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# Monogenic Autoimmunity

**Mickie H. Cheng** and **Mark S. Anderson**
Diabetes Center; Department of Medicine, Division of Endocrinology and Metabolism, University of California at San Francisco, San Francisco, California 94143; manderson@diabetes.ucsf.edu

## Abstract

Monogenic autoimmune syndromes provide a rare yet powerful glimpse into the fundamental mechanisms of immunologic tolerance. Such syndromes reveal not only the contribution of an individual breakpoint in tolerance but also patterns in the pathogenesis of autoimmunity. Disturbances in innate immunity, a system built for ubiquitous sensing of danger signals, tend to generate systemic autoimmunity. For example, defects in the clearance of self-antigens and chronic stimulation of type 1 interferons lead to the systemic autoimmunity seen in C1q deficiency, SPENCDI, and AGS. In contrast, disturbances of adaptive immunity, which is built for antigen specificity, tend to produce organ-specific autoimmunity. Thus, the loss of lymphocyte homeostasis, whether through defects in apoptosis, suppression, or negative selection, leads to organ-specific autoimmunity in ALPS, IPEX, and APS1. We discuss the unique mechanisms of disease in these prominent syndromes as well as how they contribute to the spectrum of organ-specific or systemic autoimmunity. The continued study of rare variants in autoimmune disease will inform future investigations and treatments directed at rare and common autoimmune diseases alike.

### Keywords

Immune tolerance; complement; interferon; apoptosis; Treg; thymus

## INTRODUCTION

The classical conundrum of immunology is the maintenance of an effective system of defenses balanced between immunodeficiency and *"horror autotoxicus."* The elegant recombinatorial mechanisms in both the T cell and B cell receptors that have evolved to generate effective adaptive immunity require an equally coordinated system to maintain self-tolerance. Given the complexity of these multiple checks and balances, syndromes of monogenic autoimmunity represent rare experiments of nature that can define the critical mechanisms of immune tolerance.

Autoimmune disease can be broadly classified into either systemic or organ-based disease, and these distinctions have implications for the likely mode of pathogenesis. Systemic

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diseases affect several tissues and sites in the body, as seen in systemic rheumatic diseases such as systemic lupus erythematosus (SLE) or polymyositis/dermatomyositis. Organ-based diseases typically affect a single system or tissue, as in the case of type 1 diabetes, Graves' disease, or multiple sclerosis. Not infrequently, individuals with one autoimmune disease, such as type 1 diabetes, may have a second autoimmune disease, such as autoimmune thyroiditis. These instances of multi-organ autoimmunity should not be confused with a systemic autoimmune condition in which common antigens are recognized throughout the body, as there is clear organ-specificity in these diseases. Here, we focus our attention on monogenic syndromes characterized by the production of autoantibodies and immune pathology and not immune deficiencies with mild autoimmunity associations or inflammatory syndromes. Certainly, similar clinical readouts may arise through self-destructive mechanisms such as autoinflammatory, metabolic, or hemophagocytosis defects seen in other monogenic syndromes; this underscores the need for a clear understanding of the underlying molecular mechanisms in disease. As outlined above, we broadly segregate these diseases into systemic and organ-specific syndromes, although the distinctions at times can be blurred.

Rapid advances in both clinical and molecular medicine in the past decade have uncovered the genetic basis of heretofore poorly characterized autoimmune syndromes. Improved phenotypic characterization of pleomorphic disease states coupled with advances in genomics have allowed identification of causative gene defects in several rare syndromes of autoimmunity. Such investigations have also revealed genetic heterogeneity within these disease states, leading to the recognition of signaling pathways and cellular processes sharing common phenotypes. Although genetic syndromes of autoimmunity are uncommon, they share many features with more common autoimmune diseases, which may also be caused by uncommon or incompletely penetrant single gene mutations in rare instances. Most importantly, such syndromes have revealed the basis for fundamental mechanisms of immunological tolerance. We highlight the insights gained from the study of prominent examples of monogenic autoimmunity, built upon clinical observations, genetics, and translational work in both human and animal models.

## MONOGENIC SYSTEMIC AUTOIMMUNITY

### Innate Immune Activation: Defenses Turned to Autoimmunity

The innate immune system forms the initial defenses against foreign insults. In contrast to adaptive immunity, the innate immune system lacks memory and recognizes potential noxious stimuli through germ-line-encoded receptors selected during evolution to recognize invariant patterns in pathogens. Thus, tolerance in innate immunity relies largely on the ability of these hardwired receptors to distinguish foreign antigens from self-antigens. Such distinctions may be challenging in the case of ubiquitous antigens such as nucleic acids. The mediators of innate immunity, which include the complement system and cells such as monocyte-macrophages and dendritic cells (DCs), are also found abundantly throughout the body. Activation of inflammation through innate immune responses can also amplify and interface with adaptive immunity. Consequently, perturbations in innate immunity, which senses ubiquitous danger signals and antigens, frequently produce systemic autoimmunity.

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Three syndromes—hereditary C1q deficiency, spondyloenchondrodysplasia with immune dysregulation (SPENCDI), and Aicardi-Goutieres Syndrome (AGS)— highlight how defects in innate immune mechanisms reveal critical steps in the pathogenesis of autoimmunity.

## Hereditary C1q Deficiency: Clearance for Tolerance

Over the past several years, a wealth of new genomic information has revealed multiple risk alleles and genes implicated in the pathogenesis of SLE. Because of the heterogeneity of the disease, it is no surprise that multiple genes with modest effects have been implicated in the development of SLE. Monogenic causes of SLE remain quite rare, however. Hereditary C1q deficiency (OMIM 613652) was one of the earliest recognized monogenic causes of SLE. The early studies of Clq-mediated autoimmunity led to an appreciation of the role of complement components in the pathogenesis of systemic autoimmunity. Although investigators have proposed several potential mechanisms, recent studies have uncovered new data that may clarify the role of C1q in tolerance through its actions on self-antigen clearance.

**Clinical and genetic features.—**Primary inherited deficiency of Clq is a rare autosomal recessive disorder first described in a single 10-year-old boy in 1977 (1). Since then, only 64 cases have been reported worldwide. The condition typically presents in childhood and is characterized by autoimmune manifestations, significant for SLE and SLE-like diseases, and an increased tendency for infections with encapsulated bacteria, especially *Streptococcus pneumoniae.* The penetrance of SLE-like disease is extremely high, affecting 88–93% of homozygous cases, and shows no gender predominance (2, 3). The decreased penetrance of SLE in recent years may reflect the younger age of diagnosis of newer patients, who have yet to manifest disease. SLE-related conditions can include lupus-like skin disease with photosensitive or malar rash, aphthous oral ulcers, angioedema, chronic glomerulonephritis, arthralgias/arthritis, and central nervous system (CNS) vasculitis. Autoantibodies are also prevalent and include antinuclear antibodies (ANAs) and antibodies to ribonucleoproteins (anti-Sm and -Ro). Despite the strong association of SLE with the syndrome, a greater degree of clinical variability has become apparent in recent years. Patients completely deficient for serum C1q may be completely asymptomatic into adulthood, and the phenotype can vary widely within families carrying the same mutation (4). Infectious complications also span a spectrum from mild recurrent otitis media to fulminant bacterial meningitis.

Hereditary C1q deficiency can arise from mutations in any of the three chains, C1qA, C1qB, and C1qC, that make up the 460-kDa macromolecule. The A, B, and C chains are encoded by genes tandemly arranged 5’ to 3’ (A-C-B) on a 24-kb region of human chromosome 1p (5). Each chain is composed of an N-terminal region, followed by a collagen-like region that mediates oligomerization of the molecule, and a C-terminal globular head. Twelve mutations have been identified to date, distributed over all three chains. Affected individuals carry homozygous or compound heterozygous mutations predicted to inactivate both alleles. The most common mutation is a premature stop in *ClqA,* Q208X, which has been described in several families of various ethnic origins. Despite an odds ratio of ~ 10 for SLE in individuals with homozygous C1q mutations (6), similar mutations have not been identified in screening of general SLE cohorts to date (7, 8). Although a translationally silent

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(synonymous) SNP in *ClqA* has been found in association with decreased C1q levels in some patients with subacute cutaneous lupus (9), the same SNP has been described in heterozygous family members of Clq-deficient patients without any apparent effect on Clq levels (10), suggesting that this variant may represent a polymorphism rather than a functional variant. This example reflects the difficulty of identifying rare functional variants through traditional genome-wide association (GWA) studies and highlights the importance of continued study of rare single-gene variants in the pathogenesis of heterogeneous disease states (11).

**The paradox of complement in SLE.—**At first blush, a deficiency in complement leading to SLE may seem unexpected. During an immune response, Clq binds immune complexes (ICs) to initiate the classical pathway of the complement cascade, triggering an inflammatory cascade of enzymatic cleavage events that culminates in the lysis of immune complex-opsonized bacteria. Two opposing views of complement activation are seen in SLE. The classical complement pathway, initiated by Clq, is activated in SLE patients and contributes to tissue damage with deposits of complement proteins readily detected in inflamed tissues of patients (reviewed in 12). However, deficiency of Clq is linked to worsened disease in SLE. Secondary acquired partial deficiency of Clq is common in SLE and arises through autoantibodies to Clq or increased turnover from complement activation. Anti-Clq antibodies, found in 30–48% of lupus patients, are associated with hypocomplementemia and more severe disease with renal involvement (13). It remains unclear, though, whether these autoantibodies have a pathogenic role in causing or amplifying inflammatory injury or whether they arise as a consequence of intense complement activation. Most likely, complement acts as a doubleedged sword, with deficiency promoting loss of tolerance and overstimulation leading to inflammation. Clarification of these opposing views of complement activation in SLE can be drawn from examination of animal models of complement deficiency.

**Molecular mechanism.—**Clq is expressed in myeloid cells, monocytes, macrophages, and DCs. The protein is assembled from 18 peptide chains to form a tulip-like structure, with tulip heads containing the globular domains, and the collagen-like regions forming the stalk. The globular heads mediate binding to a wide range of ligands, especially Igs, and conformational change of Clq upon binding leads to activation of Cl and initiation of the classical complement cascade (reviewed in 14). In this way, Clq functions as the pattern-recognition component of the complement defense system by binding IgG and IgM ICs.

The increased susceptibility to bacterial infections of some Clq-deficient patients seems clear given that complement-mediated activation of the membrane-attack complex is important in the killing of encapsulated bacteria. However, the role of Clq deficiency in SLE pathogenesis remains less straightforward. Several complementary and nonexclusive models have been proposed for the contribution of complement to SLE and systemic autoimmunity (reviewed in 15). Substantial data support a role for complement in the clearance of apoptotic material in the prevention of autoimmunity, and newer studies suggest a novel cellular mechanism in mediating this effect.

