AUTOIMMUNE ENDOCRINE DISORDERS

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INTRODUCTION

The establishment and maintenance of immunologic tolerance to “self” is a key feature of a healthy immune system. Immune responses directed against self-structures have been considered potentially harmful since the inception of modern immunology. Initially, it was believed that autoimmune responses did not occur (the “horror autotoxicos” of Erhlich), but this idea soon needed modification. Autoimmune phenomena were subsequently found to be common, albeit often of insufficient intensity to induce clinical disease.

Several levels of tolerogenic mechanisms are required to prevent the emergence of autoimmunity, while retaining an ability to react with vigor against foreign organisms. Thus autoimmune diseases represent a breakdown of normal self-tolerance, often at several sites, disrupting the complex balance of immuno-regulation maintained by multiple mechanisms within the central and peripheral components of the immune system.The occurrence of autoimmunity reflects an imperfect state of tolerance generated in T- and/or B-cell antigenic repertoires. Since autoimmune disorders represent inherited breakdowns in immune tolerance, they often occur in clusters or aggregations in individual patients and/or their family members. In autoimmunities affecting endocrine glands, we have named these clusters the autoimmune poly-glandular syndromes or the APSs. These dysfunctions in tolerance may occur “centrally” at the level of the thymus, whereby there is escape of self-reactive T cells into circulation instead of being eliminated there. Data shows that intra-thymic tolerance is of potential importance in establishing tolerance to nuclear antigens (1). B-cell tolerance to protein antigens may be abnormal in certain autoimmune disease models (2, 3). Tolerance also occurs in the “periphery”, such that responses by naive T cells against antigens not present in the thymus, generally do not induce significant responses against the self in health but may do so in disease. Furthermore, T cells with low affinities to self-antigens normally escape from the thymus into circulation, where they require active down-regulation to prevent them from inducing disease. In many autoimmune diseases, it is this regulatory property of T cells (Tregs) that is recognized as being defective. Among these Tregs are those bearing the markers of CD4,

CD25, and forkhead box protein P3 (FoxP3). The rare but lethal autoimmune IPEX disease ("immune dysregulatory, polyendocrinopathy, x-linked") is likely caused by a genetic defect in the CD4+ CD25+ FoxP3 Tregs [4] . The ability of Tregs to suppress effector T cells is markedly impaired in IPEX patients [5] .

It is typical of systemic autoimmune diseases, that rather than a global loss of tolerance, there is a selective autoimmune response directed primarily against a specific set of autoantigens because they are structurally related and/or they are present in an organ or tissue type undergoing attack. This can readily be seen when autoimmunities occur against components of the nucleus (6), and it characterizes the APSs.

Autoimmune diseases have traditionally been divided into: organ-specific (e.g. autoimmune diseases of the pancreatic islets, brain, thyroid, parathyroid, anterior pituitary gland, cortex of the adrenals, skin, ovaries, gastrointestinal tract, and liver); or non-organ-specific (e.g. systemic lupus erythematosus or SLE, rheumatoid arthritis or RA) types, depending on whether autoimmune responses are directed to an antigen confined to a particular organ (organ specific) or to an antigen that is widely distributed in the body (non-organ specific). The characteristic feature of organ-specific autoimmunity is the selective targeting of a single organ or individual cell type where gross abnormalities of the immune system are absent. It should be pointed out, however, that this division is artificial, as systemic and organ-specific autoimmune diseases over-lap and appear to share many common pathogenic mechanisms. The fact that in both humans and animals, one organ-specific disease is frequently associated with another (e.g. type-1 diabetes (T1DM), chronic gastritis/pernicious anemia, vitiligo and thyroiditis as in the common APS type 2 (APS-2) raises the probability that common immunologic, genetic, or environmental factors are playing critical roles in the induction of pathology of all affected organs. Organ-specific autoimmune diseases are increasingly believed to result in part from a deficiency in peripheral tolerance induction mechanisms, resulting in their failure to deactivate self-reactive lymphocytes. These autoantibodies could arise as part of a bystander immune response to self after some type of damage to the target organ. An international conference was held in April of 2007 in order to discuss the role of cellular and tissue damage in the pathogenesis of autoimmune disease. [7] Inflammation, infection, apoptosis, environmental exposure, and genetics were all reviewed as possible causes of this damage. All were deemed as key players which still need further systematic review through well-designed studies over the coming years.

