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Monogenic autoimmune diseases – lessons of self-tolerance

Ismo Ulmanen¹, Maria Halonen^{1,2}, Tanja Ilmarinen¹ and Leena Peltonen^{1,2}

The molecular defects recently identified in the rare monogenic autoimmune diseases (AIDs) have pinpointed critical steps in the pathways that contribute to the development of normal immune responses and self-tolerance. Recent studies of autoimmune polyendocrinopathy syndrome type 1, autoimmune lymphoproliferative syndrome, immunodysregulation, polyendocrinopathy and enteropathy, X-linked, IL-2 receptor α -chain deficiency, and, in particular, their corresponding mouse models, have revealed the details of the molecular mechanisms of normal immune tolerance, and exposed how defects in these mechanisms result in human autoimmunity. In addition to a deeper understanding of the immune system, detailed molecular characterization of monogenic AIDs will help us to understand the mechanisms behind common polygenic AIDs and, furthermore, to develop novel therapies and intervention strategies to treat them.

Addresses

¹ National Public Health Institute, Department of Molecular Medicine, Biomedicum, Helsinki, Finland

² University of Helsinki, Department of Medical Genetics, Helsinki, Finland

Corresponding author: Peltonen, Leena (leena.peltonen@ktl.fi)

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Introduction

Autoimmune diseases (AIDs) are a clinically important entity, consisting of at least 70 known or suspected diseases. Altogether 3–5% of the general population is affected by these diseases. Many AIDs are common in most populations and characteristically women are more frequently affected than men (typically 3:1 sex ratio) [1]. The aetiology of most AIDs is multifactorial, with a complex polygenic genetic background interacting with triggering environmental factors, most of which are unknown.

Only a few AIDs have monogenic traits [2*]. These include autoimmune polyglandular syndrome type 1 (APS1; also called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy [APECED]), autoimmune lymphoproliferative syndromes (ALPS) type 1, 2 and 3

immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX) and IL-2 receptor α -chain deficiency (IL-2R α deficiency). Table 1 shows the major characteristics of the monogenic AIDs. The genetic characterization of defects behind these monogenic traits is a straightforward process with the currently available genome-wide tools when compared to gene-finding efforts in polygenic or complex AIDs. As the defect in each monogenic AID should expose a critical component of the pathways important for the normal development of immunological tolerance, the research of these diseases should provide essential lessons of complex AIDs.

In this review, we describe how recent research into monogenic autoimmune diseases has provided us with valuable information on central and peripheral tolerance mechanisms.

APS1 – a defect in negative selection

Genetics and clinical phenotype

APS1 (Online Mendelian inheritance in man [OMIM] number 240300) has a low overall global prevalence, similar to the other monogenic AIDs. APS1, however, is more frequent among some isolated populations such as the Finns (1:25 000), Sardinians (1:14 400) and Iranian Jews (1:9000). The patients develop multiple organ-specific autoimmune diseases, often starting in childhood or during teenage years (Table 1; [3,4]). Hallmark symptoms include chronic *Candida* infection followed by autoimmune hypoparathyroidism and Addison's disease. At least two of the three aforementioned components should be present for diagnosis [3,5]. The wide diversity in clinical phenotype of APS1 patients suggests that factors other than the diversity of mutations in the *AIRE* gene affect the phenotype. Associations to specific HLA-haplotypes have been found for APS1 trait components, including alopecia, Addison's disease and type 1 diabetes [4]. APS1 patients have high levels of serum autoantibodies that react specifically with components of the affected organs, the known antigens including amino acid decarboxylases and many enzymes involved in the synthesis of steroids [6,7].

Autoimmune regulator

A gene encoding the novel transcription factor *AIRE*, which is defective in APS1 was identified by positional cloning in Finnish APS1 families [8,9]. *AIRE* expression is found in several tissues, the highest expression being evident in medullary epithelial cells (MECs) of the thymus, where *AIRE* gene activity is regulated by lymphotoxin signalling [10–14]. In the periphery, *AIRE* expression is obvious in blood monocytes and differentiated dendritic cells [15,16*].