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The earliest insight into the role of complement in the maintenance of tolerance comes from the study of Clq-deficient mice. Like patients with hereditary Clq deficiency, *C1qa*$^{-/-}$mice develop features of SLE, chiefly high-titer ANAs and glomerulonephritis with IC deposition within the kidneys (16). The expression of autoimmunity, however, varies significantly between strains and is not completely penetrant, indicating the importance of additional genetic modifiers (16, 17). Notably, among *C1qa*$^{-/-}$mice unaffected by glomerulonephritis, the authors made the surprising observation of increased numbers of apoptotic bodies within the glomeruli consistently across all strains. Because of the broad penetrance of the apoptotic phenotype, the authors predicted that this underlying Clq-dependent defect was the predisposing factor for the subsequent generation of autoimmunity in genetically susceptible strains. Walport and colleagues (16) proposed that deficiency of C1q might lead to decreased clearance of apoptotic material, leading to an increased source of autoantigens capable of stimulating an autoimmune response. Ahearn and colleagues (18, 19) had also proposed a link between apoptotic clearance and complement based on their in vitro studies of C1q. They demonstrated that C1q specifically binds surface blebs of apoptotic cells through its globular heads in a manner independent of Ig, suggesting direct C1q recognition that is not IC mediated. Subsequent in vivo studies in C1q-deficient mice also revealed defective macrophage uptake of apoptotic cells (20). Similarly, the authors found that cultured monocyte-derived macrophages from hereditary C1q-deficient patients also demonstrated defective phagocytic uptake of apoptotic cells that could be restored with the addition of purified C1q in a dose-dependent manner. These lines of investigation support a role for C1q deficiency in promoting systemic autoimmunity and SLE through defective clearance of apoptotic material in what has been termed the “waste disposal hypothesis.”

The mechanism of C1q-mediated clearance remains unclear but likely involves specific recognition by receptors on macrophages and DCs for C1q or downstream ligands generated by complement activation. In addition to phagocytic clearance by macrophages, a role for DC-mediated clearance of complement- bound apoptotic cells has emerged in recent years. The opsonin iC3b, the major product of C3 cleavage, mediates tolerogenic uptake of apoptotic cells by macrophages and immature DCs. Opsonization of apoptotic cells with iC3b led to increased efficiency of uptake by human monocyte-derived macrophages (21) and immature DCs in vitro (22). Moreover, the exposure of immature DCs to opsonized apoptotic cells inhibited DC maturation, as shown by downregulation of MHC class II and CD86 as well as by DC resistance to subsequent maturation stimuli in the form of CD40L and lipopolysaccharide activation (22). These findings suggest that tagging of apoptotic material by complement components downstream of C1q can generate potentially tolerogenic DCs. Regarding C1q specifically, two recent studies have described C1q-mediated inhibition of IFN-α production through slightly different mechanisms. In both studies, C1q was required for serum-mediated inhibition of IFN-α production induced by RNA-associated ICs in human peripheral blood mononuclear cells; this inhibition was not induced when C1q-deficient patient serum was used (23, 24). Whereas Lood et al. (23) found that C1q could inhibit IFN-α production in purified plasmacy- toid DCs (pDCs), Santer et al. (24) observed that C1q attenuation of IFN-α production by pDCs required a monocyte-dependent mechanism through preferential binding of ICs by monocytes in the presence of C1q. Although the discrepancy in the mechanism of pDC inhibition requires

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future clarification, the common finding of a C1q-dependent reduction in IFN- α production remains promising. Thus, C1q- mediated clearance of apoptotic material may function at multiple levels to prevent induction of systemic autoimmunity by altering availability and presentation of potential self-antigens.

Normal apoptosis is thought to be an important mechanism in the generation and maintenance of normal immune tolerance (25). Thus, complement-mediated uptake of self-antigen might contribute importantly to tolerance through negative selection of autoreactive T and B cells (reviewed in 26, 27). One study has addressed this using transgenic expression of an intracellular neo-self-antigen (mHEL-KK) and a corresponding high-affinity antigen-specific Ig (MD4 $Ig^{HEL}$) to examine B cell selection in C1q-deficient mice. In the absence of C1q, increased positive selection of autoreactive B1 B cells by intracellular self-antigen with an increased number of plasma cells and IgM autoantibody secretion was observed along with a concomitant reduction in negative selection of conventional B cells (28). Because self-antigen in this model is intracellular and becomes exposed during apoptosis, this example underscores another potential mechanism by which C1q-mediated clearance may protect against autoimmunity.

Although C1q clearly contributes to clearance of apoptotic debris, several questions still remain unanswered within this model. How does the binding of complement invoke clearance of apoptotic material rather than activation of an inflammatory response? Why does C1q deficiency lead to systemic autoimmunity and SLE in comparison with deficiency of the common complement pathway component C3, where immunodeficiency dominates? The potential for autoantigen generation in apopto- sis has been well established (reviewed in 29), and apoptotic cleavage, especially of ubiquitous nuclear self-antigens, could present neo-selfantigens capable of stimulating an immune response. Future studies aimed at the biology of C1q-mediated antigen uptake and presentation will be important in determining the relative contributions of these potential mechanisms.

## Induction of Type 1 Interferons and Systemic Autoimmunity

Type 1 interferons have gained increasing recognition in the pathogenesis of several autoimmune disorders, most notably in SLE, where a characteristic “interferon gene signature” is shared among the majority of lupus patients (30). The model of disease induction proposes that several mechanisms can lead to overproduction of type 1 interferons, which subsequently contributes to peripheral tolerance breakdown through the chronic activation of DCs. Activated or interferon-matured DCs can then mediate expansion of autoreactive B cells and activation of autoreactive T cells, including activation of cytotoxic $CD8^+$ cells with resultant increase in available apoptotic selfantigens. Uptake of apoptotic contents and nucleic acid-containing ICs by DCs and B cells leads to further amplification of the cycle and disease manifestations (reviewed in 31). How the overproduction of type 1 interferons is initiated in SLE, though, remains somewhat unclear.

A brief reflection on the activation of type interferons in normal host immunity may provide some insight into how defects in this system could lead to induction of autoimmunity. To combat viral infection, the innate immune system detects exogenous nucleic acids through two complementary systems to coordinate antiviral responses through induction of type 1

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interferons (Figure 1) (reviewed in 32). Within pDCs, Toll-like receptors (TLRs) can detect nucleic acids within endosomes of infected cells or in endosomal cargos from phagocytosed apoptotic cells to induce copious production of type 1 interferons. Independent of TLRs, intracellular detection of viral nucleic acids through the ubiquitous retinoic acid inducible gene-1 (RIG-1) and melanoma differentiation antigen 5 (MDA5) system of cytosolic RNA helicases as well as a currently unknown sensor of intracellular DNA also lead to activation of type 1 interferons. Because of the inherent difficulty in distinguishing exogenous viral nucleic acids from endogenous self-nucleic acids, these systems are vulnerable to breakdown, leading to the generation of autoimmunity. As we discuss below, two rare diseases have recently shed light on the relevance of these molecular mechanisms of interferon activation in SLE.

## Spondyloenchondrodysplasia with Immune Dysregulation: Bones, Brains, and Antigen-Presenting Cells

First described as primarily a disorder of bone development, spondyloenchondrodyspla- sia with immune dysregulation (SPENCDI, OMIM 607944; formerly SPENCD, OMIM 271550) is a rare skeletal dysplasia associated with a syndrome CNS and autoimmune manifestations (33).

**Clinical and genetic features.—**Individuals with SPENCDI develop persistent islands of cartilaginous tissue within the bone and have short stature. Frequently, the bony phenotype is accompanied by neurological signs with cerebral calcifications, atrophy, spasticity, or mental retardation and also by an increased incidence of autoimmunity. Autoimmune manifestations include thrombocytopenia, hemolytic anemia, and florid SLE or features of SLE such as ANAs, anti-DNA antibodies, and lupus nephritis. Recognition of the significant overlap in this syndrome with SLE prompted investigation into the genetics of this disorder.

Although only 24 cases have been reported to date, genome-wide genotyping and linkage mapping of homozygosity within families led to the independent identification of the *Tartrate-resistant acid phosphatase* gene *(TRAP,* also known as *ACP5)* as the causative defect of SPENCDI by two groups (34, 35). Mutations in affected cases were present in homozygous or compound heterozygous states and segregated with disease within families with parents heterozygous for the corresponding mutations. Notably, none of the patient mutations identified in the gene was found in either 210 and 228 alleles from controls in the two studies (34, 35). The presence of biallelic loss-of-function mutations in *TRAP* points to loss of TRAP activity as the cause of disease in SPENCDI patients.

**Molecular mechanism.—**TRAP is an abundant metalloenzyme phosphatase expressed in osteoclasts and in myeloid-lineage macrophages and DCs. Because these cells share a common myeloid origin, a single mutation in TRAP could mediate the seemingly unrelated bony and immune phenotypes of SPENCDI. TRAP is found intracellularly in lysosomes as well as secreted in two isoforms: TRAP 5a, produced by macrophages and DCs, and TRAP 5b, produced by osteoclasts. Well studied for many years in bone biology, TRAP is a marker of osteoclast bone remodeling activity and is elevated in the serum during states of increased

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bone resorption (reviewed in 36). The identification of *TRAP* as the cause of the skeletal abnormalities in SPENCDI is supported by the highly similar skeletal dysplasia in Trap-deficient (*Acp5*$^{-/-}$) mice (37).

Although Trap-deficient mice do not develop overt autoimmunity, two series of observations suggested a possible role for TRAP in the generation of autoimmunity in SPENCDI. First, Trap-deficient mice display disturbances in macrophage function with increased secretion of proinflammatory cytokines (IL-12 and IL-1|3) by Trap-deficient macrophages upon lipopolysaccharide stimulation (38). Second and more importantly, the major substrate of TRAP activity in vivo is osteopontin (OPN), a highly phosphorylated multifunctional protein that has gained recognition as an immune modulator. Extracellular secreted OPN acts as a matrix protein in bone and undergoes thrombin cleavage to allow binding of integrins and CD44 molecules. In this way, extracellular OPN can act as a cytokine/chemokine to regulate adhesion, migration, and activation in osteoclasts and immune cells (39). Significantly, the intracellular isoform of OPN is also active in several cell processes and was recently shown to be required for IFN-α production by pDCs through TLR9-MyD88-mediated signaling (40).

