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Clues to immune tolerance: The monogenic autoimmune syndromes

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Abstract

Autoimmune disease affects a significant proportion of the population. The etiology of most autoimmune diseases is largely unknown, but it is thought to be multifactorial with both environmental and genetic influences. Rare monogenic autoimmune diseases, however, offer an invaluable window into potential disease mechanisms. In this review, we will discuss the autoimmune polyglandular syndrome (APS1), the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), and autoimmune lymphoproliferative syndrome (ALPS). Significantly, the information gained from the study of these diseases has provided new insights into more common autoimmune disease and have yielded new diagnostics and therapeutic opportunities.

Keywords

IPEX; ALPS; APS1; autoimmunity; immune tolerance

Introduction

Autoimmune disease results from a breakdown in the mechanisms that maintain tolerance to self tissues. The exact cause of most autoimmune diseases is unknown and is thought to be due to a combination of genetic and environmental factors. The multifactorial nature of autoimmune diseases has made it difficult to determine their underlying pathogenesis. Genome-wide association studies have yielded correlations between multiple small nucleotide polymorphisms (SNPs) and autoimmune diseases. However, for most of the associated loci, the signals are not strong enough to directly implicate a specific gene, and the role of the candidate genes in a particular locus can only be speculated.¹ The loci that have been potentially linked with autoimmune diseases involve genes that regulate many aspects of the immune system including lymphocyte activation, microbial recognition, cytokines and cytokines receptors. Moreover, many of these gene variants are associated with multiple autoimmune diseases.² For example, similar major histocompatibility locus (*MHC*) haplotypes have been linked to multiple autoimmune disorders and SNPs in the protein tyrosine phosphatase inhibitor gene, *PTPN22*, have been connected to rheumatoid arthritis, type I diabetes, and Crohn's disease. Although polymorphisms in the *MHC* and *PTPN22* have been linked to autoimmune disease a direct genotype to phenotype correlation is lacking.^{1–2} In contrast, studies with rare monogenic autoimmune diseases have yielded

invaluable insights into the underlying mechanisms of immune tolerance because of their strong penetrance.

Here we highlight a handful of disorders including the autoimmune polyglandular syndrome (APS) type I (OMIM 240300) also known as autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (APECED); the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (OMIM 304790); and autoimmune lymphoproliferative syndrome (ALPS) (OMIM 601859) (Table I). Studies of these disorders have advanced our knowledge of the underlying mechanisms of autoimmunity, and how the breakdown of tolerance can lead to autoimmune disease. In this review, we will cover the clinical manifestations, genetics, diagnosis/treatment and in-depth pathophysiology of these disorders.

Autoimmune polyglandular syndrome type I

APS1 clinical features

APS1 is a rare disease that usually manifests in childhood. The clinical diagnosis of APS1 is based on the presence of at least 2 of 3 major features: mucocutaneous candidiasis (CMC), adrenal insufficiency, or hypoparathyroidism, with the most common presenting symptom being mucocutaneous candidiasis in infancy (Table 1).³ In a Finnish cohort of patients with APS1, 50% of patients had developed CMC by age 5, 70% by age 10, and 94% by age 20.⁴ The infection usually begins in the mouth, but may spread to other regions of the GI tract including the esophagus, vagina, stomach, and intestines. The severity of the mucositis caused by CMC infection varies greatly with some patients having only minor buccal redness and ulcerations to other patients developing esophageal strictures. Patients with CMC are at increased risk of squamous cell carcinoma of the esophagus and require periodic screening.⁵

Hypoparathyroidism is the second most common manifestation of APS1. In a Finnish cohort the prevalence was 33% by age 5, 66% at age 10 and 85% at age 30.⁴ Interestingly, if patients develop adrenal insufficiency prior to hypoparathyroidism they are somewhat protected from subsequently developing hypoparathyroidism.⁶ Clinically, hypoparathyroidism has a wide range of symptoms from muscle cramps and paresthesia to frank seizures secondary to very low serum calcium levels. Adrenal insufficiency is the third most common manifestation with 40% of Finnish patients showing symptoms by age 10 and 78% of patients symptomatic at age 30.⁴ Symptoms of hypoadrenalism include fatigue, salt craving, hypotension, weight loss and increased skin pigmentation and arise due to loss of adrenocortical dysfunction.

APS1 can have multiple other associated autoimmune manifestations including autoimmune hepatitis, type I diabetes, keratitis, autoimmune gonadal failure, pernicious anemia, interstitial lung disease, or celiac disease to name a few.^{3,7} These manifestations can vary widely in APS1 patients and overall show low penetrance. Additionally, patients with APS1 may have enamel dysplasia or other signs of ectodermal dystrophy.⁷⁻⁸ Among patients with APS1 there is great variability in the timing of the disease. In the Finnish cohort, the youngest patient presented at 2 months and the oldest at 18 years, while in a Norwegian cohort, the youngest patient presented at a few months of age and the oldest at 43.^{4,9} To further complicate the diagnosis, some patients will suffer from the less common disease manifestations before presenting with the major disease-defining manifestations.^{4,7} There are reports of APS1 patients manifesting symptoms such as chronic diarrhea, fevers, periodic rash, or autoimmune hepatitis for several years before displaying clinical features of the diagnostic triad.

The heterogeneous nature of disease manifestations and the significant associated morbidity and mortality, make it imperative that APS1 patients are followed closely by experienced providers. Treatment of APS1 depends largely on the organ-specific manifestations. CMC in APS1 is treated with antifungals and replacement therapy is used to treat endocrinopathies, with cortisol and fludrocortisone given for adrenal insufficiency and calcium and vitamin D replacement for hypoparathyroidism.³ Immunosuppression is used for some APS1 disease manifestations such as autoimmune hepatitis, however, immunosuppression is not widely used for all patients with APS1.³

