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Insights into type 1 diabetes from the autoimmune polyendocrine syndromes

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Abstract

Purpose of review: Advances in human genetics and investigations in animal models of autoimmune disease have allowed insight into the basic mechanisms of immunologic tolerance. These advances allow us to understand the pathogenesis of Type 1 diabetes and other autoimmune diseases as never before. Here, we discuss the tolerance mechanisms of the autoimmune polyendocrine syndromes and their relevance to Type 1 diabetes.

Recent findings: Defects in central tolerance with alteration of self-antigen expression levels in the thymus are a potent cause of autoimmunity. Peripheral tolerance defects that alter T cell activation and signaling also play an important role in the pathogenesis of diabetes and other associated autoimmune disorders, with multiple modest defects working in concert to produce disease. Regulation of the immune response through the action of regulatory T cells is a potent mode of tolerance induction in autoimmunity that is important in Type 1 diabetes.

Summary: Rare syndromes of autoimmunity provide a valuable window into the breakdown of tolerance and identify multiple checkpoints that are critical for generation of autoimmunity. Understanding the application of these in Type 1 diabetes will allow the development of future immunomodulatory therapies in the treatment and prevention of disease.

Keywords

AIRE; IPEX; immune tolerance; polyglandular autoimmunity

INTRODUCTION

It has been observed that patients who develop a single autoimmune disease, often go on to develop additional autoimmune disorders. Type 1 diabetes (T1D) is a prime example, with increased incidence of thyroid, adrenal and celiac disease in affected patients [1]. What accounts for this increased susceptibility and what does this tell us about the pathogenesis of autoimmune disorders? Can a single break in tolerance checkpoints allow multiple forms of autoimmune disease or do these signify multiple defects in self-tolerance? Moreover, can this give us insight into mechanisms that can impact how we care for patients with T1D? The answers to these questions can be found in the study of rare syndromes of multiple autoimmunity.

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Autoimmunity arises from a breakdown of immunologic tolerance. Though autoimmune destruction of pancreatic beta-cells is central to T1D, much remains unknown about the causes of disease. Environmental and stochastic factors, such as the stochastic generation of the immune repertoire, play a large role in addition to genetics, as evidenced by twin concordance rates of only 40-50% in T1D [2,3]. To understand the complex etiologies in T1D, we can turn to uncommon extremes of disease that illustrate the key genes and the molecular mechanisms that are critical to the maintenance of immunologic tolerance.

The autoimmune polyendocrine syndromes (APS) comprise a varied group of rare clinical disorders characterized by the autoimmune destruction of several organ systems, with a propensity to target endocrine organs, resulting in functional end-organ insufficiency. Though these syndromes are designated as “polyendocrine,” nonendocrine organs are also affected, and they are alternatively referred to as autoimmune polyglandular syndromes. Notably, three of the syndromes are characterized by a frequent association with T1D: APS type 1 (APS1), APS type 2 (APS2) and Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) disease. Organ-directed autoimmunity in these syndromes is mediated by a destructive T-cell response with generation of organ-specific autoantibodies, similar to the process seen in in T1D. Despite the differences between these disorders, much is known about the etiologies and molecular mechanisms of these syndromes. Because of the severity of the disease phenotypes and the link to known genes, these rare disorders highlight the critical pathways in the breakdown of tolerance leading to autoimmunity [4]. Such insights from these rare syndromes can be applied to the pathophysiology of type 1 diabetes and its clinical care.

An Introduction to Immune Tolerance

Variable rearrangement of antigen-specific receptors allows the generation of a robust and diverse adaptive immune system, but also generates cells that recognize selfantigens. Tolerance to self is maintained through several checks and balances to prevent the escape and activation of auto-aggressive cells that can lead to autoimmune disease (Figure 1). Central tolerance comprises mechanisms within the thymus and bone marrow that help to delete or edit autoreactive T and B cells, respectively, as they develop. Peripheral tolerance consists of mechanisms within the peripheral lymphoid organs or local tissue environments to provide an additional layer of regulation, since deletion mechanisms can be incomplete (reviewed in [5,6]). Cellular ignorance or sequestration, anergy, phenotypic skewing, or activation-induced cell death (AICD) comprise cellintrinsic mechanisms to directly regulate autoreactive T or B cells. Tolerance induction can also be mediated by additional cell subsets like tolerogenic dendritic cells or regulatory T cells that act on autoreactive lymphocytes to suppress their activation. The syndromes we describe below affect key components of these tolerance mechanisms and identify three different linchpins of immune tolerance.