Building on the observations in the mouse model, two lines of evidence suggested that inputs from innate immunity could drive the autoimmunity seen in SPENCDI. As a major downstream target of a loss of TRAP activity, changes in OPN levels or activation pose an attractive explanation for the immune phenotype. Lausch and colleagues (35) noted an increased frequency of highly phosphorylated forms of OPN in the urine of SPENCDI patients. On closer examination, they found that TRAP-deficient DCs from patients displayed reduced ability to dephosphorylate OPN and secreted increased amounts of OPN in culture. The authors also found an increase in proinflammatory cytokine production by DCs derived from SPENCDI patients compared with controls. Noting the increase in cytokine production of Trap-deficient mice in light of the known role for IFN-α in promoting SLE, the complementary study of Briggs et al. (34) investigated the activation of type 1 interferons in SPENCDI patients. They found that all subjects exhibited not only increased serum levels of IFN-α but also an “interferon signature” of type 1 interferon-stimulated genes on whole-transcriptome microarray that was virtually identical to those of SLE patients (34).

Taken together, the two studies provide a model of TRAP function to mediate both the bony and immune phenotypes of SPENCDI (41). Loss of TRAP function leads to decreased ability to dephosphorylate and inactivate OPN. Accumulation of extracellular phosphorylated OPN and the inability of osteoclasts to remodel bone lead to bone and cartilage abnormalities. At the same time, accumulation of intracellular phosphorylated OPN leads to tonic or exaggerated signaling through TLR9 in pDCs and increased IFN-α production. As hypothesized in SLE, the increase in IFN-α production can induce systemic autoimmunity by increasing antigen presentation, stimulating differentiation of myeloid and B cells, and enhancing autoantibody production to produce the autoimmune manifestations seen in SPENCDI. Aside from its role in SPENCDI, TRAP may have a role in the pathogenesis of other autoimmune diseases such as rheumatoid arthritis and multiple sclerosis in which OPN has been implicated (reviewed in 36). Additionally, the observation of defective antigen

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presentation by TRAP-deficient DCs highlights other potential mechanisms of autoimmune pathogenesis in the absence of TRAP that awaits further investigation.

## Aicardi-Goutières Syndrome: Nucleotide Overload and Innate Immune Activation

Named for the two neurologists who reported the first case series in 1984, Aicardi-Goutières Syndrome (AGS; OMIM 225750) is a devastating neurologic condition of infancy that can rapidly progress to death. Originally described as a syndrome of severe brain atrophy with basal ganglia calcifications, the presence of "inflammatory" signs of chronic lymphocytosis in the cerebrospinal fluid (CSF) and elevations of IFN-a mimicked congenital viral encephalitis despite the lack of detectable viral infection (42, 43). However, the pattern of recessive inheritance and familial consanguinity of many affected subjects suggested a genetic cause of the disease. Intriguingly, several individuals presented with chilblains, areas of painful inflammation in small skin blood vessels of the extremities due to rapid warming following cold exposure. The presence of chilblains, which are seen in cutaneous lupus, as well as other lupus-like symptoms in these cases suggested a link to SLE and autoimmune disease (44).

**Clinical and genetic features.—**AGS is diagnosed by onset within the first year of life, with progressive neurological dysfunction, intracranial calcifications involving the basal ganglia, and lymphocytosis or IFN-α elevation of the CSF (reviewed in 45). Neurologic dysfunction can include progressive microcephaly, spasticity, dystonic posturing, and profound psychomotor retardation. Aside from symptoms affecting the nervous system, subjects may also display thrombocytopenia, hepatosplenomegaly, elevated transaminases, and intermittent fevers. The extraneurologic manifestations, especially the observation of frank SLE and lupus-associated conditions, such as ANAs, hemolytic anemia, and chilblains, suggested that disease pathogenesis could arise from an aberrant immune response.

Despite the strong familial pattern of inheritance, the syndrome shows both phenotypic and genetic heterogeneity (reviewed in 46). The autosomal recessive inheritance prompted the search for a causative gene defect, and in 2006 Crow and colleagues (47, 48) defined complementary mutations in two sets of genes in AGS patients. Using an impressive combination of linkage analysis, genome-wide SNP arrays, familial homozygosity mapping, and positional cloning, the authors defined four loci that all give rise to AGS. The *TREX1* gene (also known as *AGS1* or *DNaseIII)* encodes a 3'→5' exonuclease (DNaseIII) that constitutes the major DNA-specific 3'-exonuclease in mammalian cells. Homozygous or compound heterozygous mutations in *TREX1* were found in AGS patients among 10 families and were allelic to Cree encephalitis, a variant syndrome now recognized to be part of AGS. The mutations segregated with disease and were absent in 160 control alleles. All mutations were predicted to inactivate TREX1 based on changes to key amino acid residues or premature truncations, and the absence of TREX1 exonuclease activity in cell lines derived from AGS patients— though not from their unaffected family members—confirmed a loss of function (48).

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In 24 other AGS families, disease was attributable to mutations in one of three subunits of the ribonuclease H2 (RNaseH2) complex, encoded by the *RNaseH2B, RNaseH2C,* and *RNaseH2A* genes *(AGS2, AGS3,* and *AGS4,* respectively). RNase H enzymes are endonucleases that catalyze the cleavage of ribonucleotides from RNA:DNA duplexes and are proposed to function in DNA replication through removal of lagging-strand RNA primers. Two major types of RNase H exist in mammals with distinct cleavage patterns and functions; RNaseH2 is the major source of enzyme activity in both yeast and mammals (reviewed in 49). As expected, affected individuals were either homozygous or compound heterozygous for mutations in one of the three subunits that segregated with disease. The spectrum of mutations identified in these genes occurred almost invariably in highly conserved amino acid residues and suggested hypomor- phic rather than complete loss of RNaseH2 function. Using a fluorometric oligonucleotide assay, the investigators demonstrated the ability of wild-type complexes of RNaseH2 to specifically cleave single and oligonucleotide RNA bases from RNA:DNA duplexes. The combination of a mutant RNaseH2A subunit found in an AGS family with other wild-type subunits had no effect on complex stability but led to a dramatic reduction in enzymatic activity to near baseline levels (47).

Since the initial identification of TREX1 and RNaseH2 mutations, further characterization of affected families have revealed a fifth causative locus, *SAMHD1,* in AGS (50). The SAM domain and HD domain-containing protein 1 (SAMHD1) was originally identified from a human DC library as the ortholog of the mouse IFN-γ-induced *(Mg11)* gene, hence its early designation as DC-derived IFN-γ- induced protein (DCIP) (51). Although the function of the protein remains unknown, it has been implicated in mediating tumor necrosis factor-α proinflammatory responses (52), and its homology to HD domain genes suggests a possible nuclease activity.

**Molecular mechanism.—**The clinical overlap of AGS with both congenital viral infections and SLE pointed to activation of the type 1 interferons as a potential unifying mechanism of disease. Because discrimination between self and exogenous viral nucleic acids is imperfect by nature, accumulation of self-nucleic acids has long been considered a potential mechanism of autoimmune disease. Given that the identified mutations include two sets of enzymes involved in nucleotide degradation, Crow and colleagues (47, 48) hypothesized that TREX1 and RNaseH2 may function in common DNA- or RNA-processing pathways. They proposed that failure of these nuclease activities leads to survival and accumulation of intracellular nucleic acid intermediates capable of triggering a viral-like innate immune response, manifested by IFN-α production.

Support for this model comes from subsequent studies of Trex1 function in the mouse. Initial analysis of *Trex1*$^{-/-}$ mice showed no increase in spontaneous mutation rates or cancer incidence that would be predicted if Trex1 is required for DNA synthesis and repair, consistent with the lack of increased cancer risk in patients with AGS (53). Unlike AGS patients, the Trex1-deficient mice do not show any neurologic defects, but instead develop an inflammatory myocarditis with death due to circulatory failure, suggesting a potential role for Trex1 in autoimmunity

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Further investigations in *Trex1*$^{-/-}$mice have provided insight into the mechanism of disease pathogenesis. Noting the affinity of Trex1 for single-stranded DNA (ssDNA) (53), Yang et al. (54) found accumulation of ssDNA in the endoplasmic reticulum of *Trex1*$^{-/-}$cells. Labeling of mouse embryonic fibroblasts with $^{32}$P revealed the presence of a discrete 60- to 65-nucleotide long ssDNA that accumulated in *Trex1*$^{-/-}$cells but was absent from wild-type cells. Examination of primary human fibroblasts from AGS patients also revealed accumulation of extranuclear ssDNA, as seen in the mouse fibroblasts. Similarly, Stetson, Medzhitov, and colleagues (55) also observed accumulation of ssDNA in *Trex1*$^{-/-}$mice through an independent approach. Strikingly, they found that the increased ssDNAs seemed to be derived from accumulation of endogenous retroviral elements in Trex1-deficient cells compared with wild-type cells. In cells transfected with retroviral reporters, cotransfection with wild-type Trex1 blocked the mobilization of these elements, whereas cotransfection with mutant alleles of AGS patients had no effect. Endogenous retro-elements exhibit species- and tissue-specific differences in expression, and accumulation of these substrates in the absence of Trex1 activity may explain the differential spectrum of phenotypes in AGS patients as well as differences between mice and humans. These elements represent a unique case of the altered recognition of self- or non-self-nucleic acids and suggest that the original function of Trex1 may be in host defense against retroviral integration events.

The intracellular accumulation of self- nucleic acids suggested possible activation of cytosolic sensors linked to IFN-α production as the cause of autoimmunity. Indeed, *Trex1*$^{-/-}$ mice lacking either IRF3, an essential factor in the cytosolic activation of the interferon response, or the type 1 interferon receptor IFNAR1 were completely protected from disease and rescued from mortality (55). Thus, Trex1 acts in a cell-intrinsic manner to degrade endogenous ssDNA substrates and negatively regulate interferon production. In the absence of Trex1, accumulation of endogenous nucleic acids triggers chronic interferon stimulation, leading to lethal autoimmunity. Although similar roles for RNaseH2 or SAMHD1 have yet to be identified, these proteins likely also mediate nuclease activities with similar effects on nucleic acid metabolism. Altogether, the studies in the mouse provide a mechanistic link from nucleic acid accumulation to induction of an interferon response and autoimmunity.

**Beyond AGS: genetic links to SLE.—**Since the initial report of AGS mutations by Crow et al. (47, 48), numerous mutations in *TREX1* have been identified in AGS patients and in several other related forms of autoimmunity. Heterozygous mutations of *TREX1* cause both a dominant form of AGS as well as familial chilblain lupus, a monogenic form of cutaneous lupus that presents with acral ulcers of the skin precipitated by cold or wet exposure (56, 57).Notably, chilblains are also found in ~40% of AGS patients, providing further evidence of lupus-like autoimmunity in the syndrome. Rare mutations in TREX1 have also been identified in 0.5–2% of SLE patients (58, 59), upholding the paradigm that rare variants cause disease in a subset of patients with common heterogeneous disorders (see sidebar).