APS1 genetics

APS1 is a rare monogenic disorder with increased frequency in certain populations including Iranian Jews (1:9000), Sardinians (1:14,000), Finns (1:25,000) and Norwegians (1:90,000).⁴⁻⁹⁻¹¹ APS1 exhibits autosomal recessive inheritance with carriers having no consistent phenotype observed to date (see below for further discussion). Because of its monogenic inheritance pattern, classic positional cloning was used to identify the gene defect. In 1997, two groups simultaneously discovered the mutated gene on chromosome 21q22.3 and named it the *Autoimmune Regulator (AIRE)*.¹²⁻¹³ Since its initial discovery more than 60 mutations have been found scattered throughout the gene (Figure 1).¹⁴ Particular mutations are more common in certain populations with the R257X mutation found among 82% of Finnish patients.⁴ The 1094-1106del13 is the most abundant mutation in British, Irish, North American, and Norwegian patients while the Y85C mutation is the most common in Iranian Jews.¹¹⁻¹⁵ Notably, two patients with the exact same disease mutation can have very different clinical manifestations indicating a very low genotypic-phenotypic correlation. It may be that other genes or environmental factors are important in the heterogeneity of the APS1 phenotype within families. In fact, two recent studies have identified the human leukocyte antigen locus (HLA) as a disease modifier in APS1.¹⁶⁻¹⁷

In addition to the autosomal recessive form of APS1, there is also a G228W point mutation found in an Italian family that is responsible for a unique autosomal dominant form of APS1.¹⁸ The clinical manifestations of the dominant form of the disease are different from the recessive form in that most patients develop autoimmune thyroiditis with or without the other clinical characteristics of APS1. In contrast, patients that are heterozygous for other APS1 mutations do not develop the clinical features of APS1, but it remains unclear if they have more subtle phenotypes. For example, one study of heterozygous relatives of patients with APS1 found that approximately 40% of these patients suffered from some other autoimmune manifestation, however, the significance of this is not yet clear.¹⁹

APS1 pathogenesis

Mechanisms of tolerance in the thymus—After discovery that *AIRE* gene mutations were the cause of APS1 in humans, the mouse homologue was identified and inactivated by several groups allowing further insight into Aire function.²⁰⁻²¹ *Aire* deficient mice are similar to humans with APS1 in that they develop multi-organ autoimmunity with inflammatory infiltrates and elevated serum autoantibodies. Studies in mice revealed that Aire is predominantly expressed in the thymus and to a lesser extent in secondary lymphoid organs.²⁰ Within the thymus, Aire is found exclusively in medullary thymic epithelial cells (mTECs).²² Aire localization within the thymus is interesting as the thymus has previously been shown to be the site of negative selection of autoreactive thymocytes.²³ Negative selection is the process by which thymocytes with T cell receptors (TCRs) specific for self-antigens are deleted (Figure 2). Moreover, experiments with mTECs revealed that they express a wide-array of tissue restricted antigens (TRAs) including insulin and thyroglobulin.²² TRAs are proteins whose expression is predominantly found in one tissue and whose expression outside that tissue would be unusual. An example of such a TRA is

insulin whose expression is mainly restricted to the β cells of the pancreatic islet. The finding of insulin expression in mTECs raised the intriguing possibility that these cells were able to express and present TRAs to developing T cells to cause their deletion. In addition, mTECs were found to express the major histocompatibility complex (MHC II) as well as the costimulatory molecule CD80 on their surface giving them features of other professional antigen presenting cells.²² The observation of autoimmunity in *Aire* deficient mice coupled with the predominant expression of Aire in mTECs generated the hypothesis that Aire function may be important in TRA gene expression.

Experimental evidence that Aire is important in TRA gene expression came from microarray profiling of mTECs from *Aire* wildtype and *Aire* deficient mice. The microarray data revealed that thousands of TRAs were under the control of Aire and that these genes were absent in *Aire* deficient mTECs.²⁰ Interestingly, the microarray data also showed that a subset of TRAs, such as CRP and GAD67, were not under Aire control raising the possibility that other Aire-like factors may exist.²⁴ Furthermore, TRAs under the control of Aire are enriched in organ-specific self-antigens, perhaps explaining why APS1 patients develop organ specific autoimmunity.^{25–27} For example, Interphotoreceptor retinoid-binding protein, IRBP, has been shown to be the TRA important in preventing eye infiltration in *Aire* knockout mice. Interestingly, loss of IRBP in the thymus results in eye autoimmunity, indicating that loss of one antigen is sufficient to result in disease.²⁵

The most accepted mechanism by which Aire-induced TRA expression prevents autoimmunity is to promote the negative selection of autoreactive T cells in the thymus (Figure 2). Several groups have confirmed this process by expressing ectopic antigens driven by TRA specific promoters and demonstrating the deletion of T cells specific for neo-self antigen occurs only in the presence of wildtype *Aire* and fails to occur in *Aire* deficient mice.^{20,28} These results indicate that in the absence of Aire-mediated expression of TRAs within the thymus T cells will escape deletion and can escape to the periphery to cause autoimmunity.

Extrathymic Aire—Initial studies indicated that *Aire* transcript is not only expressed in the thymus, but also at low levels in other lymphoid organs including the spleen and lymph nodes.^{20,29} The expression and function of extrathymic *Aire* is controversial, however, a recent study utilizing a unique Aire reporter mouse that expresses GFP driven by the Aire promoter has identified GFP⁺ extrathymic-Aire expressing cells (eTACs) in peripheral lymphoid organs, specifically at the B cell-T cell boundary zone of the lymph node follicle.³⁰ eTACs express nuclear Aire protein by immunofluorescence and were found to be radioresistant stromal cells that were CD45[−], Class II⁺, PD-L1 positive. Importantly, a other studies have also identified peripheral Aire expression in CD45[−] stromal cells and also in human lymph nodes.^{31–32} Similar to mTECs, eTACs are able to interact with and delete autoreactive T cells in a transgenic system.³⁰

Of note, microarray profiling of eTACs show that they also express TRAs, but Aire-dependent TRA expression by eTACs shows very little overlap to mTECs.³⁰ The ability of eTACs to delete autoreactive T cells combined with their expression of TRAs indicates that eTACs may be critical mediators of peripheral tolerance. Furthermore, the finding of differential TRA expression in eTACs and mTECs indicates that these cells may be vital in deleting a different array of autoreactive T cells. The importance of eTACs in preventing the disease manifestations seen in APS1 and *Aire* deficient mice has yet to be fully delineated.