Another classification is that of autoimmunities that are predominantly autoantibody mediated versus those that are predominantly mediated by T cell effectors. This latter category may involve cell-damaging cytotoxic T-cells, cytokines, or T-cell activated macrophages. An association has been made between diseases such as Graves ' disease and Type 1 diabetes (T1DM) and a polymorphism of the protein tyrosine phosphatase non-receptor 22 gene (PTPN22). This mutation has been linked to hyper-responsiveness of T-cells. [8]

The role that autoantigens themselves may play in initiating and maintaining autoimmunity is incompletely understood in most models of spontaneous autoimmune disease. The three main classes of organ-specific self-antigens to which auto-antibodies are produced in APS are as follows: (1) organ-specific surface receptor molecules; (2) key intracellular enzymes; and (3)

secreted proteins such as hormones produced by autoimmunity targeted organs. [9] Examples of surface receptor molecules affected by autoimmunity include the thyrotropin receptor, which is involved in autoimmune thyroid disease and a component of the pancreatic beta cell, which has been implicated as a target in T1DM. Important enzymes acting as auto-antigens include thyroid peroxidase in Hasimoto's chronic lymphocytic thyroiditis and the P-450 steroidogenic enzyme 21-hydroxylase (21-OHase) in Addison's disease. Additionally, antibodies to P-450 enzymes are also prevalent in primary biliary cirrhosis. [10] Thyroglobulin, as targeted in Hashimoto's thyroiditis, and insulin, as involved in T1DM, are examples of autoantigenic endocrine cell products important to their respective autoimmune diseases.

The following chapter is divided into three sections. The first describes the clinical features of the major organ-specific autoimmune diseases of humans and their pertinent animal models. The second section analyzes common immunologic features of these diseases that have relevance to both etiology and pathogenesis. The third section is devoted to issues related to the immunotherapy of organ-specific autoimmune diseases.

CLINICAL FEATURES OF ORGAN SPECIFIC AUTOIMMUNE DISEASES

Immune mediated (Type-1) diabetes (T1DM)

During the past two decades, researchers have been accumulating a large body of evidence indicating that T1DM, as well as other endocrine diseases associated with it, all have an autoimmune pathogeneses (11-13).

The evidence that T1DM has an autoimmune pathogenesis is considerable, e.g. disease associated autoantibodies specific for islet cells and islet cell constituents, cell-mediated immune abnormalities detectable in peripheral blood, the chronic lymphocytic infiltration of the pancreatic islets (insulitis lesions), and an immunogenetic susceptibility reflected mainly by HLA-DR/DQ gene associations and linkages (14-15). Several population studies show that the MHC class II genes on the short arm of chromosome 6 provide the largest genetic contribution to the heritability of common autoimmune diseases. Transgenic mice have developed autoimmune diseases such as celiac disease and T1DM when their MHC was replaced with a human susceptibility HLA haplotype. [16]

Historical Background

In 1849, Addison (17) first described the clinical and pathologic features of adreno-cortical failure in nine autopsied patients, some of whom also appeared to have pernicious anemia, a disease that also initially received his name. Ogle (18) reported the first instance of coexisting diabetes and adrenal insufficiency in 1866. In 1908, Claude and Gourgerot (19) suggested a common pathogenesis for the simultaneous expression of polyglandular insufficiencies involving pancreatic islets, thyroid, gonads, adrenals, and the anterior hypophysis, a fascinating and correct assertion. Parkinson (20) in 1910 noted an association between pernicious anemia and