Autoimmune Polyendocrine Syndrome Type 1

APS1 (Autoimmune Polyendocrine Syndrome Type 1, OMIM 240300) is a rare, mongenic, autosomal recessive disorder with about 500 cases reported worldwide. The syndrome typically presents in childhood and is defined clinically by the presence of at least two of

three clinical manifestations: hypoparathyroidism, primary adrenal insufficiency (Addison's disease) and mucocutaneous candidiasis. Additionally, a variable array of other organ-based diseases can develop in affected individuals over time, including involvement of the thyroid, lung, liver, skin, and gonads (see Table 1), due to autoimmune-mediated tissue damage [7,8]. Disease presentation is quite pleiomorphic with variability in the autoimmune disorders seen among affected siblings as well as differences in timing and severity of disease (reviewed in [9,10]).

Despite the low incidence in the general population, APS1 occurs with increased frequency in certain ethnic groups, including Iranian Jewish, Sardinian, Finnish, Norwegian, and Irish populations. Based on the pattern of inheritance in a large cohort of Finnish patients, a massive positional cloning effort was successful in identifying the *Autoimmune Regulator* (*AIRE*) gene identified as a the causative defect in APS1 [11,12].

Mechanisms of disease: Thymic Expression of Self-Antigen is Key for Central Tolerance—The *AIRE* gene encodes a putative transcriptional regulator based on its structural similarity to other DNA binding transcription factors [13], though notably Aire is not thought to directly bind DNA. Unlocking the mechanism for the variable spectrum of autoimmune diseases in this syndrome came from critical work in animal models following identification of the gene in humans. Inactivation of the *Aire* gene in mice gives rise to a remarkably similar syndrome of multi-organ autoimmunity with lymphocytic infiltrates and tissue-specific autoantibodies to several sites shared with humans, including the stomach, liver, pancreas, adrenal and thyroid [14-16]. Further analysis of the phenotype in mice revealed that the immunologic breakdown of tolerance mapped to the thymus [14].

Aire is most highly expressed within the thymus, primarily in specialized medullary thymic epithelial cells (mTECs) that are the chief mediators of central thymic tolerance [14]. In addition to its expression in the thymus, there has also been detection of Aire expression in the lymphoid organs in rare cells termed extrathymic Aire-expressing cells (eTACs), however, the exact contribution of eTACs to tolerance remains controversial [17,18]. Central tolerance is established in the thymus through the "promiscuous" or ectopic expression of thousands of tissue-specific self-antigens (TSAs) that are normally found only in peripheral tissues [19,20]. As T cells develop in the thymus, they encounter self-antigens presented on the surface of mTECs in the context of MHC Class II. T cells that recognize self-antigen are triggered to undergo cell death and are deleted in a process termed negative selection, the primary mode of deletional tolerance within the thymus. Based on the expression of Aire in mTECs, investigators hypothesized that Aire functions to promote the expression of TSAs. Microarray analysis of purified mTECs showed that hundreds of TSAs were dependent upon Aire for their expression, including known autoantigens like insulin, P450scc, and the acetylcholine receptor [14,21]. Thus, with the loss of Aire, a host of tissue-specific self-antigens are no longer expressed in mTECs, allowing autoreactive T cells to escape negative selection and exit to the periphery with ensuing autoimmunity [22-24]. Thus, the array of autoimmunity seen in APS1 reflects the TSAs that are dependent upon Aire for their expression. Notably, Aire is not the only regulator of TSAs since several self-antigens like GAD65 and thyroglobulin were found to be Aire-independent, implying the existence of other regulatory factors that modulate different subsets of TSAs.