Molecular mechanism of Aire—While Aire is critical for the expression of TRAs, the molecular mechanism it uses to induce TRA gene expression is unknown. The ability of one protein to induce the “promiscuous” transcription of thousands of genes from diverse tissues

is remarkable and an area of active research interest. After its initial discovery, Aire was hypothesized to be a transcriptional regulator for several reasons. First, Aire is located in nuclear speckles which are thought to be sites of active transcription.³³ Second, the structure of the Aire protein contains many functional domains common to transcriptional regulators.^{14,34–36} The Aire protein contains a SAND domain which shows homology to the DNA binding domain of Sp100 family members.³⁷ Evidence that Aire's SAND domain is a functional DNA-binding domain comes from previous studies using Aire recombinant protein, however this result has been called into question as typical Sp100 proteins bind DNA through a K(D/N)WK motif and Aire does not contain this motif.^{35,37} Additionally, genomic studies of Aire target genes have failed to find an Aire consensus sequence among the thousands of Aire target genes. In vitro chromatin immunoprecipitation (ChIP) experiments, using transfected Aire have shown Aire to be targeted to the promoters of TRA genes, however it is unclear if Aire binds directly to DNA or is part of a large macromolecular complex.³⁶ These results indicate that Aire may not act as a sequence-specific transcription factor. Additional studies are needed to address this issue and to determine the relevance of Aire-DNA binding in vivo.

One observation noted about Aire regulated genes is that they show clear chromosomal clustering.^{38–39} Due to the fact that the Aire genes are clustered, it may be that Aire uses epigenetic mechanisms to coordinately de-repress and upregulate an entire cluster of genes. This potential mechanism appears to be more complex, however, in that within an Aire-regulated cluster of genes some of Aire's targets are repressed, some are activated, and for some genes within the cluster Aire has no effect at all.^{40–41} For example, the mouse *casein* locus is expressed in the mouse mammary epithelium and is also a TRA gene cluster expressed in the thymus. In the mouse mammary epithelium, all of the *casein* locus genes are coordinately upregulated. In the thymus, however, some *casein* genes within the cluster are upregulated and others are repressed with activated and repressed genes interspersed within the cluster. Furthermore, single cell sorting followed by qPCR on mTECs shows that a given mTEC will only express a few *casein* genes and that the distribution is random within the locus.^{40–41} This stochastic nature of TRA gene expression within mTECs, in that all mTECs do not uniformly express all TRA genes, argues against Aire functioning simply by globally de-repressing a given cluster of genes and suggests that both gene-specific interactions and other cofactors may be important for Aire's mechanism of action.

Further clues to Aire function come from its known binding partners. Similar to Aire's promiscuous gene regulation, Aire has been shown to bind “promiscuously” to a large number of proteins.⁴² Recent in vitro studies have shown that the first plant homology domain (PHD1) in the Aire protein binds to an unmethylated form of histone 3 (H3K4me0).^{34,36} Additional experiments showed that Aire binding is methylation sensitive and that Aire does not bind to the methylated form of histone 3 (H3K4me3). The finding that Aire binds to histones in a methylation-sensitive manner is additional evidence that epigenetics may be important in Aire function. Interestingly, H3K4me3 is a global mark of active transcription whereas the H3K4me0 is a mark of repression.⁴³ Thus, Aire may target areas of gene repression and it has been speculated that part of its function may be to change a gene's state from a “repressive” to “active” landscape.⁴⁴ The importance of a functional PHD domain is highlighted by the fact that disease causing mutations are found in this region.¹⁴

Other proteins found to bind Aire indicate a potential function in the transcriptional process. Initial studies showed that Aire binds to P-TEFb (positive transcription factor elongation factor b complex), CBP (CREB-binding protein), and DNA-PK (DNA-dependent protein kinase).^{45–47} Both P-TEFb and CBP have well known functions in transcription with P-TEFb important in transcriptional elongation and CBP a well-characterized transcriptional

co-activator. A recent study using large scale immunoprecipitation from Aire-transfected cells followed by mass spectrometry further defined Aire binding partners and identified over 40 proteins that interact with Aire.⁴² The group proceeded to confirm the binding of a large proportion of these proteins with reciprocal coimmunoprecipitation and the function of each binding partner with RNA interference.

The approximately 40 interacting partners could be divided into four functional groups: chromatin structure, gene transcription, nuclear transport, and RNA processing. In this study, the group focused on further characterizing the function of Aire with regard gene transcription and RNA processing. One complex of Aire binding partners contains DNA-PK (DNA-dependent protein kinase), PARP-1 (poly ADP ribose polymerase 1), FACT (facilitates chromatin transcription), and Ku (binds to DNA_double-strand break ends and is required for the non-homologous end joining (NHEJ) pathway of DNA repair). This complex is thought to aid in the transcriptional elongation process by introducing double-stranded breaks into DNA and unwinding DNA to allow access to general transcriptional machinery.⁴⁸ RNAi knockdown of individual members of the complex was shown to disrupt the expression of TRA genes in 293 cells indicating this complex may have functional importance in gene transcription.

A second complex involves members of the mRNA processing machinery including the splicing factors SFRS and SFRS3, the putative DEAD box helicases DDX5 and DDX17, MYB-binding protein 1a, and poly(A) binding protein C.⁴² Members of this complex were also knocked out in 293 cells and were found to be important in Aire mediated TRA gene expression. Interestingly, the group went on to show that Aire was important in mediating mRNA processing by showing selective upregulation by Aire of spliced mRNA, but not pre-mRNA in wild-type mTECs but not in *Aire* deficient mTECs. This result is in contrast to the role of other classic transcription factors such as Foxp3 which are important for the transcription of pre-mRNA.⁴² The finding that Aire does not affect pre-mRNA levels and only affects spliced mRNA raises questions about Aire's role in the general transcriptional process. Specifically, why does Aire bind to factors such as DNA-PK that are thought to be involved in transcriptional elongation if Aire does not affect pre-mRNA and only affects RNA splicing? It may be that Aire has an affect on transcriptional elongation that is beyond our level of detection. Further work needs to be done to characterize the mechanisms of action of these diverse binding proteins with regard to Aire-mediated TRA gene expression.