## Homeostasis of Peripheral Lymphocytes: Life, Death, and Regulation

Innate immunity constitutes one of the most ancient forms of defense and relies on recognition of pathogen-associated and damage-associated molecular patterns (PAMPs and

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DAMPs) to signal danger. The generation of systemic autoimmunity secondary to defects in innate immunity seems reasonable because these mechanisms and the self-antigens recognized are relatively ubiquitous. Although the development of adaptive immunity provides an enormously robust and dynamic defense system, the cost of recombinatorial diversity is an elaborate system of regulation from birth to selection and from activation to death to maintain tolerance. Central tolerance acts in the thymus and bone marrow through negative selection and receptor editing, resulting in clonal deletion, inactivation, or diversion of autoreactive T and B cells. Peripheral tolerance includes mechanisms of clonal deletion and apoptosis, anergy, and diversion as well as active suppression and ignorance to regulate autoreactive lymphocytes. With the unique specificity of B and T cells, specific defects in the regulation of adaptive immunity would allow the generation of organ-specific autoimmunity without incurring immunodeficiency. The following syndromes—autoimmune lymphoproliferative syndrome (ALPS); immune dysregulation, polyendocrinopathy, enteropathy, and X- linked (IPEX); autoimmune polyendocrine syndrome type 1 (APS1)—highlight how defects in processes of lymphocyte selection and homeostasis reveal critical mechanisms in the maintenance of tolerance.

## Autoimmune Lymphoproliferative Syndrome: The Good Death

First described by Canale & Smith (60) in 1967, autoimmune lymphoproliferative syndrome (ALPS; OMIM 601859) is a rare but classic disorder of immune tolerance breakdown. Patients with ALPS typically develop lymphadenopathy, hepatosplenomegaly, and autoimmune cytopenias, such as hemolytic anemia and thrombocytopenia. Although the marked lymphoproliferation of the original cases suggested lymphoma or leukemia, Canale & Smith recognized that the condition was not malignant but likely arose from “an immune response to a recognized auto-antigen, and the proliferation of abnormal cells, ‘immunocytes,’ might be responsible for the production of antibodies” (60).

Recognition of a possible genetic cause for ALPS did not come until later investigators recognized the phenotypic similarity of affected patients to *Ipr* and *gld* mutant mouse strains, which demonstrated lymphoproliferation, and coined the name for this novel syndrome (61). The discovery of the causative genes in the mouse led to rapid identification of mutations in similarly affected patients and highlighted for the first time the importance of apoptosis in the maintenance of immunologic tolerance. Continued investigations in humans and mouse models have led to rapid growth in our understanding of the clinical, genetic, and immunologic aspects of ALPS in the last 20 years.

**Clinical and genetic features.—**As our understanding of the molecular basis of ALPS has grown, we have gained increased recognition of the sophisticated and variable clinical spectrum of disease encompassed by this syndrome. Based on the growth of information in this field, the criteria for diagnosis of ALPS were redefined in a2009 NIH InternationalWorkshop (Table 1) (62). Onset typically occurs in infancy or childhood (median age 24 months); however, later onset of disease has been described as well. The most prominent feature of the syndrome is persistent lymphadenopathy and/or splenomegaly in the absence of infectious or malignant etiologies. Lymphadenopathy is nontender and chronic, though some regression can be seen with age. Lymphoproliferation is generally

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benign, but 10–20% of ALPS patients go on to develop cancers with a 14-fold and 51-fold increased risk of non-Hodgkin's and Hodgkin's lymphoma, respectively (63).

Autoimmunity is the second most prominent feature of ALPS, with autoantibody production (80%) or overt autoimmune disease (47%) in most patients (64). Autoantibodies can include anticardiolipin, ANAs, and anti-red blood cell (positive direct Coombs') antibodies. Autoimmune cytopenias are the most common autoimmune disorder, but several other systems can be affected including the eye (uveitis), kidney (nephritis), liver (hepatitis), joints (arthritis), nervous system (Guillain- Barre), and skin (dermatitis, urticarial rash) (64, 65). Thus, ALPS can potentially cause systemic and multiple types of organ-specific autoimmunity in a single patient.

Several diagnostic markers are associated with ALPS. The hallmark of the disease is the elevation in an unusual population of mature polyclonal $CD3^+TCR\alpha\beta^+CD4^-CD8^-$T cells termed double-negative T (DNT) cells (66). DNT cells represent a unique population of peripheral lymphocytes that are distinct from γδ T cells or double-negative thymocytes that are also $CD4^-$ $CD8^-$. Typically representing < 1–1.5% of lymphocytes in normal individuals, mild elevation of these cells is not pathognomonic for ALPS and can be seen in other autoimmune diseases such as SLE (62).

However, elevations of DNTs > 3% of lymphocytes are rarely seen outside ALPS patients, in whom levels can sometimes reach >40% (61, 62). Serum IL-10 is elevated in ALPS and is thought to arise from increased production by the expanded DNT compartment as well as from a skewed Th2 cytokine profile (67, 68). Serum vitamin B12 levels are also a surprisingly good screening test, as they are elevated in ALPS for unclear reasons (69, 70).

ALPS is a classic example of how a genetic syndrome can arise through a heterogeneous array of mutations resulting in a common phenotype, thus revealing the critical components of a signaling pathway. Following the identification of *Fas* and *FasL* mutations in *Ipr* and *gld* mice, respectively (71, 72), mutations in the corresponding human genes were identified as the cause of ALPS (73–76). Subsequently, several other members of the Fas-mediated apoptosis pathway were identified as causative mutations in ALPS (reviewed in 77). Thus, ALPS is a syndrome that can arise from more than one Mendelian trait.

Mutations in the *FAS* gene (also known as *TNFRSF6)* are the most common cause of ALPS, with >70 mutations scattered throughout the gene. Unlike the *Ipr* mouse, inheritance of ALPS in most *FAS* mutants follows a heterozygous autosomal dominant pattern. Because FAS signaling relies on formation of a homotrimeric cell surface receptor, one defective allele can produce a dominant negative effect (by generating only one in eight fully wild-type trimers). Disease severity and penetrance vary depending on an individual's *FAS* genotype (78), and even vary between family members carrying identical mutations. Heterozygous mutations in the intracellular domain of FAS affect the canonical death domain required for initiating the apoptotic cascade and are associated with a higher penetrance of ALPS (80%) than heterozygous mutations of the extracellular domain (30% incidence of ALPS). Accordingly, homozygous mutations in *FAS* usually result in loss of function as well as earlier and more severe disease. Notably, both germ-line and somatic

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mutations in *FAS* can lead to ALPS, with the latter specifically affecting the DNT cell compartment (70, 79).

Mutations in other members of the apoptotic signaling pathway are also responsible for ALPS, including *FAS Ligand (FASLG)* (75, 76) and *Caspase-10 (CASP10)*(80), highlightingthe central role of defective apoptosis in the syndrome. Still another 20% of patients that meet ALPS criteria remain without a known mutation. As genetic testing is becoming a common diagnostic tool, it is increasingly important to distinguish between pathogenic and polymorphic gene changes. Given the variable clinical presentation and penetrance of mutations in ALPS, confirmation of a link to disease in newly described mutations is critical. A database of known pathogenic *FAS* mutations is maintained online through the National Institutes of Health (http://www3.niaid.nih.gov/ topics/ALPS). With increased understanding of this pleomorphic syndrome, ALPS classification has been revised to center on the genes involved (Table 2).

A long-standing puzzle in the study of ALPS is the wide clinical heterogeneity seen with various mutations and the variable penetrance of disease among family members carrying the same genetic defect. Even in inbred mouse lines, the phenotype of *lpr* and *gld* mice varies by genetic background. Thus, much of this variability has been ascribed to other genetic or environmental modifiers. New data have begun to define other mechanisms that also contribute to the clinical heterogeneity of ALPS. Further reduction of FAS expression in patients carrying extracellular domain mutations can arise through nonsense-mediated RNA decay or protein instability (81), indicating that hap- loinsufficiency of FAS may produce the variable spectrum of clinical disease. Recently, ALPS patients carrying a heterozygous germ-line mutation of *FAS* were found to have acquired a second somatic *FAS* mutation within their DNT cells, demonstrating a loss of heterozygosity (82). Clinical expression of ALPS may follow a two-hit model, as has been well described in cancer. Thus, autoimmunity likely arises from accumulation of multiple genetic defects leading ultimately to a breakdown of tolerance mechanisms (83).

**ALPS-related disorders.—**Previously, several other lymphoproliferative conditions were included as subtypes of ALPS. With further characterization of genotypic-phenotypic associations seen with these ALPS-like conditions, there has been a move to reclassify these ALPS-related disorders based on recognition of infectious or malignant complications not seen in ALPS (Table 3). Mutations in *Caspase-8 (CASP8)* or *NRAS* genes were originally described as potential causes of ALPS (84, 85) and share the lymphoproliferation and apoptotic defects characteristic of ALPS. However, germ-line mutations of *CASP8* causing the Caspase-8 deficiency state (CEDS) exhibit immunodeficiency rather than autoimmunity, and somatic *NRAS* mutations causing Ras-associated autoimmune leukoproliferative disease (RALD) are associated with hematopoietic malignancies not seen in ALPS. Included now in this class are Dianzani autoimmune lymphoproliferative disease (DALD, unknown cause) and X-linked lymphoproliferative disease (XLP1, due to *SH2D1A* and *SAP* mutations) (86–88). Given their similarity to ALPS, these disorders likely reflect alternative and uncharacterized molecular defects of apoptosis in immune homeostasis and provide fertile ground for future investigation.

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### Molecular mechanism.

Like many monogenic syndromes of human disease, the identification of the causative genes in ALPS arose from the recognition of similar phenotypes in mouse models. Although the *lpr* and *gld* mice are often considered mouse models of SLE, they share the lymphoproliferation and DNT cell elevation seen in ALPS patients (reviewed in 89). A wealth of studies in these mouse models and others have dissected the immunologic mechanisms of ALPS and illuminated the role of apoptosis in the maintenance of peripheral tolerance.

Appropriate cessation and contraction of the immune response is necessary to maintain homeostasis and prevent autoimmunity, as discussed in a recent review (77). In the case of T cell activation, the presence of high antigenic load can stimulate Fas-mediated cell death by upregulation of the expression of FasL on the T cell, leading to the ligation of Fas receptors and triggering active apoptosis. Expression of FasL on T cells can in turn induce death in Fas-expressing B cells and antigen-presenting cells (APCs). After the clearance of antigen and decrease in cytokine production, the remaining T cells can undergo passive apoptosis through the intrinsic pathway (lymphokine withdrawal). Thus, antigen-driven T cell death provides a cell-autonomous mechanism of negative feedback control to balance the proliferative response to antigen. Loss of this feedback control can lead to activated T cell accumulation and the potential for autoimmunity, especially in the face of persistent stimulation by self-antigens.