Table 1**Major features of monogenic autoimmune diseases.**

Disease	Affected gene and function	Number of mutations	Main clinical features	Affected pathway	Mouse model
APS1	<i>AIRE</i> transactivator	>50	Hypoparathyroidism, Addison's disease, Candida, diabetes, ovarian failure	Self-antigen presentation and negative selection in thymus	KO
ALPS 1a	<i>TNFRSF6</i> membrane receptor	>40	Splenomegaly, lymphadenopathy, hypergammaglobulinemia, autoimmune diseases	Fas-mediated apoptosis	lpr/lpr-mouse KO
ALPS 1b	<i>TNFSF6</i> membrane-bound ligand	1	Systemic lupus erythematosus, lymphadenopathy	Fas-mediated apoptosis	gld/gld-mouse
ALPS 2	<i>Caspase 10</i> cysteine protease	2	Adenopathy, hepatosplenomegaly, haemolytic anaemia	Lymphocyte apoptosis cascade	None presently available
IPEX	<i>Foxp3</i> transcription factor	10	Polyendocrinopathy, haemolytic anaemia, chronic diarrhoea, eczema	CD4 ⁺ CD25 ⁺ regulatory T cell development	Scurfy-mouse
IL-2R α deficiency	<i>IL-2Rα</i> cytokine receptor	1	Lymphadenopathy, chronic diarrhoea, hepatosplenomegaly, chronic lung disease, recurrent infections	CD4 ⁺ CD25 ⁺ regulatory T cell development	KO

KO, knockout.

The numerous predicted domains of the AIRE polypeptide suggest that this protein might play a role in transcriptional regulation [8,9]. Transfection experiments have proven that AIRE, particularly the zinc-finger domains, acts as a powerful transcriptional transactivator *in vitro* [17,18]. The amino-terminal homogeneously staining region domain is another established functional domain of AIRE, and is responsible for the homo- and hetero-multimerisation needed for the assembly of functional AIRE-containing complexes [18,19^{*}]. To date, more than 50 APS1-resulting *AIRE* mutations have been found, and founder mutations characteristic for the populations showing enrichment of APS1 have been identified, providing a basis for reliable DNA diagnostics and carrier detection [19^{*},20,21].

AIRE characteristically shows two types of distribution in both cells and tissues: nuclear matrix-associated dots and cytoplasmic filaments. Human and mouse cells show similar distribution [10,11,22–24]. The functional significance of AIRE nuclear dots is still unknown; however, it is unlikely that the AIRE dots are directly involved in transcription, as they do not co-localize with transcriptionally active chromatin [25], suggesting that AIRE dots might actually be transient storage sites of inactive AIRE and other proteins involved in transcription regulation.

Mouse models reveal defects in negative selection

Recently, three different Aire-deficient mouse models have been produced [26–28]. The models differ in the targeting site of gene disruption. All *Aire*^{-/-} mouse models expose some, although slightly variable, autoimmune features of APS1, including multiorgan lymphocytic infiltration, circulating autoantibodies and infertility. The overall phenotypes of the *Aire*^{-/-} mice are clearly milder

and somewhat different from those of human APS1 patients.

In intercrossing experiments of *Aire*^{-/-} mice with transgenic mice expressing T cell receptor genes against tissue-specific (pancreatic islet cell) antigens, the mice completely fail to delete organ-specific T cells in the thymus [29]. This finding links the Aire mutations and APS1 directly to problems in the thymic deletion of autoreactive T cells. Similar intercrosses have provided evidence for the critical, dose-dependent role of *Aire* in autoimmunity to organ-specific gene products [30^{*}]. The sensitivity of Aire-dependent thymic deletion of autoreactive T cells to even small reductions in Aire function makes this pathway a prime candidate for subtle autoimmune quantitative trait loci (QTLs) linking Aire to tissue-specific symptoms of common autoimmune diseases.

Transcription profiles of *Aire*^{-/-} thymic epithelial cells are of special interest. These cells are known to express small amounts of numerous peripheral antigens creating 'tolerization' against them during the maturation of lymphocytes [31^{*},32,33]. This is a critical process for the development of self-tolerance. MECs of *Aire*^{-/-} mice revealed distinct downregulation of numerous genes, implying that Aire is a transcriptional activator of some 200–1200 genes. Specifically, 30 of the most strongly downregulated genes were genes encoding tissue-restricted antigens, which were expressed ectopically in *Aire*^{+/+} mice [26,33]. Recent analysis on the expression patterns of wild-type and *Aire*^{-/-} mice suggests that Aire is able to both downregulate and upregulate genes [16^{*},31^{*},34]. Furthermore, those Aire-responsive genes are located in the genome as multiple upregulated or

downregulated clusters in different chromosomes. How these findings relate to the multiple tissue symptoms of patients remains an unanswered question.