Novel autoantigens in APS1 – aides in diagnosis

The definitive diagnosis of APS1 can be met if an individual meets one of the following three criteria: 1) presence of at least two of the major components chronic mucocutaneous candidiasis, hypoparathyroidism, or adrenal insufficiency, 2) one major component and a sibling with a definitive diagnosis, or 3) *AIRE* mutations in both genes. APS1 patients also develop autoantibodies which can both aid in diagnosis and also give insights into disease pathogenesis. For example, multiple independent groups found autoantibodies to type I interferons in the sera of APS1 patients with the correlation between APS1 and autoantibodies to type I interferons close to 100%.^{49–50} The specific interferon subtypes that APS1 patients have reactivity to include interferon alpha and/or interferon omega. Patients with the G228W mutation also produce autoantibodies to type I interferons, potentially making them a useful diagnostic tool for all forms of the disease.⁴⁹ The major in vivo function of type I interferons is thought to be in protection from viral infections, however, APS1 patients do not show increased susceptibility to viral infections. Thus, the clinical relevance of type I interferon autoantibodies, other than as a diagnostic maker, is unclear.

In addition to autoantibodies against Type I interferons, a recent set of studies have discovered autoantibodies that may explain the susceptibility to candida infection in patients with APS1. A consortium of researchers has discovered autoantibodies to T helper 17 (Th17) cytokines specifically IL-17A, IL-17F, and IL-22 in sera from APS1 patients.^{51–52} The Th17 subset of T cells is thought to be important for host protection against fungi at epithelial surfaces. Thus, these studies have proposed that the CMC seen in APS1 patients is secondary to autoantibodies targeting these cytokines. While the data shows a significant correlation between autoantibodies to Th17 cytokines and CMC, further study needs to be performed to directly link a Th17 defect to CMC.

Another study, using sera from APS1 patients with hypoparathyroidism identified the parathyroid specific protein NALP5 (NACHT leucine-rich repeat protein 5) as a putative parathyroid autoantigen.⁵³ Autoantibodies to NALP5 are specifically found in APS1 patients with hypoparathyroidism making the autoantibody response to NALP5 a potential diagnostic marker for hypoparathyroidism in these patients. With regard to adrenal insufficiency, APS1 patients also have autoantibodies to P450-containing enzymes that are important in steroid biosynthesis. Similar to patients with isolated autoimmune adrenal insufficiency (Addison's disease), APS1 patients have autoantibodies to 21-hydroxylase (a key enzyme in adrenal steroid biogenesis).³ In addition, APS1 patients with adrenal sufficiency can also have autoantibodies to other enzymes involved in steroid biogenesis such as side-chain cleavage enzyme and aromatic L-amino acid decarboxylase.^{54–55}

Research is currently underway to identify novel autoantibodies that could be used to help diagnose specific APS1 clinical manifestations or potentially other autoimmune diseases. Studies in *Aire* deficient mice have shown that autoantibodies result directly from failed antigen presentation in the thymus.^{25–27} For example, *Aire* deficient mice make autoantibodies to lung epithelium and have an inflammatory infiltrate within the lung. The autoantibody target is the lung-specific protein vomeromodulin and the autoantibody arises secondary to failed thymic presentation of vomeromodulin. Similar to *Aire* deficient mice, there are also APS1 patients with interstitial lung disease (ILD) that shows nearly identical pathology to the disease in mice and interestingly, these patients also make autoantibodies to LPLUNC1 a vomeromodulin homologue.²⁷

Thymic antigen expression in other autoimmune diseases

The importance of thymic antigen presentation may extend beyond APS1 and be relevant to other autoimmune diseases. For example, in type I diabetes thymic expression of insulin has been linked to disease. Population studies have shown that individuals with polymorphisms in the variable number of tandem repeat (VNTR) region upstream of the insulin promoter are more likely to develop disease secondary to lower thymic expression of insulin.^{56–57} A similar situation occurs with myasthenia gravis and expression of the TRA *CHRNA1* (α chain of the acetylcholine receptor).⁵⁸ A single-nucleotide polymorphism in the promoter of the *CHRNA1* gene has been associated with early-onset myasthenia in two populations. Interestingly, this polymorphism is associated with decreased *CHRNA1* antigen expression in ex vivo human mTECs. Furthermore, a correlation seems to exist between thymic *CHRNA1* expression and AIRE expression, indicating that AIRE may play a role in *CHRNA1* expression in the human thymus. Finally, it is interesting to note that the known predisposition to autoimmunity in patients with thymic tumors may be acquiring autoimmunity through a defect in appropriate AIRE expression.⁵⁹

Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome

IPEX clinical features

IPEX is a rare, X-linked, syndrome that results in a severe systemic autoimmune disorder. Most patients with IPEX have the clinical triad of enteritis, endocrinopathy, and dermatitis (Table 1).^{60–61} The presenting symptom is often intractable diarrhea with failure to thrive (FTT) requiring parenteral nutrition.⁶² Histologically, the bowel shows villous atrophy and extensive lymphocytic infiltrate within the mucosa. Insulin-dependent type I diabetes and hypothyroidism are the most common endocrinopathies seen in IPEX. Similar to the GI disease, patients with endocrinopathies have inflammatory infiltrates within the pancreas and thyroid and will often have autoantibodies to pancreatic islet cells.^{60–61} The dermatitis that affects IPEX patients is most commonly an eczematous rash that in some patients can be very severe affecting a large percentage of their body surface area. In addition to the diagnostic triad, IPEX patients can have multiple other autoimmune manifestations including cytopenias, renal disease, and hepatitis.^{60–61} The symptoms in IPEX can fluctuate with exacerbations precipitated by infections, dietary allergens, or vaccinations. Laboratory studies often show eosinophilia and hypergammaglobulinemia. IPEX patients often display increased susceptibility to a wide range of infections including bacterial, viral, and fungal, a phenomenon which is ascribed to decreased barrier function secondary to severe gastritis and dermatitis.^{61–62} Treatment of IPEX is with either immunosuppressive agents or bone marrow transplantation.^{63–64} Aggressive treatment is warranted as this disease can be lethal if left untreated.