In ALPS, lymphoproliferation and accumulation of DNT cells result from the loss of Fasmediated apoptosis. However, the relative contribution of these defects to the generation of autoimmunity is not clear, and the role of DNT cells in disease pathogenesis has been debated. DNT cells are thought to arise from previously activated polyclonal $CD8^+$ cells (66). Although the cells demonstrate resistance to apoptosis in vivo, they are paradoxically unresponsive to proliferative and activating stimuli in vitro and often die in culture. The best evidence for the pathogenic nature of these cells is the presence of somatic mutations of *FAS* in ALPS patients that are confined to the DNT population; these patients exhibit the same lymphoproliferative and autoimmune features of individuals with germ-line *FAS* mutations (70, 79, 90). New evidence has recently emerged to clarify the role of these cells in autoimmunity. Eomeso- dermin (Eomes) is a T-box transcription factor that plays critical roles in Th differentiation and CD8 effector cell function. Expression of Eomes is markedly elevated in DNT cells from both *lpr/lpr* mice and humans with ALPS, and specific deletion of *Eomes* in the T cell lineage of Fas-deficient (pr//pr)micemarkedly reduces lymphoproliferation and restores DNT cells to wild-type levels (91). Significantly, though, the mice continue to develop humoral autoimmunity, indicating that other Fas-deficient immune compartments also contribute to disease.

The role of APCs in ALPS disease has gained increasing recognition in recent years. Apoptotic defects can be seen in DCs of ALPS patients (80), and mice with conditional inactivation of Fas in B cells or DCs exhibit evidence of autoimmunity but lack DNT cell expansion (92, 93). Inactivation of Fas in DCs only or in B cells only is sufficient to produce systemic autoimmunity, with high-titer ANAs, hyper- immunoglobulinemia, and splenomegaly (93). Although defective apoptosis of autoreactive B cells can contribute to

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humoral autoimmunity, the common phenotype of the two mouse lines suggests that defective apoptosis of APCs is sufficient to mediate autoimmunity. Thus, chronic antigen presentation, from either activated DCs or B cells that fail to undergo apoptosis, can lead to a breakdown in tolerance.

## MONOGENIC ORGAN-SPECIFIC AUTOIMMUNITY

As outlined above, organ-specific autoimmune syndromes are characterized by an autoimmune response to tissue-specific self-antigens (TSAs). Although organ-specific autoimmunity is a relatively common clinical problem, monogenic forms are exceedingly rare. This likely reflects the wide number of checks and balances that help prevent such untoward responses. Here we highlight two of the known monogenic forms of organ-specific autoimmunity: IPEX and APS1.

### Immune Dysregulation, Polyendocrinopathy, Enteropathy, and X-Linked: Tolerance Dominates

Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX, OMIM 304790) was first described in 1982 by Powell and colleagues (94), who identified a large kindred in which eight males developed a syndrome characterized by diarrhea, eczema, diabetes, thyroid autoimmunity, and exaggerated responses to viral infections. Inheritance of the syndrome in this large kindred strongly suggested an X-linked pattern, with an additional males having died early in life. Multiple groups later reported similar male patients from different ethnic backgrounds (95–98).

**Clinical and genetic features.—**Recently, Ochs et al. (99) compiled the common features of this syndrome from 12 independent reports involving 39 patients. The most common presenting symptoms are the triad of diarrhea, autoimmune diabetes, and skin disease. Watery diarrhea appears in nearly 100% of patients, whereas diabetes and skin disease occur in about 75% and 60% of patients, respectively. In addition to these major features, autoimmune thyroiditis and susceptibility to recurrent infection are also relatively common. Infectious disease susceptibility is not a unilateral feature and may be related to barrier dysfunction in the skin and gut, rather than to a direct immune deficit. On laboratory testing, patients frequently demonstrate elevated IgA and IgE and also often have eosinophilia (98, 100, 101). Autoantibodies are frequently detected in patients and often include those targeting pancreatic islets, thyroid, erythrocytes, platelets, and the small intestine (98, 99, 101).

In 2000, two groups mapped the IPEX syndrome to the Xp11.23-Xq.13.3 region of the X chromosome (98, 102). Further inroads toward identifying the defective gene came from the observation of a somewhat similar phenotype in the *Scurfy* mutant mouse strain that also demonstrated X-linked inheritance. Through a positional cloning approach, Ramsdell and colleagues (103) identified *Foxp3,* a forkhead/winged-helix family transcription factor, as the defective gene in the *Scurfy* strain. Extending these findings into the human syndrome, two groups identified the human ortholog of *Foxp3* as the defective gene in IPEX (104, 105). As shown in Figure 2, the human FOXP3 protein has several structural domains seen in transcription factors, including a fork- head domain (FKH), a leucine zipper, a zinc finger

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domain, and a repressor domain. Since the identification of *FOXP3*, numerous mutations have been described in affected patients (100, 101, 104–107). Missense mutations associated with the disorder cluster in the FKH, leucine zipper, and repressor domains of the protein, supporting the likely functional importance of these domains for gene function (107, 108). No reliable phenotype has been established in female carriers of mutations; however, these carriers do exhibit skewing of gene expression to the wild-type allele of FOXP3 in their $CD4^+CD25^+$ T cells (109).

**Molecular mechanism.—**The IPEX syndrome contributed to the identification of Foxp3 as a key regulator of dominant tolerance. The severe symptoms ofthe disease emphasize the importance of the actions of Foxp3 in maintaining tolerance and have helped improve our understanding of the molecular control of regulatory T cells. Patient mutations have also proven useful in furthering our understanding of molecular interactions in FOXP3.

Several years after the identification of *FOXP3* as the defective gene in IPEX, a clearer picture of its role in immune tolerance was revealed through several landmark studies establishing its critical role in $CD4^+CD25^+$ regulatory T cells (Tregs) (110–112). Suppressor activity by this T cell population had already been appreciated, and these studies mapped high levels of expression of Foxp3 to Tregs and also demonstrated that Foxp3-deficient *(Scurfy)* $CD4^+CD25^+$ T cells lost suppressor activity. Furthermore, transduction of *Foxp3* into $CD4^+CD25^-$ T cells could confer suppressor activity, supporting a model in which Foxp3 is a master regulator of Treg function. In mice, adoptive transfer of Foxp3-sufficient Tregs into Foxp3-deficient hosts helps ameliorate autoimmunity and lymphoproliferation, demonstrating a dominant form of tolerance regulated by Foxp3. Interestingly, the most promising treatment to date for IPEX patients appears to be allogeneic bone marrow transplantation, which is consistent with this model (113–118). Moreover, Treg activity seems to be required throughout life, as selective ablation of Tregs, even in adulthood, can lead to lymphoprolifera- tion and autoimmunity in mouse models (119). The discovery of Foxp3 in Tregs has opened a large and intensive field of study too broad for any single review (108, 120, 121). In discussing the molecular mechanisms of this unique gene, we highlight here the areas relevant to human Tregs and the IPEX syndrome.

Several studies have demonstrated that FOXP3 expression maps to $CD4^+CD25^+$ Tregs in both humans and mice and is considered a key marker for this subset. In humans, expression of the IL-7 receptor (CD127) can discriminate between activated $CD4^+CD25^+$ T cells that are $CD127^{hi}$ and $FOXP3^+$ Tregs that are $CD127^{low}$ (122, 123). It is important to note, however, that FOXP3 is also transiently expressed in activated T cells in humans (124, 125). The significance of this transient expression is unclear, and the suppressive activity of such cells remains an area of debate (126). One tool that has been used to distinguish such activated cells from Tregs is the methylation status of the *Foxp3* promoter (127, 128), as Tregs appear to have more complete demethylation of the *Foxp3* promoter than do activated T cells. Whether defects in the activated population of T cells in the IPEX syndrome are also in play in the clinical disease remains to be determined, and further study is warranted. Although Tregs express high levels of CD25 (the IL-2 receptor), they do not produce IL-2 and are thus strongly dependent on exogenous IL-2 for their maintenance in vivo (129, 130). Interestingly, defects in IL-2 production or IL-2 blockade can lead to autoimmunity (131–

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134). This observation is further bolstered by the recent description of a patient with the clinical features of IPEX but with a mutation in *CD25* rather than *FOXP3*(135).

Further complexity within human Treg populations that express FOXP3 is likely. Using the combination of CD45RA and FOXP3 expression, Sakaguchi and colleagues (136) have suggested that humans may harbor several distinct $FOXP3^+$ populations. They describe $CD4^+FOXP3^{hi}CD45RA^-$ cells as effector Tregs (eTregs) that suppress efficiently in culture but demonstrate low proliferative capacity. In contrast, a second Treg population that is $CD4^+FOXP3^{low}CD45RA^+$ is designated as natural Tregs; these cells can also suppress in vitro but proliferate and expand in culture and appear to differentiate toward the eTreg phenotype. Distinct Treg populations also arise both through development in the thymus and through peripheral mechanisms (137, 138). Recently, Helios has been identified as a potential marker that distinguishes the two populations in both mice and humans (139). Additionally, inflammatory conditions may affect peripheral Treg generation, given that recent initial studies have demonstrated IFN-γ-secreting $FOXP3^+$ cells in patients with multiple sclerosis or type 1 diabetes (140, 141). The significance of the functional and phenotypic plasticity of these Treg lineages in humans has yet to be discovered. Currently, the exact contribution of adaptive (peripheral) and thymically derived Tregs to maintaining tolerance in humans is unclear, as are the conditions under which each population is critical. Further development of cell surface markers that distinguish the populations will be needed to help answer this question.

Treg suppression of immune responses appears to be a complex process, and numerous mechanisms have been proposed (142). The mechanisms in play are likely to involve both soluble factors and direct contact mechanisms on other cells. Soluble factors that have been implicated in Treg suppression include the cytokines IL-10, TGF-β, and IL-35 (143— 145). IL-35 expression in human Tregs was initially discounted (146); however, a recent report has demonstrated that IL-35 expression can be found in human Tregs, where it plays a role in Treg suppression and potentially induces infectious tolerance (147). IL-2 consumption mediates Treg suppression in the mouse (148) but does not appear to play the same role in human Tregs (149). Regarding contact-dependent mechanisms, recent studies in mice have shown that CTLA-4 expression on Tregs plays a role in suppression by competing for costimulatory ligands (150, 151). Human Tregs also express high levels of CTLA-4, and it will be interesting to determine if a similar mechanism is in play in human Tregs.

