leukemia* **13**, 196–205.
- Szczepanski, T., Pongers-Willemsse, M. J., Langerak, A. W., Harts, W. A., Wijkhuijs, A. J., van Wering, E. R., and van Dongen, J. J. (1999b). Ig heavy chain gene rearrangements in T cell acute lymphoblastic leukaemia exhibit predominant DH6-19 and DH-27 gene usage, can result in complete V-D-J rearrangements, and are rare in T cell receptor alpha beta lineage. *Blood* **93**, 4079–4085.
- Tai, X., Cowan, M., Feigenbaum, L., and Singer, A. (2005). CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. *Nat. Immunol.* **6**, 152–162.

- The Finnish-German APECED Consortium (1997). An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. The Finnish-German APECED Consortium. Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dys trophy. *Nat. Genet.* **17**, 399–403.
- Thornton, A. M., Donovan, E. E., Piccirillo, C. A., and Shevach, E. M. (2004). IL-2 is critically required for the in vitro activation of CD4⁺CD25⁺ T cell suppressor function. *J. Immunol.* **172**, 6519–6523.
- Tommasini, A., Ferrari, S., Moratto, D., Badolato, R., Boniotto, M., Pirulli, D., Notarangelo, L. D., and Andolina, M. (2002). X chromosome inactivation analysis in a female carrier of FOXP3 mutation. *Clin. Exp. Immunol.* **130**, 127–130.
- Uchida, D., Hatakeyama, S., Matsushima, A., Han, H., Ishido, S., Hotta, H., Kudoh, J., Shimizu, N., Doucas, V., Nakayama, K. I., Kuroda, N., and Matsumoto, M. (2004). AIRE functions as an E3 ubiquitin ligase. *J. Exp. Med.* **199**, 167–172.
- Villa, A., Santagata, S., Bozzi, F., Giliani, S., Frattini, A., Imberti, L., Benerini Gatta, L., Ochs, H. D., Schwarz, K., Notarangelo, L. D., Vezzoni, P., and Spanopoulou, E. (1998). Partial V(D)J recombination activity leads to Omenn syndrome. *Cell* **93**, 885–896.
- Villa, A., Sobacchi, C., Notarangelo, L. D., Bozzi, F., Abinun, M., Abrahamsen, T. G., Arkwright, P. D., Baniyash, M., Brooks, E. G., Conley, M. E., Cortes, P., Duse, M., Fasth, A., Filipovich, A. M., Infante, A. J., Jones, A., Mazzolari, E., Muller, S. M., Pasic, S., Rechavi, G., Sacco, M. G., Santagata, S., Schroeder, M. L., Seger, R., Strina, D., Ugazio, A., Valiaho, J., Vihinen, M., Vogler, L. B., Ochs, H. D., Vezzoni, P., Friedrich, W., and Schwarz, K. (2001). V(D)J recombination defects in lymphocytes due to RAG mutations: Severe immunodeficiency with a spectrum of clinical presentations. *Blood* **97**, 81–88.
- Wada, T., Takei, K., Kudo, M., Shimura, S., Kasahara, Y., Koizumi, S., Kawa-Ha, K., Ishida, Y., Imashuku, S., Seki, H., and Yachie, A. (2000). Characterization of immune function and analysis of RAG gene mutations in Omenn syndrome and related disorders. *Clin. Exp. Immunol.* **119**, 148–155.
- Wada, T., Toma, T., Okamoto, H., Kasahara, Y., Koizumi, S., Agematsu, K., Kimura, H., Shimada, A., Hayashi, Y., Kato, M., and Yachie, A. (2005). Oligoclonal expansion of T lymphocytes with multiple second-site mutations leads to Omenn syndrome in a patient with RAG1-deficient severe combined immunodeficiency. *Blood* **106**, 2099–2101.
- Walker, M. R., Kasprovicz, D. J., Gersuk, V. H., Benard, A., Van Landeghen, M., Buckner, J. H., and Ziegler, S. F. (2003). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4⁺CD25⁺ T cells. *J. Clin. Invest.* **112**, 1437–1443.
- Walport, M. J., Davies, K. A., Morley, B. J., and Botto, M. (1997). Complement deficiency and autoimmunity. *Ann. N. Y. Acad. Sci.* **815**, 267–281.
- Wang, C. Y., Davoodi-Semiromi, A., Huang, W., Connor, E., Shi, J. D., and She, J. X. (1998). Characterization of mutations in patients with autoimmune polyglandular syndrome type 1 (APS1). *Hum. Genet.* **103**, 681–685.
- Ward, L., Paquette, J., Seidman, E., Huot, C., Alvarez, F., Crock, P., Delvin, E., Kampe, O., and Deal, C. (1999). Severe autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy in an adolescent girl with a novel AIRE mutation: Response to immunosuppressive therapy. *J. Clin. Endocrinol. Metab.* **84**, 844–852.
- Wildin, R. S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J. L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., Perroni, L., Bricarelli, F. D., Byrne, G., McEuen, M., Prohl, S., Appleby, M., and Brunkow, M. E. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* **27**, 18–20.

- Wildin, R. S., Smyk-Pearson, S., and Filipovich, A. H. (2002). Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J. Med. Genet.* **39**, 537–545.
- Wirt, D. P., Brooks, E. G., Vaidya, S., Klimpel, G. R., Waldmann, T. A., and Goldblum, R. M. (1989). Novel T-lymphocyte population in combined immunodeficiency with features of graft versus host disease. *N. Engl. J. Med.* **321**, 370–374.
- Zlotogora, J., and Shapiro, M. S. (1992). Polyglandular autoimmune syndrome type I among Iranian Jews. *J. Med. Genet.* **29**, 824–826.
- Zuklys, S., Balciunaite, G., Agarwal, A., Fasler-Kan, E., Palmer, E., and Hollander, G. A. (2000). Normal thymic architecture and negative selection are associated with Aire expression, the gene defective in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *J. Immunol.* **165**, 1976–1983.