The expression of self-proteins within the thymus as mechanism for tolerance induction has been noted previously for various self-antigens, especially in the case of T1D. One of the major genetic susceptibility in T1D maps to the variable nucleotide tandem repeat (VNTR) region upstream of the *insulin* gene [25], with increased risk seen in alleles with shorter repeat lengths and a protective effect with longer repeat alleles. Examination of insulin expression within the thymus showed that increased expression was seen with long-repeat VNTR alleles, suggesting that increased thymic antigen expression could promote tolerance and explain the protective nature of this genetic variant [26]. Examples such as this and the dramatic loss of tolerance in the absence of Aire suggest that significant tolerance induction in T1D depends upon regulating the expression of islet-specific antigens like insulin within the thymus and that defects in central tolerance are likely to be part of the pathogenesis of T1D in humans.

Autoimmune Polyendocrine Syndrome Type 2

With a prevalence of 1 in 100,000, APS2 represents the most common of the polyendocrine syndromes and is estimated to be 3 times more common than APS1. In contrast to APS1, APS2 presents in adulthood and shows a female predominance of 3:1 [27,28]. APS2 has gone by several names based on the manifestations in earlier historical descriptions (Schmidt's syndrome, Carpenter's syndrome) and some systems that split the disease into further subtypes. Generally, a more broad, categorical approach has become accepted in including such earlier subdivisions under the umbrella of "APS2." APS2 is diagnosed by the presence of two of three autoimmune disorders: Addison's disease, autoimmune thyroid disease, and T1D. The most prevalent of these is Addison's disease, which is found in upwards of 70% of APS2 patients [27,28]. As in APS1, multiple other organ-specific autoimmune disorders are also seen in APS2 (Table 1) with variable penetrance, including celiac disease, pernicious anemia, vitiligo, and hypophysitis.

The syndrome shows a familial basis but is a multigenic disorder with a complex inheritance pattern. Several genes have been implicated in contributing to disease risk (Table 1), chief among these being the *HLA* locus, as is seen in many autoimmune disorders. Several *HLA* haplotypes are associated with APS2 with DR3 haplotypes being the most common. Other non-*HLA*-linked genes have been implicated through their risk association with individual disease states within the syndrome (see Table 1). Some of these, like *CTLA-4* and *PTPN22*, have been seen in association with all three major components of APS2 ([29-36], while others, like the *IL-2Rα*, have only been identified in association with T1D [37-39]. The influence of HLA on genetic risk and the lack of GWA studies in large cohorts of APS2 patients has limited the ability to further define significant causative genes in this syndrome. Much remains to be learned about the genetic determinants of APS2, but clues from the known genes associated with this syndrome suggest that multiple defects in peripheral tolerance contribute to disease pathogenesis in a combinatorial fashion.

Mechanisms of disease: Combinatorial Defects in Peripheral Tolerance—The genes implicated in the development of APS2 point to the breakdown of peripheral tolerance mechanisms as important in this syndrome. Association with *HLA* haplotypes indicates that antigen recognition and T cell activation are intimately connected to the loss of immune

tolerance; this has been noted in several autoimmune disorders and is not surprising since HLA-restricted antigen presentation determines how T cells see the antigenic world. For example, whereas the *DRB1*0401* haplotype is associated with isolated T1D, the *DRB1*0404* haplotype is seen with both T1D and Addison's disease, likely due to the preferential ability of this molecule to efficiently present epitopes of the adrenal 21-hydroxylase autoantigen [40].

Non-*HLA* genes associated with APS2 highlight the importance of several mechanisms in immune tolerance. Peripheral tolerance, which includes checkpoints in the peripheral tissues and lymphoid organ, is maintained on several levels through the inactivation (anergy), sequestering, death, or suppression of effector T cells [5,6]. T cell activation is tightly regulated can be modulated at several levels, including access to antigen, strength of activation through the T cell receptor (TCR), as well as via positive and negative co-receptor modulation of TCR signaling. *CTLA-4* and *PTPN-22* are two examples of genes implicated in T cell signaling that have been linked to multiple autoimmune diseases. CTLA-4 is a co-receptor on T cells that acts as a negative regulator of T cell signaling. Variants in the 3' noncoding region of the gene have been associated with APS2 disorders and result in lower expression levels of this negative co-stimulatory molecule with reduction in T cell activation and proliferation [31]. PTPN22 is a protein tyrosine phosphatase acts downstream of the T cell and B cell receptors to modulate signal strength. A single C1858T variant is linked to risk for several autoimmune disorders (reviewed in [36]) and causes a gain-of-function mutation in the molecule to impact T cell and B cell signaling ([41-43].