How Foxp3 operates at a molecular level and how it helps confer a suppressive phenotype to T cells have been areas of intense investigation (108). Genome-wide chromatin immunoprecipitation (ChIP) experiments have shown that Foxp3 directly binds to a number of important target genes, including *CD25, CTLA-4, IL-10,* and *IL-2* (152, 153). Foxp3 also binds to several other transcription factors, including NFAT, Runx1, RORγ, and RORα (154–158). Foxp3 appears to coordinate with NFAT to target a number of genes, including *IL-2,* for repression (159, 160). Consistent with this, Marson et al. (152) found that NFAT targets were enriched in their genome-wide ChIP experiments. Recently, the study of IPEX-associated missense mutations illuminated a unique property of the FOXP3 FKH to participate in domain swapping through the formation of a domain-swapped multimer capable of bridging large regions of DNA (161). Although these disease-associated

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mutations lie in the FKH, they appear not to affect the ability of the domain to bind to DNA, but rather diminish formation of domain-swapped dimers that appear critical to FOXP3's suppressive function. Thus, higher-order complex formation of FOXP3 could be involved in recruitment and tethering of widely separated DNA regions, and further study in this area is warranted.

IPEX arises essentially from the loss of a transcription factor that encodes a general program of cell-nonautonomous tolerance, yet it displays remarkable organ-specific autoimmunity. The spectrum of autoimmunity in IPEX is distinct, and, interestingly, the common autoimmune features of the disorder differ from those seen in APS1. For example, why IPEX patients have such a strong penetrance of type 1 diabetes is not completely understood. Future studies on molecular mechanisms of FOXP3 as well as investigations into the normal Treg repertoire may hold the answers.

## Autoimmune Polyendocrine Syndrome Type 1: Tissue-Specific Tolerance Mechanisms

As shown for the syndromes discussed so far, the development of systemic autoimmunity or generalized autoimmune manifestations can be explained by defects in genes that play a role in widespread processes of self-antigen degradation, immune cell death, and immune cell regulation. Many autoimmune diseases affect only a single site or organ system, however, and most individuals have only a single disease. How do we explain a specific hole in tolerance?

One of the early clues to how tissue-specific loss of tolerance could arise came from the studies in type 1 diabetes. Investigators noted that rat insulin-promoter-driven transgenes showed low levels of expression within the thymus (162–164)—levels similar to endogenous insulin expression within the thymus. Studies of human genetics in diabetes have identified a variable number of tandem repeats (VNTR) element associated with the insulin gene (*INS*) promoter as one of the highest risk alleles for disease aside from the HLA itself (165, 166). Longer repeats correlated with increased levels of insulin expression within the thymus and reduced risk of diabetes. Surprisingly, the expression levels of a peripheral antigen within the thymus seemed linked to disease. These observations led Hanahan (167) to propose that the ectopic expression of peripheral antigens within the thymus could mediate antigen-specific tolerance and protect against autoimmunity.

**Clinical and genetic features.—**Autoimmune polyglandular syndrome type 1 (APS1, formerly known as APECED, OMIM 240300) is a highly variable syndrome of autoimmunity affecting a wide range of organ systems. The first published cases of the syndrome come from the early 1940s (168–170), although records of cases dating to 1910 can be found in Finland (171). Classically, APS1 is diagnosed by the presence of at least two of three hallmark conditions: autoimmune hypoparathyroidism, autoimmune adrenal insufficiency, and mucocutaneous candidiasis. Development of multiple organ-based autoimmune diseases begins in childhood and can involve a diverse array of conditions, including thyroiditis, type 1 diabetes, gastritis, ovarian failure, hepatitis, and vitiligo (reviewed in 172, 173). Disease is quite pleomorphic, and affected siblings carrying the same mutations can develop divergent spectrums of autoimmune disease. The classic

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evolution of disease, as described in the large Finnish cohort of patients, begins with early signs of mucocutaneous candidiasis as early as 1 year of age that can wax and wane, followed by onset of hypoparathyroidism in early childhood, and subsequent additional autoimmune manifestations such as adrenal insufficiency (172). However, in other populations disease onset and pattern of presentation are more variable. Autoimmunity is manifested by lymphocytic infiltrates of affected organs as well as by autoantibodies to antigens specific to affected tissues (174). Notably, APS1 patients do not typically exhibit conditions associated with systemic autoimmunity or SLE, such as ANAs.

Although only approximately 500 cases have been reported worldwide, APS1 occurs with increased frequency in certain ethnic populations of Iranian Jews (1:9,000), Finns (1:25,000), Sardinians (1:14,000), Norwegians (1:90,000), and Irish (1:130,000), likely reflecting individual founder mutations (175–179). True prevalence is difficult to assess because clinical diagnosis can be challenging with the recognition of atypical cases and the evolution of disease over time. Surprisingly, high-titer autoantibodies to type 1 interferons (chiefly IFN-α and IFN-ω) are found in almost 100% of APS1 patients, regardless of age or ethnic background (180,181), and can provide a diagnostic marker in patients who do not meet classical criteria (182, 183).

With gradual identification of these rare patients, an autosomal recessive pattern of inheritance (176) became clear, and linkage analysis of 14 affected Finnish families mapped the causative gene to chromosome 2lq22.3 (184). Through a massive positional cloning effort, in 1997 two groups simultaneously identified the *Autoimmune Regulator* gene *(AIRE)* as the responsible gene (185, 186). *AIRE* encodes a 545 amino acid protein that contains several domains seen in traditional transcription factors (Figure 3), including a homogeneously staining region (HSR) domain, followed by a SAND domain and two plant homeodomain (PHD) domains. Additional transcriptional coactivator motifs (LXXLL) are interspersed along the protein, and a putative caspase-recruitment domain (CARD) lies within the N-terminal HSR domain. AIRE is expressed primarily in lymphoid organs, with the highest expression in the thymus; however, low levels of expression can be found in the lymph node, spleen, and even gonads (187, 188). Within the thymus, AIRE is found almost exclusively within medullary thymic epithelial cells (mTECs), one of the first clues about its role in tolerance (189).

Almost all APS1 patients bear homozygous or compound heterozygous mutations scattered throughout the gene (a database of known mutations is maintained at the Institute of Biomedical Technology, http://bioinf.uta.fi/AIREbase) (190). More than 60 distinct mutations have been identified to date, but no clear genotypic-phenotypic associations have emerged (178, 187, 191, 192). A single autosomal dominant variant has been reported in an atypical Italian kindred displaying a high penetrance of thyroiditis without the classic presentation of APS1 (193). The mutation is predicted to disrupt the SAND domain of AIRE, which is thought to mediate both protein dimerization and recruitment to DNA. The presence of a single copy of the novel G228W mutation in both humans and mice results in multi-organ autoimmunity through a dominant-negative effect (193, 194), highlighting the importance of this domain in normal AIRE function.

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The diverse range of autoimmunity seen in APS1 patients raises questions about other genetic and environmental influences in Aire- mediated tolerance. Expression of disease varies within families carrying the same mutations and among *Aire*$^{-/-}$mice on different inbred genetic backgrounds (195), indicating a role for other genetic modifiers. Studies of genetic interactions have consistently identified the MHC as the strongest modifier of APS1 in mice and humans (195–197), but other significant risk alleles await future discovery (see sidebar, A Fresh Look at Rarity in the Genetics of Autoimmunity). Environmental effects can be difficult to assess, but multiple environmental danger signals known to activate innate immunity failed to have an effect on Aire-mediated autoimmunity (198).

**Molecular mechanism.—**The discovery of AIRE as the cause of APS1 has stirred intense interest and investigation into its cellular and molecular mechanisms, as described in recent in-depth reviews (199, 200). Our knowledge of AIRE's function stems from critical studies in mouse models that have revealed its role in the negative selection of autoreactive T cells to maintain immunologic tolerance. Inactivation of *Aire* in the mouse mirrors the disease seen in APS1 patients. *Aire*$^{-/-}$mice develop multi-organ autoimmunity characterized by lymphocytic infiltrates and tissue-specific autoantibodies targeting several sites shared with humans, including the stomach, liver, pancreas, adrenals, and thyroid (201–203). Substantial emerging data supported the thymus as a site of "promiscuous" or ectopic expression of thousands of TSAs to represent the peripheral self to developing T cells (204, 205). In this way, autoreactive T cells encountering peripheral self-antigens could be deleted through negative selection (reviewed in 206). Because Aire expression within the thymus is restricted to mTECs, investigators hypothesized that Aire promotes the expression of self-antigens to mediate deletional tolerance of self-reactive developing thymocytes. Microarray analysis of purified mTECs revealed that expression of a multitude of TSAs is dependent on Aire and virtually absent from *Aire*$^{-/-}$mTECs (201, 205). These TSAs include transcripts such as insulin, P450scc, and the acetylcholine receptor, known targets of human organ-specific autoimmunity. Other TSAs such as CRP and GAD remained unaffected by loss of Aire, suggesting that other factors may also mediate TSA expression. Indeed, variable expression from the *CHRNA1* locus is both AIRE- and IRF8-dependent and correlates with risk of myasthenia gravis in humans (207).

Linkage of organ-specific autoimmunity to loss of Aire-dependent antigen expression in the thymus has been demonstrated for the stomach (208), lung (209), and eye (210), confirming Aire's role in promoting tissue-specific tolerance. Studies utilizing TCR-transgenic mice that express neo-self-antigens further demonstrated that Aire-dependent expression of the neo-self-antigen leads to clonal deletion of developing antigen-specific thymocytes (201–213). Consistent with its role in thymic tolerance, autoimmunity in *Aire*$^{-/-}$miceispre-dominantly T cell mediated, although generation of antigen-specific autoantibodies through cognate T cell help is also observed (214, 208). Additional roles for Aire within the thymus have centered on its contribution to antigen presentation by thymic DCs and to a potential effect on generation of Tregs, discussed in detail elsewhere (200). Altogether, studies in mice and humans point to a central role for Aire in the regulation of TSA expression to mediate negative selection of self-reactive T cells, thus preserving immune tolerance.

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The specific mechanisms by which Aire promotes TSA expression remain the topic of intense study and are more thoroughly reviewed elsewhere (199, 215). Briefly, several lines of evidence suggest that Aire is recruited to specific regions of chromatin (205, 216, 217) to mediate transcriptional activation. Although a direct DNA-binding capability for Aire remains unclear, Aire appears to interact with multiple components of transcriptional machinery, including nuclear transport, chromatin binding/structure, transcription, and pre-mRNA-processing proteins, to facilitate gene expression (218). Additionally, Aire recognizes TSA target genes through interactions with histones and histone marks mediated through binding of the PHD1 domain to hypomethylated histone H3 tails (H3K4Me0) that are associated with transcriptionally inactive loci (217, 219–221). Much work is still needed to flesh out these mechanisms, and further investigations to define the molecular actions of Aire will be important for this uniquely promiscuous transcriptional activator.