In summary, the studies of APS1 have exposed a novel transcriptional regulator, AIRE/Aire, and revealed the critical role of this molecule in the regulation of ectopic antigen expression in thymus, triggering the deletion of T cells, which are otherwise reactive against these self-antigens (Figure 1). Future research is urgently needed to reveal the role of genetic variation of *AIRE* as a factor predisposing for autoimmunity and common autoimmune diseases. Furthermore, unravelling the mechanisms of AIRE-dependent gene expression at the whole genome level will answer some of the basic questions regarding the transcriptional regulation of well-orchestrated genes.

ALPS – defects in apoptotic pathways

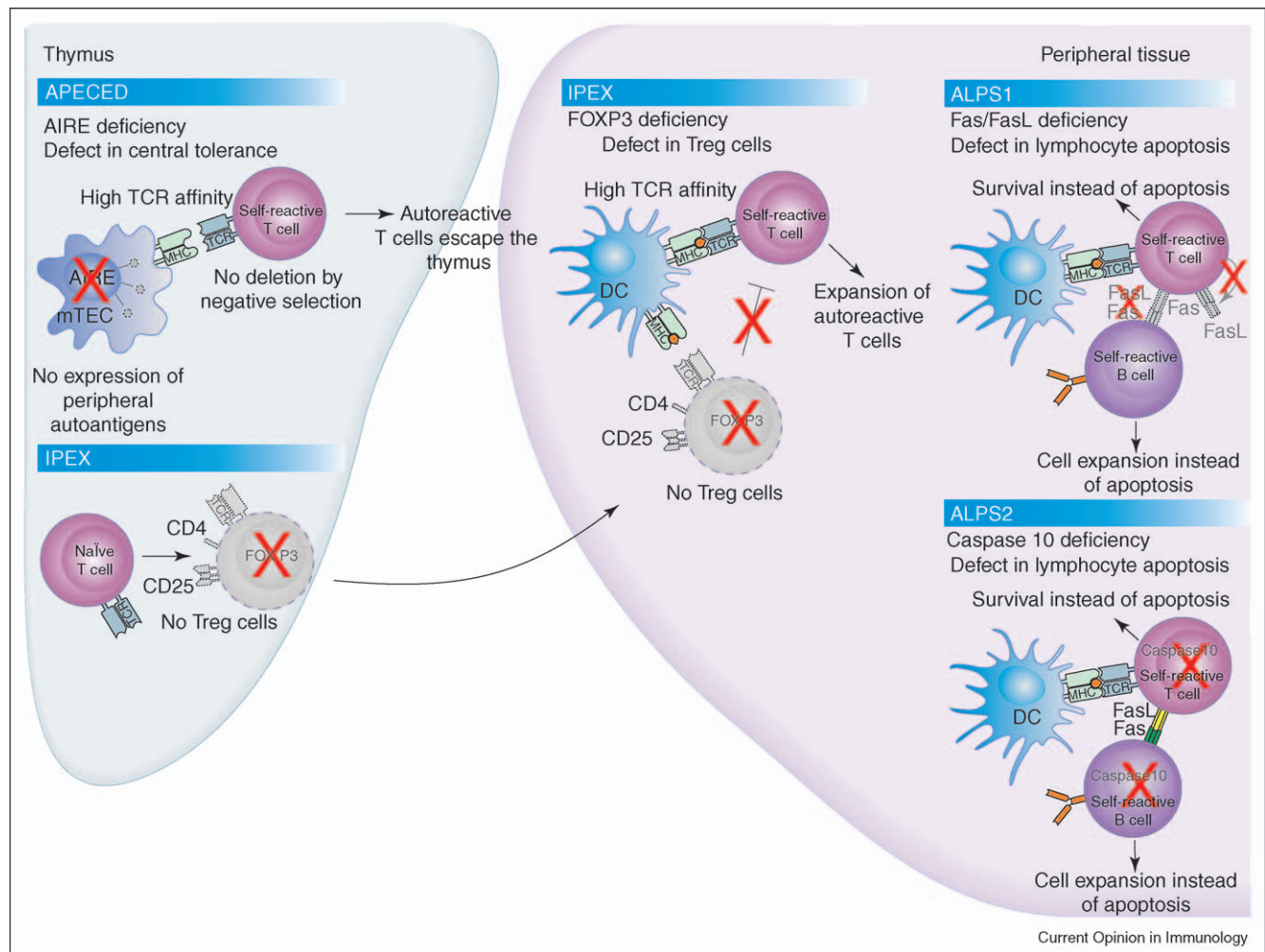
Genetics and clinical phenotype

ALPS (OMIM 601859) is inherited as an autosomal dominant trait [35] and is characterized by the accumulation of a polyclonal population of double-negative T cells ($CD3^+ TCR\alpha\beta^+ CD4^- CD8^-$) [36]. The phenotypic components of ALPS include lymphocytosis of $CD4^- CD8^-$ T cells, non-malignant lymphadenopathy, splenomegaly, hypergammaglobulinemia and autoimmune manifestations, such as autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura and autoimmune neutropenia (Table 1).

Molecular defects in apoptosis

Three different forms of ALPS have been established (type 1 [a and b], type 2, and type 3) each with slightly

Figure 1



A schematic model of the pathways targeted by the monogenic AIDs APS1, ALPS and IPEX in the central (thymus) and peripheral immunological organs. The red crosses pinpoint the molecules or functions hit by the disease mutations. In APECED, defects in AIRE lead to impaired expression of ectopic antigens in mTECs, causing inefficient negative selection of T cells. In IPEX, the mutations in *Foxp3* prevent the normal development of regulatory T cells (Treg), and in ALPS 1 and 2 Fas/Fas ligand mutations result in the defective apoptotic elimination of autoreactive T cells. Abbreviations: DC, dendritic cell; mTEC, medullary thymic epithelial cell; TCR, T-cell receptor; Treg cell, regulatory T cell.

different genetic defects and disease components, [37]. In all of the disease forms, the different genetic defects lead to impaired apoptosis and, consequently, into loss of restriction of autoimmune T and B cells [38].

The causative gene for ALPS type 1a was identified with the help of a spontaneous animal model for the disease, the *lpr* mouse, which lacks expression of Fas (then called Apo-1) due to retrotransposon insertion [39]. Subsequently, mouse strains with missense mutations in the *Fas* gene have been described [40]. The human homologue for Fas, the *TNFRFS6* gene, has also been cloned [41], and its mutations in the heterozygous state were found to cause defective apoptosis in patients with ALPS type 1a [42,43].