IPEX genetics

The discovery of the genetic mutation in IPEX was made possible secondary to observation of a mouse mutant called *scurfy* which has severe autoimmunity and lymphoproliferation similar to IPEX. The mutated gene in *scurfy* mice was found to be the *Foxp3* gene located on Xp11.3-q13.3.⁶⁵ After *Foxp3* was identified in mice, two groups sequenced the *FOXP3* gene in families with IPEX and discovered mutations within the human homologue.^{66–67} In these families, only males are affected and female carriers with one functional copy of *FOXP3* are asymptomatic. A wide range of mutations have been found in the *FOXP3* gene of IPEX patients including point mutations, deletions, and splicing mutations.^{60–61} *Foxp3* is a zinc-finger transcription factor required for the development and function of regulatory T cells (T_{reg} cells). T_{reg} cells are $CD4^{+}$, IL-2R α ($CD25^{+}$), and *Foxp3* $^{+}$ T cells that are functionally anergic and upon activation produce cytokines and cell surface molecules that are important in diminishing immune responses in a variety of settings.^{68–69} These cells make up approximately 10–15% of $CD4^{+}$ T cells and have proven to be critical mediators of peripheral tolerance.

IPEX pathogenesis

Prior to the discovery of *Foxp3*, the identity of T_{reg} cells was suspected when a seminal study showed lymphocyte suspensions depleted of $CD4^{+}CD25^{+}$ cells induced autoimmunity when injected into lymphopenic mice.⁷⁰ Interestingly, the autoimmunity could be prevented with immediate reconstitution of the mice with $CD4^{+}CD25^{+}$ T cells and the effects of the $CD4^{+}CD25^{+}$ T cells were dose dependent. This important study was the first to suggest a population of suppressor cells within the $CD4^{+}CD25^{+}$ T cell lineage. After the discovery of *Foxp3*, several groups determined that *Foxp3* functions specifically in $CD4^{+}CD25^{+}$ T cells and is critical for the prevention of autoimmunity.^{71–72} Bone marrow chimeras using bone marrow from *Foxp3* deficient mice into wild-type hosts induced an autoimmune syndrome similar to *scurfy* mice indicating that *Foxp3* functions in hematopoietic cells.⁷¹ Furthermore, using retroviral vectors that express *Foxp3*, it was shown that $CD4^{+}CD25^{-}$ T cells could be transformed into T_{reg} cells by expression of *Foxp3* and these cells are able to

suppress T effector cells both *in vitro* and *in vivo*.⁷² T_{reg} cell lineage specification was further defined using a Foxp3^{GFP} reporter mouse that expresses functional Foxp3.⁷³ This mouse showed that Foxp3 is exclusively expressed in CD4⁺CD25⁺ T_{reg} cells and in no other tissues or hematopoietic cells. In addition, the importance of T_{reg} cell function throughout life is illustrated with the Foxp3^{DTR} mouse and a study utilizing cre-mediated ablation of Foxp3 in mature T_{reg} cells.^{74–75} In both mouse models, selective ablation of T_{reg} cells in adult mice leads to death in three weeks secondary to autoimmunity. The ability of a specialized set of cells to enforce tolerance is defined as dominant tolerance, as this cell type is able to suppress self-reactivity by acting in a dominant fashion.^{68–69} The model of dominant tolerance explains why female carriers of a mutated Foxp3 are not affected. Despite random X-chromosome inactivation and only one-half of T cells harboring a functional Foxp3 gene, carrier females are normal because the T_{reg} cell population carrying the wild-type allele is able to control autoimmunity in a dominant fashion.⁷⁶

Significant research has been performed to determine the exact mechanism by which T_{reg} cells are able to dampen the immune system. It is likely that multiple mechanisms are involved including both contact dependent mechanisms (direct killing of activated T cells/antigen presenting cells (APCs)) and soluble factors (production of inhibitory cytokines such as IL-10 or TGF- β).^{68–69} Two well characterized mediators of T_{reg} cell suppression are the cytotoxic T lymphocyte antigen (CTLA-4) and the cytokine IL-10.^{77–78} CTLA-4 is a surface molecule on T cells which is in the same family as the co-stimulatory molecule CD28. Selective ablation of CTLA-4 on T_{reg} cells in mice results in severe autoimmunity. CTLA-4 is hypothesized to function on T_{reg} cells by sending an inhibitory signal to APCs and reducing their production of proinflammatory cytokines.^{69–78} Additionally, loss of IL-10 production by T_{reg} cells results in gut and lung inflammation suggesting a role for specific cytokines in T_{reg} cell function.⁷⁷

T_{reg} cell differentiation and Foxp3 expression

The extracellular signals required for T_{reg} cell differentiation in the thymus and Foxp3 expression is an area of intense research. It has been hypothesized that T_{reg} cells express self-reactive TCRs and that TCR specificity for self may guide T_{reg} cell differentiation. Several studies have shown that T_{reg} cell TCRs are skewed toward self-peptides and that TCR signaling is likely important for Foxp3 expression.^{79–80} Several transcription factors downstream of the TCR have been implicated in Foxp3 expression including NFAT, NF- κ B, CREB, AP1, and ATF.^{81–84} IL-2 has also been implicated in T_{reg} cell differentiation because mature T_{reg} cells express high levels of the CD25. Experiments utilizing a Foxp3^{GFP} reporter mouse crossed onto the IL-2 and IL-2 receptor knockout background showed that IL-2 is not required for Foxp3 induction in the thymus, but it is required for the peripheral homeostasis of T_{reg} cells and the continued expression of Foxp3 in the periphery.⁸⁵ In support of this finding, mice lacking IL-2 or IL-2R α have a severe lymphoproliferative syndrome.⁸⁶ Notably, recent case reports have described patients that have mutations in both copies of the IL-2 receptor gene and these patients present with severe autoimmunity similar to IPEX.^{87–88} Additional signals implicated in T_{reg} cell differentiation include TCR co-stimulatory molecules such as CD28 and the cytokine TGF- β .^{89–90}

To add a layer of complexity, T_{reg} cell differentiation can be further divided into natural and adaptive regulatory T cells.⁹¹ Natural T_{reg} cells originate in the thymus while adaptive T_{reg} cells are induced in the periphery from a population of CD4⁺CD25⁺ T cells. Adaptive T_{reg} cells are mainly located in lymph nodes and chronically inflamed tissue.^{92–93} The exact signals required for adaptive T_{reg} cell development are controversial, however, there is thought to be considerable overlap with the signals required for natural T_{reg} cell development including TCR signaling, costimulation with CTLA-4, TGF- β , and IL-2.⁹¹

Additionally, retinoic acid produced by CD103⁺ dendritic cells in the lamina propria of the gut has been implicated in the development of adaptive T_{reg} cells.^{94–95} Furthermore, it has been hypothesized that adaptive T_{reg} cells may be particularly important in the gut to dampen the immune response to commensal bacteria and food antigens.