In recent years, evidence has emerged describing a potential role for Aire in the maintenance of tolerance outside the thymus (222). Initial studies in the mouse found Aire expression at low levels in peripheral lymphoid tissue, including lymph nodes and spleen (201, 223, 224). Although the expression pattern and function of Aire in the periphery remain controversial, two groups have recently reported further evidence for a role of peripheral Aire action. Using an Aire-promoter-driven GFP reporter, Gardner et al. (225) identified $GFP^+$ extrathymic Aire-expressing cells (eTACs) within $CD45^{lo}$ MHC class $II^+$ lymphoid stromal tissue, localizing in part to the T-B cell zone of lymph node follicles. Using an independent approach, Lee et al. (226) found a heterogeneous $CD45^{lo}$ stromal population in the lymph node cortex that includes Aire- expressing cells. Notably, characterization of lymph node stromal populations using gp38 and CD31 markers identifies four major subsets (227, 228), including fibroblastic reticular cells ($gp38^+$ $CD31^-$) and lymph node endothelial cells ($gp38^+CD31^+$), as well as double-negative ($gp38^-CD31^-$) cells that are most enriched for Aire expression. Through use of transgenic expression of self- or neo-self-antigen in the peripheral stromal cells, both groups demonstrated the deletion of antigen-specific $CD8^+$ cells mediated by eTACs (225) or the fibroblastic reticular cells (226, 228), respectively. Like mTECs, both eTACs and the lymph node stromal cells exhibited promiscuous self-antigen expression, and gene-expression profiling of $GFP^+$ eTACs revealed a set of Aire-dependent. TSAs distinct from those found in the thymus (225). The differential expression of Aire- regulated TSAs between mTECs and eTACs suggests that these systems may be complementary in the deletion of a different range of autoreactive T cells. Remarkably, similar $CD45^{lo}$ MHC class $II^+$ AIRE-expressing cells have been described in human peripheral lymphoid tissue; these cells express some TSAs as well as indoleamine 2,3-dioxygenase and IL-10, markers suggestive of a tolerogenic phenotype (229). Although it remains to be determined whether endogenous expression of self-antigens in the periphery can similarly induce immune tolerance, these results suggest that Aire may act in a subset of lymphoid stromal cells to mediate peripheral T cell tolerance.

**Aire and APS1 as a model disease system.—**The study of Aire and APS1 has provided novel insights into fundamental mechanisms of immune tolerance and highlighted the importance of central or thymic tolerance in the prevention of autoimmunity. With understanding of the underlying immune defect, focused study of the individual diseases

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within the broad spectrum of APS1-mediated autoimmunity has become possible. Three notable examples of such study provide new clinical tools in the diagnosis and understanding of APS1 disease. Screening of sera from a large cohort of APS1 patients has identified the NACHT leucine-rich repeat protein (NALP5, also known as NLRP5) as a putative parathyroid autoantigen (230). High-titer autoantibodies to NALP5 were found only in APS1 patients with hypoparathyroidism, presenting a new diagnostic tool in APS1 disease. Autoantibody studies in APS1 patients have also identified antibodies to Th17 cytokines that are linked to the development of candidiasis (231, 232). Autoantibodies to IL-17A, IL-17F, and IL-22 were found in all APS1 patients affected with candidiasis, suggesting that they may directly mediate disease susceptibility, given that Th17 responses are essential for fungal immunity (233). Finally, because APS1 encompasses a broad spectrum of individual organ-based autoimmunity, autoantigens identified in these rare patients may provide insight into similar disease in non-APS1 populations. Shum et al. (209) recently reported the identification of a lung-specific antigen in *Aire*$^{-/-}$mice linked to the onset of lung autoimmunity dependent on loss of Aire-mediated thymic tolerance. Homology to the mouse antigen identified the LPLUNC1 protein as a putative lung autoantigen in APS1 patients with interstitial lung disease. Thus, study of disease in the Aire/AIRE system may serve as a translational model for more common autoimmunity.

**Links for AIRE to other disease states.—**Aire-mediated autoimmunity highlights the importance of thymic antigen presentation in establishment oftolerance. The examples ofthe insulin gene *(INS)* VNTR and *CHRNA1* locus discussed above show that the thymic TSA expression level for each gene is a significant risk factor for type 1 diabetes and myasthenia gravis, respectively. As such, several studies have sought to link genetic variation in *AIRE* or *AIRE* haplotypes (and reduced thymic TSA expression) to more common autoimmune diseases, such as Addison's disease, type 1 diabetes, and vitiligo, without success (234–236). Interestingly, a rare patient presenting with onset of APS1 in adulthood had a thymoma (a tumor of thymic epithelial cells) deficient for AIRE. Although thymomas are frequently linked to autoimmunity, this case suggests that somatic loss of AIRE in these tumors may be one mechanism of acquired autoimmunity (237). Further investigations are needed in larger patient populations to adequately address a role for AIRE in other autoimmune states.

## CONCLUSION

Although rare, monogenic autoimmune syndromes have helped clarify our understanding of mechanisms that govern immune tolerance. The rarity of these syndromes likely reflects the multiple complementary mechanisms that regulate tolerance. Interestingly, these syndromes can be broadly segregated into systemic and organ-specific subtypes. This division helps highlight how innate immune response mechanisms are tied to systemic autoimmunity, whereas adaptive immune response mechanisms are tied to organ-specific autoimmunity. Despite these distinctions, the differences are sometimes unclear, and some phenotypes cross this boundary. For example, the organ-specific autoimmune phenotype of autoimmune thyroiditis can be observed in both monogenic and polygenic forms of SLE. Indeed, this

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crossover reinforces the important links between innate and adaptive immune pathways for the maintenance of tolerance.

Looking forward, several interesting unanswered questions remain surrounding this topic. For instance, what are the underpinnings of the propensity for type 1 diabetes in IPEX when compared with the propensity for hypoparathyroidism and adrenal autoimmunity in APS1? Although thymic selection mechanisms certainly contribute in both syndromes, a more clear understanding of why certain phenotypes emerge could be an important clue for future work. Recent advances in deep sequencing have allowed for the determination of defective genes in isolated rare families, as evidenced by *TRAP* in the SPENCDI syndrome. Further discovery of novel genes in other rare families with autoimmune features is extremely likely. Although much attention has been placed on the study of common genetic variants in common autoimmune diseases, the study of rare variants in rare Mendelian autoimmune diseases has clearly made a significant contribution to our understanding of tolerance and factors that protect against autoimmunity.

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### A FRESH LOOK AT RARITY IN THE GENETICS OF AUTOIMMUNITY

Advances in the fields of genomics and gene sequencing are changing our approach to human genetics. The genetics of autoimmunity has focused in the past on rare monogenic disorders or on the discovery of high-risk alleles in more common autoimmune diseases. Traditional linkage analysis in large families or diseased cohorts have revealed the contribution of several key genetic risk factors, most notably the MHC molecule. As the figurative interface through which the immune system sees the antigenic world, the MHC molecule and its variants remain the most frequent risk alleles in almost all autoimmune disorders. Large-scale GWA studies have identified genes—such as *PTPN22, CTLA-4,* and *IL2/IL2RA*—that significantly increase risk in several autoimmune diseases (reviewed in 238). These approaches require studies in large families or populations of affected individuals, and the detection of rare variants with significant effects in common diseases has remained challenging. The advent of exome sequencing in human genetics may be changing this. Using this technology, a consortium of investigators has recently identified rare mutations in sialic acid acetylesterase (SIAE) that are associated with an eightfold increased risk of autoimmunity in both rheumatoid arthritis and type 1 diabetes (235). The identification of rare phenotypes coupled with more powerful genetic technologies holds potential for the characterization of novel mechanisms of autoimmunity.

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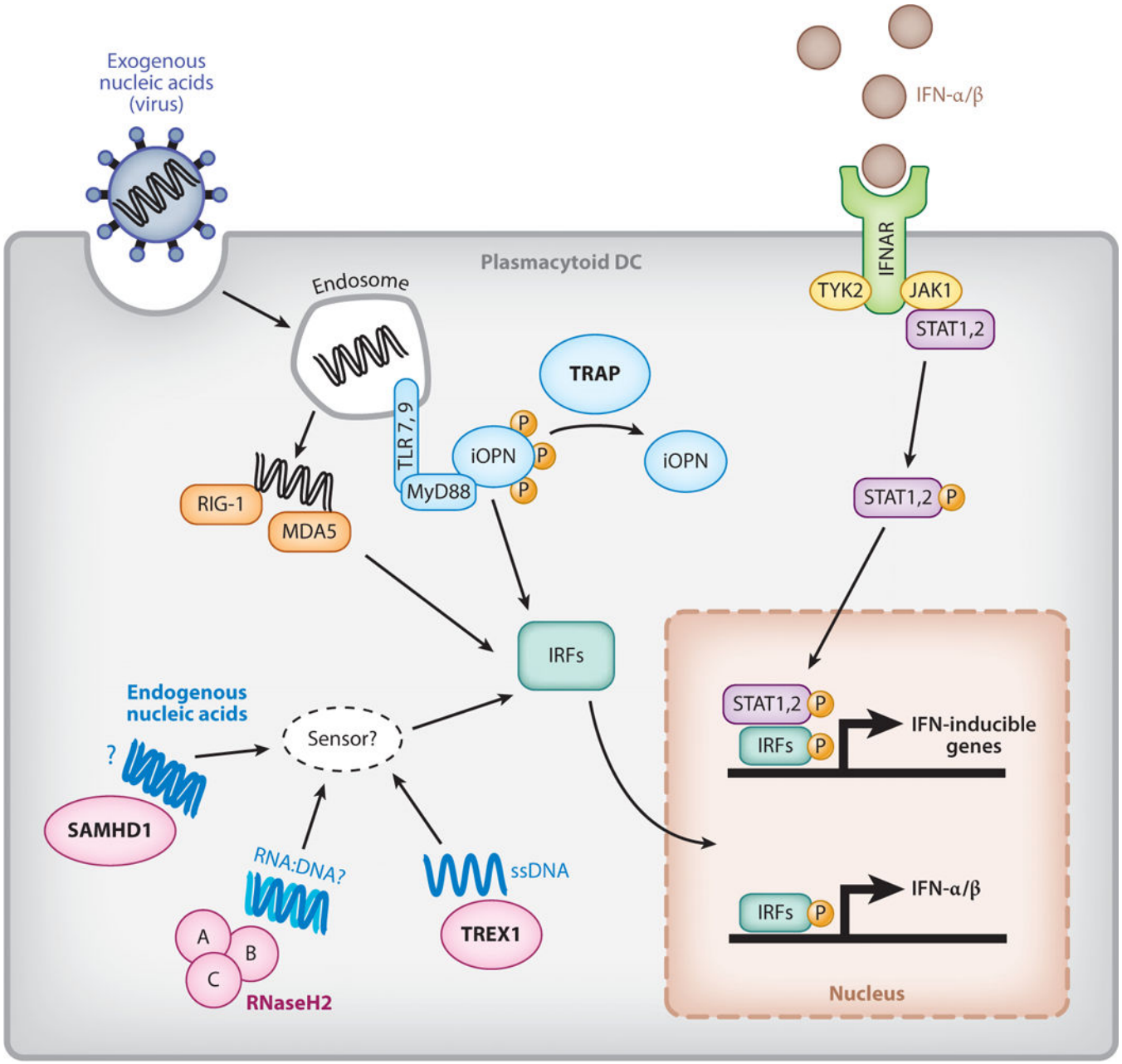