Additionally, cell-extrinsic mechanisms, such as growth factor (IL-2) deprivation and suppression by regulatory T cells, can also enforce tolerance through their action on effector T cells. Variants in the *IL-2Rα* are linked to risk in both T1D and Graves' disease ([37], Brand 2007) and thought to play an important role in T cell homeostasis and in the generation of regulatory T cells that are highly IL-2 dependent [44-47]. The genetic associations are supported by functional studies of peripheral blood lymphocytes of APS2 patients. $CD4^+CD25^+$ regulatory T cells from affected patients seem to show a defect in suppressive function despite lack of differences in their frequency or expression level of marker such as CD25 (the IL-2Rα) [48]. Moreover, T cells from APS2 subjects express decreased levels of caspase-3 and exhibited defective activation-induced cell death, suggesting that defective regulation of T cell death may contribute to disease generation [49]. Much work remains to be done to further characterize the molecular contributions of individual risk genes in APS2, but the genetics illustrates how multiple hits to immune tolerance culminate in organ-based autoimmunity.

Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked disease

IPEX (Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked disease, OMIM 304790) is an extremely rare X-linked recessive disorder first described by Powell in 1982[50]. Classically, onset of disease is seen infancy, and the syndrome is often fatal within the first two years of life unless aggressively treated. IPEX disease is characterized by a triad of enteropathy, Type 1 diabetes and skin disease, as well as variable association with several other autoimmune disorders, including autoimmune thyroid disease, hemoloytic

anemia, thrombocytopenia, hepatitis and nephritis (Table 1). Early onset of Type 1 diabetes, often at birth, is almost pathognomonic for the syndrome, as diabetes is found in 75-80% of IPEX patients [51]. Aside from supportive or replacement treatment for the individual disorders, at this time the mainstay of therapy in IPEX consists primarily of immunosuppression and hematopoietic stem cell transplant. Bone marrow transplantation is life-saving and can be curative with remission of autoimmunity [52].

In 2001, the *FOXP3* gene was identified as the causative gene in IPEX [53,54]. Insight into the identification of the causative gene came from parallel studies of the *Scurfy* mouse mutant strain that had a remarkably similar phenotype to IPEX patients that also arose from mutations in the *Foxp3* gene [55]. Since the identification of *FOXP3*, less severe and late-onset presentations of the syndrome have also described due to the availability of genetic testing [56]. Notably, recent clinical reports have also described cases of clinical IPEX disease without detectable mutations in *FOXP3* [57-59] suggesting a role for other genes that act in concert with *FOXP3*. Indeed, one of these cases harbored mutations in the IL-2Rα subunit (CD25), a critical growth factor for the survival and stability of Tregs, illustrating the central role of Tregs in this syndrome [57].

Mechanisms of disease: Regulatory T cells are Critical Mediators of Tolerance —*FOXP3* encodes a forkhead-winged helix transcription factor. Studies in the *Scurfy* mouse and other animal models demonstrated that Foxp3 plays a critical role in the function of CD4+CD25+ regulatory T cells (Tregs) [46,60,61]. Mice that lack Foxp3 do not develop Tregs and quickly fall ill due to uncontrolled proliferation and activation of effector T cells. Remarkably, transfer of Tregs in deficient animals is able to completely rescue disease, underscoring the critical role of these cells in enforcing immune tolerance.

Given the critical role of $FoxP^+$ Tregs in maintaining immune tolerance, the study of this specialized cell population has been an area of intense investigation over the last decade that is beyond the scope of this review (reviewed in [62-64]). Of note, it appears that Tregs are a preferentially self-reactive cell that can develop in the thymus or in the immunological periphery after exposure to self-antigen. In addition, it also appears that, at least in animal models, Tregs can suppress and reverse autoimmune diabetes in an antigen-specific fashion [65,66]. These findings demonstrate that the manipulation of Tregs may be an attractive target for the treatment of T1D. Administration of islet-specific Tregs would allow antigen-specific blockade of the autoreactive islet response without the use of global immunosuppression. In fact, there have been recent reports of clinical trials in new onset T1D utilizing *ex vivo* expanded Tregs [67]with other studies in progress.