The causative gene of ALPS type 1b was found to be the gene encoding for the ligand of Fas, the *TNFSF6* gene, in one patient [44]. Fas–FasL ligation is an important regulator of T cell homeostasis, but it can also induce apoptosis in B cells, antigen presenting cells (APCs) and target tissues [45]. During the immune response, a negative feedback loop regulates excess effector lymphocytes by Fas-mediated apoptosis. Consequently, defective Fas results in the massive accumulation of lymphocytes in lymphoid organs and also in a failure to delete autoreactive naïve T cells (see Figure 1).

ALPS type 2 is caused by mutations in the *Caspase 10* gene, which is involved in apoptotic pathways used by various different death receptors including Fas [46]. Recently, *Caspase 8* mutation has been shown to cause ALPS in two siblings [47]. Interestingly, Caspase-8-deficient mice display a lethal phenotype [48].

Finally, ALPS type 3, which is sporadic and mild, is caused in some cases by somatic mutations of Fas in T cells, most often in polyclonal double-negative T cells, or mononuclear cells [49]. It has been suggested that, in some ALPS type 3 patients, pathways other than Fas-mediated apoptotic pathways would be defective. For more details of the manifestations of different gene defects in ALPS, see the comprehensive review of Rieux-Laucat [38]. Altogether the different ALPS diseases have revealed us the mutation-sensitive steps of lymphocyte apoptosis pathways in man and thus essentially increased our understanding of the critical importance of maintenance of lymphocyte homeostasis in normal self-tolerance (Figure 1).

IPEX – a developmental defect of regulatory T cells

Genetics and clinical phenotype

IPEX (OMIM 304790) is a monogenic X-linked recessive syndrome [50], also known as XLAAD (X-linked autoimmunity-allergic dysregulation syndrome). The defective gene, *Foxp3* (Xp11.23; also called *JM2*) was identified

by the positional candidate approach [51]. Recently, involvement of an unidentified autosomal locus in IPEX has been reported, which suggests genetic heterogeneity in this syndrome [52]. The disease is very severe and usually results in early death. The disease phenotype includes severe allergic inflammation, autoimmune polyendocrinopathy, secretory diarrhoea, haemolytic anaemia and thrombocytopenia (Table 1; [53]). Interestingly, the critical DNA region in a mouse model for dysregulated lymphocyte activation, *scurfy*, overlapped with the syntenic human region for IPEX [54,55]. The clinical and immunological characteristics of the *scurfy* mice resemble IPEX in humans [56]. The critical region of the mouse locus was used to isolate a novel human gene encoding a putative transcription factor, Scurfin, which belongs to a family that includes the forkhead transcription factor *Foxp3*. In addition to the putative DNA-binding forkhead homology domain, a C2H2 zinc finger domain and a leucine zipper motif, as well as a putative nuclear localization signal, are found in the *Foxp3* polypeptide.