**Figure 1.**

Type 1 interferons are activated in the systemic autoimmunity seen with SPENCDI and AGS. The plasmacytoid dendritic cell (pDC) is one of the primary sensors and producers of type 1 interferons (IFN-α/β), providing self-amplification of interferon-mediated signaling. Exogenous nucleic acids in the form of viral infection activate TLR signaling through endosomal processing coupled to TLR7 and TLR9 signaling. Intracellular nucleic acids from exogenous viral sources can also be sensed through the intracellular RIG1/MDA5 system. These pathways converge on the activation of interferon regulatory factors (IRFs) that then translocate to the nucleus to activate transcription of type 1 interferons. These events occur in concert with IFN-a/p activation of the IFNAR (IFN-a receptor), resulting in

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JAK1-STAT-mediated signal transduction to upregulate several interferon-inducible genes through the combined action of IRFs and STAT1,2. The AGS and SPENCDI syndromes highlight mechanisms at two levels that can lead to chronic activation of type 1 interferon signaling with potential for systemic autoimmunity. In AGS, the TREX1, RNaseH2 complex, and SAMHD1 proteins are thought to mediate degradation of endogenous nucleic acids, such as ssDNAs (single-stranded DNAs) and RNA:DNA duplexes. In the absence of these functions, endogenous nucleic acids can accumulate and, through a yet-to-be-identified sensor, lead to activation of IRFs. In SPENCDI, deficiency of TRAP (tartrate-resistant acid phosphatase) leads to accumulation of hyperphosphorylated forms of iOPN (intracellular osteopontin). In pDCs, phosphorylated iOPN acts as an adaptor molecule in complex with MyD88 to activate TLR9 signaling. Notably, iOPN selectively couples TLR9 signal to IRF7 activation instead of NF-KB. Thus, accumulation of phosphorylated iOPN can lead to skewing of TLR9 signaling to type 1 interferon activation. As sensors of exogenous and endogenous nucleic acids as well as apoptotic debris (not shown in figure), pDCs can induce and amplify type 1 interferon signals to produce systemic autoimmunity. The resulting production of type 1 interferons can also act upon T cells, B cells, and DCs to promote a network of responses, leading to autoimmunity (reviewed in 31).

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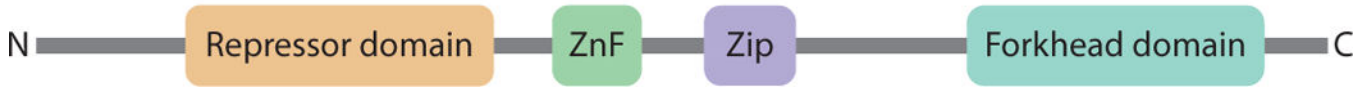


**Figure.2.**

Domain structure of the FOXP3 protein. The N-terminal half of the protein houses a repressor domain important for the binding and inhibition of the NFAT (nuclear factor of activated T cells) transcription factors. Consistent with its function as a transcription factor, the protein also contains a zinc finger domain (ZnF) and leucine zipper (Zip), followed by a C-terminal canonical forkhead domain that mediates DNA binding

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**Figure 3.**

Domain structure of the AIRE protein. Several domains within the protein share homology with components of transcriptional regulatory elements. The N terminus of AIRE consists of a homogeneously staining region (HSR) that is thought to mediate homodimerization of the protein and is followed by a nuclear localization sequence (NLS). Overlapping the HSR is a putative caspase-recruitment domain (CARD) that is also predicted to mediate homotypic protein oligomerization with other CARDs, although other CARD-carrying partners of AIRE have yet to be identified. It remains to be seen if these two domain designations mediate all the same functions. The SAND domain, so named for its homology to the DNA-binding domain of the Spl00 family of proteins (Spl00, AIRE, NucP4l/75, and DEAF-1), occupies the central portion of the protein. Four LXXLL (X being any amino acid) transcriptional coactivation motifs are distributed throughout the protein ( *gray bars).* The C terminus consists of two plant homeodomains (PHD1, PHD2) thought to mediate differential binding to histone marks and a proline-rich region (PRR) that is hypothesized to mediate transactivation as seen in other transcription factors. Over 60 mutations in AIRE have been documented, scattered throughout the protein. The G228W mutation lies within the SAND domain, as shown (*asterisk*).

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## Table 1

Diagnostic criteria for ALPS[a]

| Diagnosis | Required criteria | Primary criteria | Secondary criteria |
|---|---|---|---|
| | 1.Chronic (> 6 mo) **lymphadenopathy and/or splenomegaly** (nonmalignant, noninfectious)<br>2.Elevated DNT cells (CD3+TCRαβ+CD4-CD8-) ≥1.5% of total lymphocytes or >2.5% CD3+ cells in setting of normal or elevated lymphocyte counts | 1.Defective lymphocyte apoptosis (in 2 assays)<br>2.Mutation in *FAS*, *FASLG*, or *CASP10* (somatic or germ line) | 1.Elevated plasma marker (sFASL, IL-10, IL-18, or vitamin B12)<br>2.Typical immunohistological findings<br>3.**Autoimmune cytopenias** (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated IgG (polyclonal hypergammaglobulinemia)<br>4.Family hx of nonmalignant lymphoproliferation +/— autoimmunity |
| **Definitive** | Required criteria (2of2) | Primary criteria (1 of 2) | — |
| **Probable** | Required criteria (2of2) | — | Secondary criteria (1 of 4) |

[a]Abbreviations: DNT, double negative cells; hx, history; sFASL, soluble FAS ligand. Boldface indicates two predominant features of the syndrome.

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**Table 2**

Revised classification of ALPS and related disorders

| **ALPS** | | |
|---|---|---|
| **Revised nomenclature** | **Gene** | **Definition** |
| ALPS-FAS | *FAS* | Germ-line homozygous or heterozygous *FAS* mutations. Previously ALPS type 0 and type Ia. |
| ALPS-sFAS | *FAS* | Somatic mutations in *FAS*. Previously ALPS type Im. |
| ALPS-FASLG | *FASLG* | Germ-line mutations in *FASLG*. Previously ALPS type Ib. |
| ALPS-CASP10 | *CASP10* | Germ-line mutations in *CASP10*. Previously ALPS type IIa. |
| ALPS-U | Unknown | Meets ALPS criteria but with undetermined genetic defect. Previously ALPS type III. |
| **ALPS-related disorders** | | |
| **Revised nomenclature** | **Gene** | **Definition** |
| CEDS: Caspase-8 deficiency state | *CASP8* | Germ-line mutations in *CASP8*. Lymphadenopathy and/or splenomegaly, marginal elevation of DNT cells, recurrent infections. Previously ALPS type IIb. |
| RALD: Ras-associated autoimmune leukoproliferative disease | *NRAS* | Somatic mutations in *NRAS*. Autoimmunity, lymphadenopathy, and/or splenomegaly, normal to elevated DNT cells, defective in vitro Fas-mediated apoptosis. Previously ALPS type IV. |
| DALD: Dianzani autoimmune lymphoproliferative disease | Unknown | ALPS-like signs but with normal DNT cells and defective in vitro FAS-mediated apoptosis |
| XLP1: X-linked lymphoproliferative disease | *SH2D1A, SAP* | Germ-line mutations in *SH2D1A* or *SAP*. Fulminant Epstein-Barr virus infections, hypogammaglobulinemia, or lymphoma. Defective apoptosis in response to TCR restimulation |

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## Table 3

Overview of monogenic autoimmune disorders[a]

| | Hereditary Clq deficiency | SPENCDI | AGS | ALPS | IPEX | APS1 |
|---|---|---|---|---|---|---|
| **Gene(s)** | *ClqA, ClqB, ClqC* | *TRAP (ACPS)* | *TREX1, RNaseH2 (A, B, C), SAMHD1* | *FAS, FASLG, CASP10* | *F0XP3* | *AIRE* |
| **Inheritance** | Autosomal recessive | Autosomal recessive | Autosomal recessive | Autosomal dominant, autosomal recessive, variable penetrance | X-linked | Autosomal recessive* |
| **Main features** | SLE and SLE-like disease Recurrent bacterial infections | Skeletal dysplasia Cerebral calcifications and CNS symptoms SLE | Basal ganglia calcifications Neurologic dysfunction SLE | Lymphoproliferation (lymphadenopathy and/or splenomegaly) Autoimmune cytopenias Malignancy | Autoimmune enteropathy Neonatal diabetes Thyroiditis Eczema | Hypoparathyroidism Adrenal insufficiency (Addison's disease) Mucocutaneous candidiasis |
| **Autoimmunity** | Systemic | Systemic | Systemic | Systemic, organ-specific | Organ-specific | Organ-specific |
| **Autoimmune features** | SLE, glomerulonephritis, angioedema, fANAs, +RNP Abs | SLE, thrombocytopenia, hemolytic anemia | SLE, chilblains, hemolytic anemia, +ANAs | Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, neutropenia) | Enteropathy Type 1 diabetes | Multi-organ disease + organ-specific autoAbs anti-IFN Abs, NALP5 Abs |
| **Tolerance defect** | Impaired clearance of apoptotic material | Activation of type 1 interferon signaling | Activation of type 1 interferon signaling | Defective lymphocyte apoptosis | Loss of Tregs | Defective deletional tolerance |
| **Central versus peripheral tolerance mechanism** | Peripheral | Peripheral | Peripheral | Peripheral | Peripheral | Central, peripheral? |
| **Innate versus adaptive immune defect** | Innate | Innate | Innate | Adaptive | Adaptive | Adaptive |
| **Immunodeficiency** | Susceptibility to encapsulated bacteria | None described | None described | Not generally described | Recurrent infections | Candidiasis |

[a]Abs, antibodies; ANA, antinuclear antibody; CNS, central nervous system; IFN, interferon; NALP5, NACHT leucine-rich repeat protein 5; RNP, ribonuclear protein; SLE, systemic lupus erythematosus;

*= single autosomal dominant variant.