CONCLUSIONS

Although the autoimmune polyendocrine syndromes are extreme forms of disease, they have been incredibly informative into the underlying pathogenesis of autoimmune disease and the control of immune tolerance. All three syndromes include T1D as a manifestation and highlight the connection between the defective tolerance pathways in these diseases and the susceptibility to T1D. The defect in *AIRE* in APS1 has helped identify the importance of self-antigen expression in the thymus as a mode of immune tolerance to self. This

mechanism also appears to be in play for isolated T1D subjects with normal AIRE function that harbor a risk allele in the VNTR of the *insuli*n gene promoter that leads to lower expression of thymic insulin for the induction of tolerance. Intriguingly, recent work shows that *AIRE* may also contribute to peripheral tolerance through the selection of self-antigen specific Tregs [68]. Building on the rapid advances in the genetics of human disease, the genetics of APS2 highlight the important observation that many autoimmune diseases share common genetic risk variants, such as in *CTLA4* and *PTPN22*. It is important to note that there are likely to be other features of APS2 that have yet to be identified to explain why these patients manifest multiple autoimmune problems. It may be that such patients harbor rare variants in genes that control important immune tolerance pathways; further study using high-throughput sequencing in these subjects could be particularly enlightening in this regard. Finally, defects in *FOXP3* have helped hone in on Tregs as a critical regulator of immune tolerance. An interesting observation in these subjects is how penentrant T1D is in this clinical disorder. This leads to the question as to why? Perhaps, low level underlying autoreactivity to pancreatic beta cells is much more prevalent than previously appreciated in humans. Furthermore, with the increasing use of bone marrow transplantation in the treatment of IPEX and the improved survival of these patients, it will be interesting to see if such an extreme manipulation of the immune system can lead to reversal of T1D in these subjects. Such results could help further inform the treatment of T1D. In conclusion, although subjects with APS are rare, they have been and continue to be a rich resource of information to guide our understanding of T1D and its treatment.

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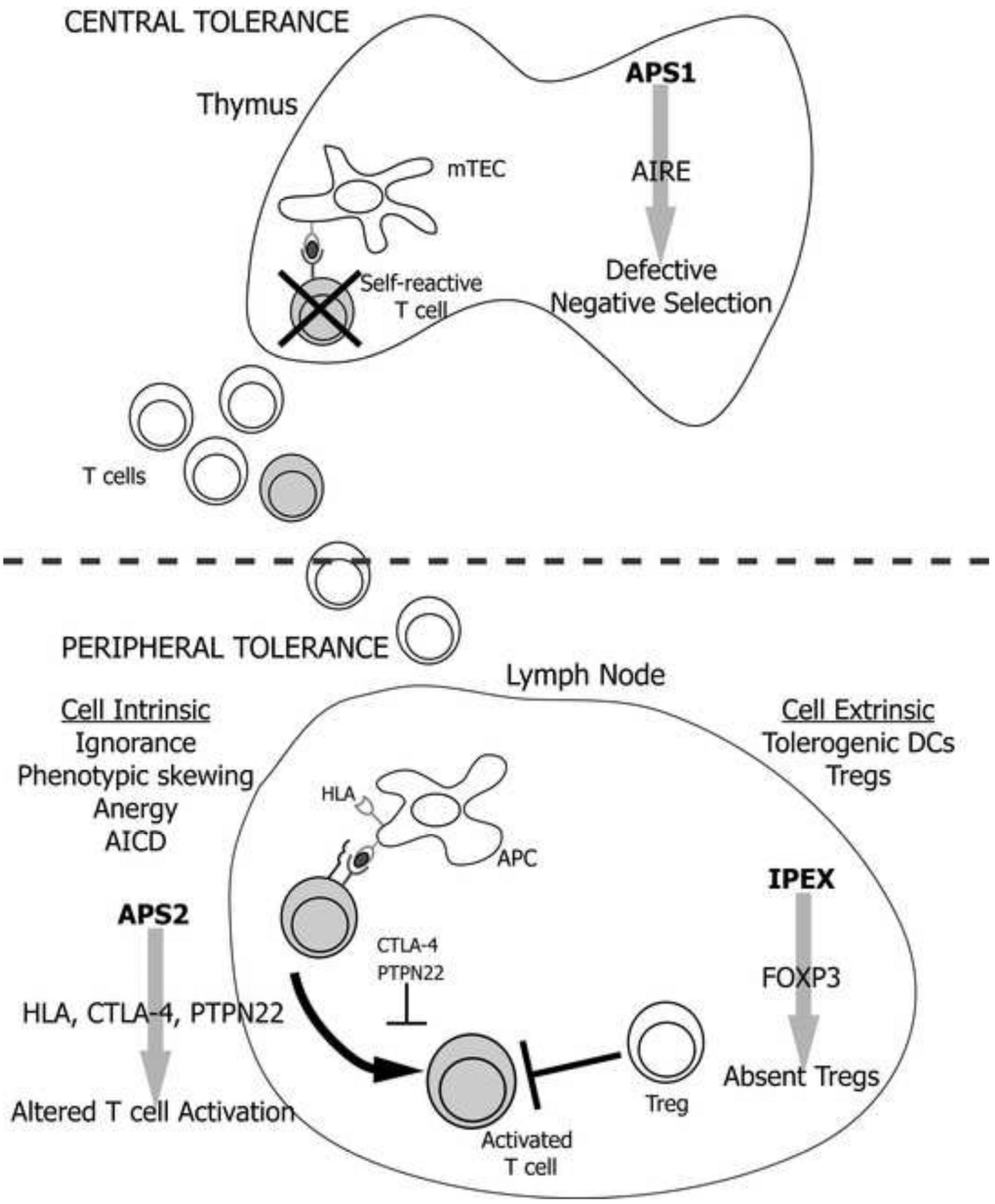