Molecular mechanism of Foxp3 function

Regulatory T cells (T_{reg} cells) represent a unique population of CD4⁺ T cells and the transcription factor Foxp3 serves as a lineage specification marker. The Foxp3 protein contains many functional domains: an N terminal proline rich region that binds the nuclear factor of activated T cells (NFAT), a central domain that contains a C2H2 zinc finger and a leucine zipper that is thought to be involved in protein-protein interactions, and a C terminal region that contains the forkhead DNA binding domain and the nuclear targeting sequences (Figure 3).⁶¹ NFAT is a transcription factor required for activation of T cells and IL-2 production. Interestingly, the Foxp3/NFAT complex acts as a corepressor of cytokine promoters such as IL-2 and changes T cell phenotype from one of activation to tolerance.⁹⁶ In addition to NFAT, Foxp3 also binds to several other transcription factors including the Runt domain transcription factor Runx1, IRF4, STAT3, and the orphan nuclear receptors ROR γ and ROR α .^{97–100}

Recently, two groups performed chromatin immunoprecipitation (ChIP) experiments to analyze Foxp3 DNA binding.^{101–102} Previous microarray profiling of Foxp3⁺ T cells compared to Foxp3[−] T cells revealed that Foxp3 induced the expression of many genes important for its function including CTLA-4, CD25, IL-10, and the TNF family member GITR.^{73,103–104} Interestingly, ChIP analysis showed that Foxp3 only directly regulated approximately 10% of these genes and induced a group of transcription factors and micro RNAs that likely play an important role in T_{reg} cell biology.^{68,101–102}

Human FOXP3⁺ regulatory T cells

The isolation of T_{reg} cells from peripheral blood in humans has proven to be much more complex than the isolation of T_{reg} cells in mice. The main reason for this complexity is that upon T cell stimulation naïve human CD4 T cells will transiently express low-levels of FOXP3 making it difficult to differentiate T_{reg} cells from effector T cells.^{105–106} The exact function of the low-level expression of FOXP3 in activated T cells is unknown, however, these cells secrete inflammatory cytokines and are unable to suppress in vitro.^{107–108} Due to the heterogeneous expression of FOXP3 in human CD4⁺ T cells, it cannot be used alone as a marker of Tregs.^{109–110} To circumvent this problem, many groups have attempted to use the level of FOXP3 protein and the methylation status of the FOXP3 promoter to identify Tregs.^{109,111–112} Human T_{reg} cells are defined by high levels of FOXP3 protein associated with a completely demethylated FOXP3 promoter while activated T cells express lower levels of FOXP3 protein and the FOXP3 promoter is incompletely demethylated.

One difficulty in using FOXP3 protein to define T_{reg} cells is that FOXP3 is a nuclear protein and T cells must be fixed prior to analyzing protein expression. Therefore, T_{reg} cells isolated using FOXP3 cannot be used for further applications. In mice, CD25 can be used as a surrogate marker as most CD25⁺ cells will express high levels of FOXP3 and will have a suppressive phenotype.⁷⁰ In contrast, activated human CD4⁺ T cells that transiently express FOXP3 also express high levels of CD25 making it difficult to solely use this cell-surface marker for the isolation of human T_{reg} cells. For this reason, many groups are using additional markers such as CD127, CD49d, and CD45 isoforms for the isolation of human T_{reg} cells to be used in further functional assays.^{109,113–114}

One marker that has been used successfully is the IL-7 receptor, CD127, which is found on both effector and memory T cells. In contrast, FOXP3 positive human T regulatory cells express low levels of CD127. Using a combination of high surface expression of CD4 and CD25 along with low levels of CD127 has allowed several groups to isolate a pure population of human T_{reg} cells that express high levels of FOXP3 protein that retain suppressive function.^{114–115} Another marker that has recently been used to aid in the purification of human T_{reg} cells is CD49d which is the α -chain of the integrin VLA-4($\alpha_4\beta_1$) and is found on the majority of proinflammatory effector cells, but is absent on FOXP3 positive T_{reg} cells. By depleting CD49d⁺ cells, one group has been able to enrich for T_{reg} suppressor cells within the CD25⁺/CD127^{lo} subset of cells.¹¹³ The ability to isolate a pure population of human T_{reg} cells is critical because T_{reg} cells may be important in the pathogenesis of several autoimmune diseases and adoptive transfer of T_{reg} cells has been proposed as a potential therapy for these diseases.

T_{reg} cells and autoimmune disease

While T_{reg} cells are clearly important in the pathogenesis of IPEX, a number of studies have recently attempted to address whether subtle changes in T_{reg} cell function or numbers are important in other polygenic autoimmune disease. In both rheumatoid arthritis (RA) and type I diabetes, there have been conflicting results as to whether T_{reg} cells are deficient. For example, in RA two groups found no difference in the ability of T_{reg} cells from RA patients to suppress effector T cell proliferation, however, another group found a striking defect.^{116–118} Similarly, for type I diabetes one group found a reduction in T_{reg} cell percentage of CD4⁺ T cells, while several other groups have found no difference in T_{reg} cell frequency.^{119–122} Further research needs to be performed to better understand the role of T_{reg} cells in more common autoimmune disease.

Autoimmune lymphoproliferative syndrome

ALPS clinical Features

The autoimmune lymphoproliferative syndrome (ALPS) is characterized by lymphadenopathy, splenomegaly, hepatomegaly, autoimmune manifestations, and an increased risk of malignancy (Table 1).^{123–125} The lymphadenopathy in ALPS is chronic and to be diagnosed a patient must have lymphadenopathy for >6 months.^{124–125} The most common autoimmune manifestation of ALPS are cytopenias secondary to autoimmune destruction of blood cells (red blood cells, platelets, or neutrophils) affecting 70% of patients.^{124–125} Destruction of hematopoietic cells is often autoantibody mediated and 92% of ALPS patients develop at least one autoantibody: anticardiolipin, direct antigen test (DAT), anti-platelet, anti-neutrophil, anti-nuclear, or rheumatoid factor.¹²⁵ Autoimmunity, however, can affect almost any organ system including the kidney (nephritis), liver (hepatitis), eye (uveitis), joints (arthritis), gut (colitis), and skin (urticaria). The exact risk of malignancy in ALPS patients is unknown, although it is postulated to be 10%–20%.¹²⁶ The most common malignancy is lymphoma and there are case reports of leukemia's as well as several solid tumors.^{124–126} The treatment of ALPS is based on the clinical manifestations with immunosuppressive agents used to treat autoimmune manifestations and chemotherapy for malignancy.^{124–126} Bone-marrow transplants have been performed in patients with severe-refractory disease.¹²⁷