T1DM. Mononuclear leukocyte infiltration of goitrous thyroid glands was observed by Hashimoto (21) in 1912, while a similar inflammatory lesion of pancreatic islets, termed insulitis, was described by von Meyenburg (22) in 1940. The association between adreno-cortical failure and thyroiditis was documented by Schmidt (23) in 1926, and the syndrome complex was extended by Carpenter et al. (24) in 1964 to include T1DM. It was not until 1956 that the autoimmune pathogenesis of these disorders could be supported by laboratory evidence, beginning with the discovery of circulating precipitating autoantibodies to thyroglobulin in patients with Hashimoto's thyroiditis by Roitt and Doniach (25).

The ability to detect organ-specific humoral autoantibodies with methods developed by Anderson et al. (26) and Blizzard and Kyle (27) confirmed the clinical association between diabetes and idiopathic (autoimmune) adrenalitis. Solomon et al. (28) demonstrated the coexistence of adrenal atrophy in diabetics with thyroid and adrenal dysfunction. Irvine et al. (29) reported that both pernicious anemia and thyroid disorders occur with significant frequency in first-degree relatives of diabetic patients.

The autoimmune polyglandular syndromes

In 1980, Neufeld and colleagues distinguished two major APSs that contained Addison's disease (APS-1 and APS-2) and one APS that was like APS-2 but without the involvement of Addison's disease, which was classified as APS-3. [9,31] In retrospect, the latter two APSs are sufficiently related that APS-2a with Addison's disease and APS-2b without Addison's disease would seem more appropriate and have been used herein. Additional work is needed to characterize these latter groups and to identify their underlying genetics and pathogenesis(Table 1).

APS-1 became to be seen as a fully penetrant recessively inherited syndrome complex while APS-2a/2b appeared biased to female patients with components expressed in successive generations, suggesting a dominant mode of transmission. Recently, various studies have identified the gene called autoimmune regulator (AIRE) responsible for APS-1 (33, 34). The thymus is a predominant site of AIRE gene transcription suggesting that defective thymic functioning disrupting central tolerance could be responsible for the widespread autoimmunities of APS-1.

Table 1. Clinical Features of the Autoimune Polyglandular Syndromes

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APS-1	APS-2a	APS-2b
Central Features		
Addison's	Addison's/adrenalitis	No Addison's/ adrenalitis
MC-Candidiasis	Not seen outside T1DM and not mucocutaneous type	
Hypoparathyroidism	Rare and mild, assoc with CLT	
T1DM occas and late onset	T1DM common to all ages	
CLT unusual	CLT common	
Other Features		
Juvenile pernicious anemia	Adult-onset pernicious anemia	
Malabsorption common	Malabsorptive problems uncommon, most celiac disease	
Hepatic autoimmunity	Chronic biliary tract autoimmunities–cirrhosis	
Dental enamel hypoplasia	Not found in APS-2	
Metaphyseal dysplasia	Not described in APS-2	
Hypogonadism frequent	Hypogonadism uncommon in ASP-2	
Alopecia universalis	Alopecia totalis/areata occasionally	
Occasional Sjögren's	Associated with CLT more frequently than in APS-1	
Vitiligo	Common, especially with Graves' disease and CLT	
Rare hypophysitis	Probably more common than realized	
	Myasthenia gravis, espec CLT/ Graves' and thymomas	

APS, Autoimmune polyglandular syndrome; *CLT*, chronic lymphocytic thyroiditis; *MC*, mononuclear cell; *T1DM*, type 1 diabetes mellitus.

Table 1.