Molecular defects in the development of CD4⁺CD25⁺ regulatory T cells

Foxp3 is expressed in CD4⁺CD25⁺ regulatory T cells in the thymus and periphery, and it has been established as a key regulatory gene for the development of regulatory T cells [57–59] thus serving as a specific marker for those cells. The functional role of *Foxp3* was confirmed by demonstrating that forced expression of the *Foxp3* gene converted murine naïve T cells to cells resembling natural CD4⁺CD25⁺ cells [57,58]. Regulatory T cells are critical for autoimmunity, as they suppress the activation and expansion of self-reactive T cells in the periphery [68]. Consequently, the transfer of CD4⁺CD25⁺ T cells into *scurfy* mice and also into mice suffering from inflammation and autoimmune disorders prevented their autoimmune symptoms [58,60]. Interestingly, *Foxp3*-transduced T cells are able to control the rejection of an allogeneic transplant [61]. In summary, studies of the molecular pathogenesis of IPEX have confirmed the importance of CD4⁺CD25⁺ regulatory T cells in the maintenance of peripheral tolerance (Figure 1) and have provided an understanding of the mechanisms behind this still incompletely known phenomenon.

The molecular targets of *Foxp3* have recently been uncovered by demonstrating that *Foxp3* inhibits the expression of IL-2, IL-4 and IFN- γ in primary helper T cells by physically associating with transcription factors of the Rel family, nuclear factor of activated T cells (NFAT) and NF- κ B, thereby blocking their activity [62]. Furthermore, it was shown that the downregulation of NFAT and NF- κ B activity by *Foxp3* suppressed the effector functions of T helper cells. These results extended our knowledge of the role of *Foxp3* beyond the control of regulatory T cell formation into monitoring of cytokine production and effector functions of T cells.

IL-2R α deficiency – a defect of regulatory T cells

IL-2R α deficiency (OMIM 606367), another rare monogenic AID affecting the peripheral immune system, was revealed when a deletion in the gene coding for the IL-2R α chain (CD25) in regulatory T cells was detected in one patient [63]. The CD25 molecule has proven to be crucial for the generation, survival and suppressive function of regulatory T cells [64]. Consequently, the IL-2R α deficiency resulted into impaired peripheral tolerance, which is normally executed by the CD4⁺CD25⁺ regulatory T cells [65–67]. The patient suffered from multiple immunological deficiencies and autoimmune symptoms, and he was susceptible for different microbial infections (see Table 1). These findings underlined the multiple roles of regulatory T cells in the immune response, in maintaining lymphoid homeostasis and in the depletion of autoreactive lymphocytes in the periphery [63,65].

Conclusions

To conclude, the research into monogenic autoimmune diseases has demonstrated how detailed characterization of the molecular background of extremely rare diseases has provided us with novel information on the aetiology and pathogenesis of autoimmunity, and on human self-tolerance in general. Firstly, the established disease genes and mutations have provided the scientific community with specific diagnostic screening tools for these monogenic diseases. Secondly, new avenues for the identification of molecular pathways involved in development and maturation of cells and tissues of human immune system have been opened. This enables us the possibility of addressing the molecular background of common autoimmune diseases. It will be interesting to define the impact of common variants of the identified genes on predisposition to different autoimmune diseases. Furthermore, unravelling the downstream genes or associated pathways may provide clues for their involvement in more common failures of self-tolerance. In the future, such findings will provide us with novel drug targets, paving the way towards development of novel treatment and therapy strategies for autoimmune diseases.

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The WHO and six medical journal publishers have launched the Access to Research Initiative, which enables nearly 70 of the world's poorest countries to gain free access to biomedical literature through the Internet.

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Gro Harlem Brundtland, director-general for the WHO, said that this initiative was 'perhaps the biggest step ever taken towards reducing the health information gap between rich and poor countries'.

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