ALPS genetics

Similar to APS1 and IPEX, the pathogenesis of ALPS has been delineated utilizing a mouse model that phenotypically resembled patients with ALPS. Mice homozygous for the *lpr* or *gld* mutations were found to have multiple autoimmune manifestations, autoantibody production, and an increase in nonmalignant TCR $\alpha\beta$ CD4⁺CD8⁺ double-negative (DN) T

cells.^{128–129} The *lpr* and *gld* mutations were determined to be in Fas or its binding partner Fas ligand (FasL) which are both critical for the apoptosis of activated lymphocytes. Similar to *lpr* or *gld* mice, ALPS patients have increased numbers of DN T cells and were found to have mutations in *FAS* (ALPS-FAS), and *FASLG* (ALPS-FASLG), demonstrating that defective apoptosis is the cause of the disease.^{130–132} A large number of *FAS* mutations (>70) have been discovered with two-thirds in the intracellular domain (ICD) and one-third in the extracellular domain (ECD). Clinical penetration is higher with ICD mutations (70–90% penetration) than ECD mutations (30% penetration).^{125,133}

The first ALPS patients described had mutations in one *FAS* gene and the syndrome was inherited in an autosomal dominant fashion in which there was a family history of ALPS. Subsequent studies revealed patients with homozygous mutations in *FAS*.^{131,134–135} Predictably, patients with homozygous mutations have severely diminished *FAS* expression and a more severe ALPS phenotype requiring early bone-marrow transplantation. Additionally, one family has been described in which two siblings are compound heterozygote's with distinct missense mutations in each *FAS* allele.¹³⁶ Interestingly, despite mutations in both *FAS* genes these patients have a milder ALPS phenotype. Also, unlike other patients with homozygous *FAS* mutations these patients did not have a family history of ALPS despite both parents being heterozygous for *FAS*. This may be due to the specific *FAS* mutation with missense mutations causing a milder phenotype.

Further work identified other mutations responsible for an ALPS or ALPS-like phenotype including Caspase 10 (ALPS-CASP10), Caspase 8 (CEDS), and the NRAS oncogene (RALD).^{137–140} Caspase 10 is a protein downstream of Fas in the apoptotic pathway. Similar to ALPS patients with *FAS* or *FASLG* mutations, *CASPASE 10* mutations are usually inherited in an autosomal dominant fashion. Analogous to Caspase 10, Caspase 8 is downstream of Fas in the apoptotic pathway, however, the inheritance of Caspase 8 mutations and the phenotype of families with Caspase 8 mutations differs from typical ALPS patients. For this reason, Caspase 8 deficiency is given a unique acronym, CEDS, standing for Caspase-Eight Deficiency State.¹⁴¹ Families with Caspase 8 mutations display autosomal recessive inheritance and also have defects in T, B, and NK activation leading to susceptibility to bacterial and viral infections. NRAS is a small GTPase and activating mutations in NRAS cause decreased activity of the proapoptotic protein BIM.¹⁴² This disease entity is now classified as RALD, for RAS-associated autoimmune leukoproliferative disease.¹⁴¹

To further complicate ALPS classification, patients with somatic mutations in Fas (not germline) have recently been discovered and have been named ALPS-sFAS.^{143–144} Patients who have the ALPS phenotype, but who do not have an identifiable mutation are called ALPS-U. Recently, an NIH consensus conference created a new ALPS classification scheme (Table 2) and diagnostic criteria (Table 3).^{124,141,145–146} Two other ALPS-like disorders are Diansani Autoimmune Lymphoproliferative disease (DALD) and X-linked lymphoproliferative disease (XLP1).^{147–148} The mutation for DALD is unknown and the mutation in XLP1 is in the *SH2D1A* gene. Patients with DALD present with lymphadenopathy, autoimmunity, and defective Fas-mediated apoptosis, however, these patients have normal numbers of DN T cells. Due to the normal number of DN T cells, DALD is given its own acronym because it is thought to be a unique genetic disorder.

ALPS pathogenesis

As discussed above, ALPS patients have a variety of defects in the apoptotic pathway. The exact mechanism, however, by which defects in apoptosis cause the autoimmunity seen in ALPS is unknown. One hypothesis is that failure of the immune system to contract after antigen stimulation may lead to the escape of autoreactive clones and autoimmunity. Upon

antigen stimulation, lymphocytes express Fas and FasL on their surface in the process of activation induced cell death (AICD). In order for Fas or FasL to induce apoptosis they each must form a trimolecular complex.¹⁴⁹ The requirement of the trimolecular complex explains the autosomal dominant inheritance seen in ALPS-FAS, ALPS-FASL, and ALPS-sFAS.^{130–132} In heterozygous patients the mutated allele functions as a dominant-negative inhibiting the function of the wild-type allele and disrupting the formation of a functional complex. In most cases, the mutated Fas protein still forms a trimolecular complex with wild-type Fas, however, the mutated Fas allele disrupts death domain signaling or binding of FasL.¹²⁵ Similarly, patients with ALPS-CASP10 also show autosomal dominant inheritance with the mutated Caspase 10 functioning in a dominant-negative fashion.

One important unanswered question regarding the pathogenesis of ALPS pertains to the unexplained heterogeneity in clinical manifestations among family members with the same ALPS genotype. Some family members will be asymptomatic while others will require significant immunosuppression. It has been proposed that the genetic background of the individual will determine the severity of the disease and that additional mutations in genes involved in tolerance may tip the balance toward autoimmunity.¹⁵⁰ Thus, similar to cancer autoimmunity may involve a multi-step process in which additional mutations in rapidly dividing immune cells promotes the growth of autoimmune clones that cause disease. Support for this hypothesis comes from ALPS patients with somatic mutations in Fas.^{143–144} These patients have normal germline FAS alleles; however, at some point in lymphocyte maturation a somatic mutation occurred in one FAS allele breaking tolerance. The finding that somatic mutations are able to break tolerance may explain the heterogeneity seen in ALPS patients as patients with more severe disease may have accumulated additional mutations promoting autoimmunity. Future research will aim to determine the other genes and environmental factors potentially modulating the ALPS phenotype and to further understand how defects in apoptosis lead to autoimmunity.