Figure 1. Tolerance Defects in the Autoimmune Polyendocrine Syndromes

A brief overview of the major mechanisms of immunologic tolerance is illustrated. Tolerance can be broken down into central (occurring in the thymus) and peripheral (occurring in the lymph nodes and peripheral organs) mechanisms. Within the thymus, specialized medullary epithelial cells (mTECs) express self-antigens bound to MHC Class II to developing T cells. Autoreactive T cells (shown in gray) are normally deleted in a process termed negative selection. In APS1, defects in the *AIRE* gene lead to loss of expression of many self-antigens in the thymus and result in defective negative selection and, thus, autoimmune disease. Once T cells reach the periphery, autoreactive cells that evade thymic negative selection can also be tolerized through cell intrinsic or cell extrinsic mechanisms.

Cell ignorance, either through sequestration or absence of antigen, prevents autoreactive T cell activation. Upon engaging a self-antigen presented by an antigen-presenting cell (APC), T cells can undergo phenotypic skewing (alteration of their profile to a less pathogenic type), anergy (functional inactivation), or activationinduced cell death (AICD or apoptosis) to preserve tolerance. Multiple risk variants in the *HLA*, *CTLA-4*, *PTPN22*, and likely other yet unidentified genes combine to produce alterations in T cell activation that produce the autoimmunity seen in APS2. Finally, cell extrinsic mechansims involving interactions with tolerogenic dendritic cells or suppression by regulatory T cells (Tregs) are a potent mechanism to induce tolerance in autoreactive T cells. Defects in the *FOXP3* gene lead to loss of Tregs in IPEX patients with ensuing autoimmunity.

Table 1

Key Features of the Autoimmune Polyendocrine Syndromes

Syndrome	APS1	APS2	IPEX
Gene(s)	*AIRE*	*HLA* *CTLA-4* *PTPN22*	*FOXP3*
Tolerance defect	Central: Negative selection	Peripheral: Multiple- T cell activation, Tregs	Peripheral: Tregs
Frequency of T1D	2-33%	40-50%	80%
Major clinical features	Hypoparathyroidism Addison's disease Mucocutaneous candidiasis	Addison's disease T1D Autoimmune thyroid disease	Enteropathy T1D Dermatitis/eczema
Other associated autoimmune disorders	Hepatitis Nephritis Gondal failure (ovarian) Lung disease Alopecia Vitiligo Sjögrens	Celiac disease Pernicious anemia Gondal failure (ovarian) Vitiligo Hypophysitis	Autoimmune thyroid disease Hemolytic anemia Thrombocytopenia Hepatitis Nephritis Lymphadenopathy