PATHOPHYSIOLOGY CELLULAR AND SEROLOGICAL AUTOIMMUNITIES

Evidence for an Autoimmune Pathogenesis

The evidence supporting the autoimmune nature of the component diseases of the APS is compelling: (1) affected organs demonstrate a chronic inflammatory infiltrate composed mainly of lymphocytes, sometimes aggregating into follicle formation; (2) some of the component diseases are associated with immune-response genes encoded by class-II lociof the HLA complex and more recently, the cytotoxic T lymphocyte antigen-4 (CTLA-4) locus [9] ; and (3) the

syndromes are replete with autoantibodies reacting to targeted tissue-specific antigens, which often are targeted organ-specific enzymes, secretory products of the cells or their receptors. Linkage to CTLA-4 gene in APS-2, as well as in T1DM, autoimmune thyroid disease, Addison's disease and celiac disease suggests that CTLA-4 is a general autoimmune locus, and susceptibility polymorphysm(s) within the gene may lead to general defects in the immune regulation, while other tissue specific (e.g. insulin gene polymorphisms) or antigen specific (e.g. MHC) genetic factors and environmental factors determine the involvement of particular target organs (180)

The inductive events that lead to the initiation of autoimmune pathogenic processes remain poorly understood. It is clear, however, that both genetic and environmental factors are involved in the disease processes. In celiac disease, the ingestion of gliadin in wheat flour induces autoimmunity to transglutaminase in intestinal cells and the clinical disease. Removal of the inciting agent (gliadin) causes remission in celiac disease. However, for the remainder, no clear inciting agent has been identified albeit many have been suspected. While APS-1 is associated with recessive and often heterologous mutations of the AIRE gene, patients with same types of AIRE gene mutations can develop different component diseases of APS-1 at various ages of onsets, suggesting the involvement of environmental factors and/or other background genetic factors, notably the HLA genotype.

THE AUTOANTIGENS IN APS

Table 2. Autoantibody Reactive Autoantigens in Patients with Immune Mediated Diabetes (T1DM) and Associated Endocrinopathies

T1DM Specific	**Autoantibodies**	**References**
ICA Group	GAD65 > GAD67	(200)
	IA-2α	(201)
	IA-2β	(202)
	? GLIMA-38	(181)
Others	IAA	(12, 182)
	? Insulin receptors	(183)
	? GLUT-2	(184)
Associated diseases		
Hashimoto's	Antimicrosomal/thyroid peroxidase and thyro-globulin	(185)
Graves'	TSH receptors, flavoprotein and G2s (eye muscle autoantigens)	(79,80, 186)
Atrophic gastritis	H+/K+ ATP-ase	(30)
Pernicious anemia	Gastrin receptors/ Intrinsic factor	(83)
Addison's disease	21-hydroxylase	(31, 187)
Vitiligo	Tyrosinase, MCHR1,	(114,115, 188)

	SOX10	
Celiac disease	Transglutaminase	(189)

As mentioned previously,there are three main classes of organ-specific self-antigens to which autoantibodies are directed in the APSs (Table 3). These are surface receptor molecules, intracellular enzymes that have central roles in vital and unique cellular functions of the target cells, and secreted proteins such as hormones produced by the affected organ.

It remains perplexing as to why the component diseases of APS co-exist. It was initially assumed that sharing of target antigenic epitopes in the affected glands could provide an explanation for the involvement of multi-organs in APS. For example, 17-hydroxylase enzyme is present in the “steroidal hormone producing cells” of testes, ovary, placenta, and adrenal cortex; all of which are involved in APS-1. In addition, partial cross-reactivity between autoantibodies to 21-OH and autoantibodies to 17-OH has been proposed since there is amino-acid sequence homology on the epitope region of these two molecules (35). This is supported by the evidence that recombinant 17OH could partially remove the reactivity of sera from patients with APS-1 that had 21-OH autoantibodies and vice versa, suggesting the presence of cross-reactive antibodies reactive with 17-OH and 21-OH, as well as separate population of autoantibodies to 21-OH and 17-OH in sera from patients with APS-1 (36). Patients with APS-1 or APS-2, however, have increased frequencies of antibodies to 21-hydroxylase (37), but patients with APS-1 are more frequently associated with autoimmune gonadal diseases than patients with APS-2, and typically have autoantibodies reactive to 17-hydroxylase and the side chain cleavage enzyme as well (38). This suggests that other mechanisms interrupting normal tolerance appears to be more likely involved in the occurrence of multiple syndrome complexes, than the sharing of common antigenic self-determinants, albeit both mechanisms may be involved.