Summary

The study of monogenic autoimmune diseases has provided invaluable insights into the underlying mechanisms of immune tolerance. Mouse models of these diseases have allowed detailed studies of the pathogenesis of these unique syndromes and have shed light on the molecular mechanisms of autoimmunity. By further dissecting the molecular pathways involved in these diseases we may gain a greater understanding not only of the pathogenesis of monogenic autoimmune syndromes, but of more common autoimmune syndromes. The downstream genes or associated pathways may provide us with novel therapies or aid in the diagnosis of autoimmune disease. Therapies are already being developed from the lessons learned in these diseases. One new therapy that has emerged is the adoptive transfer of Tregs for the treatment of type I diabetes based on studies of a mouse model of diabetes showing that the transfer of a small number of antigen-specific Tregs could reverse diabetes.¹⁵¹ A number of groups are in the process of moving toward using the adoptive transfer of Tregs for diverse autoimmune diseases such as type I diabetes and graft vs host disease.^{152–154} Similarly, the novel autoantibodies discovered from the study in APS1 may aid in the diagnosis of more common autoimmune disease such as interstitial lung disease or autoimmune gonadal failure. In summary, the exciting research advances from the study of monogenic autoimmune diseases are making vital contributions to the diagnosis and treatment of more common autoimmune diseases.

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Figure 1.

Functional domains of the Aire protein. The N terminus contains both a homogenously staining region (HSR) thought to be involved in dimerization and a nuclear localization sequence. The middle of the Aire protein contains a SAND domain which shows homology to the DNA binding domain of Sp100 family members. The C terminus contains a proline rich region (PRR) and two plant homeodomains (PHD). The PRR may be important in complexing DNA bound transcriptional coactivators. The figure shows a few of the important mutations found in patients with APS1. Over 60 mutations have been identified scattered throughout the gene.

Figure 2.

Negative selection of T cells in thymus. Immature T cells (thymocytes) that express T cell receptors (TCRs) specific for tissue restricted antigens (TRAs) are deleted in the thymus in the process of negative selection. The expression of TRAs by medullary thymic epithelial cells (mTECs) is dependent on Aire. In Aire knockout mTECs, TRAs are not expressed and autoreactive T cells are not deleted. The molecular mechanism Aire uses to promote the expression of TRA genes is an area of active research.

Figure 3.

Functional Domains of Foxp3 protein. The N terminus contains a repressor domain important for binding the nuclear factor of activated T cells (NFAT) and inhibiting its function. The middle of the Foxp3 protein contains a zinc finger domain and a leucine zipper. The C terminus contains the forkhead DNA binding domain and a nuclear localization sequence.

Table I

Major Characteristics of Monogenic Autoimmune Diseases

	APS1	IPEX	ALPS
Defective Gene(s)	<i>AIRE</i>	<i>FOXP3</i>	<i>FAS</i> <i>FasL</i> <i>Caspase 10</i> <i>NRAS</i>
Inheritance	Autosomal Recessive	X-linked	Autosomal Dominant
Major Symptoms	Hypoparathyroidism Mucocutaneous candidiasis Addison's disease	Diarrhea Type I diabetes Thyroiditis Eczema	Lymphadenopathy Splenomegaly Autoimmune Cytopenias Malignancy
Treatment	Hormone replacement Antifungals	Bone marrow transplant	Immunosuppression

Table IIDiagnostic Classification of ALPS and ALPS-like disorders¹⁴⁰

Previous Nomenclature	Revised Nomenclature	Gene
ALPS Type 0	ALPS-FAS	Germline Homozygous <i>FAS</i> mutations
ALPS Type Ia	ALPS-FAS	Germline Heterozygous <i>FAS</i> mutations
ALPS Type Im	ALPS-sFAS	Somatic <i>FAS</i> mutations
ALPS Type Ib	ALPS-FASLG	Germline <i>FAS Ligand</i> mutations
ALPS Type IIa	ALPS-CASP10	Germline Caspase 10 mutations
ALPS Type III	ALPS-U	Unknown mutation
ALPS Type IIb	CEDS	Germline Caspase 8 mutations
ALPS Type IV	RALD	Somatic <i>NRAS</i> mutations
DALD	DALD	Unknown mutation
XLP1	XLP1	Germline <i>SH2D1A</i> mutations

Table III**Diagnostic Criteria for ALPS****(a)Original Diagnostic Criteria¹²⁶**

- 1** Chronic non-malignant lymphoproliferation
- 2** Elevated peripheral blood DNT's
- 3** Defective *in vitro* Fas-mediated apoptosis

Supporting: genetic mutations; autoimmunity

Diagnosis: Must meet all three criteria

(b)Revised diagnostic criteria¹⁴⁰Required

- 1** Chronic (>6 months), non-malignant, non-infectious lymphadenopathy and/or splenomegaly
- 2** Elevated CD3⁺ TCRαβ⁺ CD4⁻CD8⁻ DNT cells (equal to or greater than 1.5% of total lymphocytes or 2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts.

AccessoryPrimary

- 1** Defective lymphocyte apoptosis (in 2 separate assays);
- 2** Somatic or germline pathogenic mutation in *FAS*, *FASLG*, or *CASP10*;

Secondary

- 1** Elevated plasma sFASL levels (>200 pg/ml) OR elevated plasma IL-10 levels (>20 pg/ml) OR elevated serum or plasma Vitamin B12 levels (>1500 ng/L) or elevated plasma IL-18 levels above 500 pg/ml;
- 2** Typical immuno-histological findings as reviewed by an experienced hematopathologist;
- 3** Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated IgG levels (polyclonal hypergammaglobulinemia);
- 4** Family history of a non-malignant/non-infectious lymphoproliferation with or without autoimmunity.

Definitive Diagnosis: Both required criteria plus one primary accessory criterion.

Probable Diagnosis: Both required criteria plus one secondary accessory criterion.