HUMORAL AUTOIMMUNITY

One of the prominent features of the APSs is the presence of circulating autoantibodies to autoantigens normally present in the endocrine organs involved in the disease. Such autoantibodies can occur long before the appearance of the evident clinical diseases and are thus predictive of the possible onset of autoimmune disease later in life. This has been demonstrated in several prospective studies of autoimmune diseases, such as in T1DM and systemic lupus erythematosus (SLE) [9,39] Patients with any one of the two types of APS may have autoantibodies against thesame antigens (Table 2). The identification of circulating organ-specific autoantibodies provided the earliest and strongest evidence for the autoimmune pathogenesis of the APSs. Whereas patients with collagen-vascular diseases synthesize immunoglobulins that recognize non-organ-specific cellular targets such as nucleic acids or nucleoproteins, the endocrine autoimmunities are associated with autoantibodies that react to organ-specific antigens. While their pathogenic relevance remains unclear, their importance as diagnostic indicators and predictive markers of future disease is well established (21-25). Indirect immunofluorescent assay is a useful and convenient method to screen for autoantibodies to autoantigens present in target organs. Procedures for procurement and

processing of fresh frozen substrate tissues for such testing must be meticulously followed in order to obtain consistent and reliable results. However, biochemical assays, such as immunoprecipitation assays using autoantigens labeled with radioisotopes or other labels, are used increasingly to measure specific autoantibodies, have shown high sensitivity and good reproductibility, and can be used to rapidly screen large numbers of serum samples.

Table 3. Autoantigens in Autoimmune Polyglandular Syndromes

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Target Organ	Enzymes	Receptors	Secreted Cell Products
Islets	Glutamic acid decarboxylase, (CAD_{65}), tyrosine phosphatases (IA2/IA-2β)	Insulin receptor Glucose transporter (GLUT2)	Insulin Proinsulin Amino acid decarboxylase
Thyroid Parathyroids	Thyroid peroxidase, thyroglobulin	TSH receptors (TBII and TSI) Calcium sensing receptor, NACHT leucine-rich repeat protein 5 (NALP5)	
Adrenals	21α Hydroxylase 17α-Hydroxylase	ACTH receptor	
Gonads	17α-Hydroxylase, side-chain cleavage enzyme	LH receptor	
Stomach and intestine	Intrinsic factor, H^+,K^+-ATPase, tryptophan hydroxylase, tissue transglutaminase		
Liver	P-450 2A6 (CYP2A6) and P-450 1A2 (CYP1A2)		
Lung	A potassium channel regulator KCNRG		
Melanocytes	Tyrosinase/binding protein		

ACTH, Adrenocorticotropic hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone.

Table 3.

Adreno-cortical and Steroidal Cell Autoantibodies

Adrenal autoantibodies (AA) detected by indirect immunofluorescent labeling have been reported in most patients with non-tuberculous Addison's disease when tested at the time of their diagnoses (27). All layers of the adrenal cortex bind AA with striking sparing of the adrenal medulla. Fluorescence of the zona glomerulosa in particular gives a distinctive pattern when viewed by ultraviolet indirect immunofluorescent microscopy. Some 15% of AA-positive patients with Addison's disease also have an autoantibody that cross-reacts with other steroid-hormone producing cells, i.e. the placental syncytiotrophoblasts, ovarian luteal cells, and/or testicular Leydig cells. These "steroidal cell" autoantibodies (SCA) are distinguished from AA by their ability to be adsorbed from serum by preincubation with adrenal, gonadal (ovarian or testicular), or placental homogenates, whereas AA are exclusively removed from positive sera by prior exposure to adrenal homogenates. When detected, SCA indicates a high risk for future gonadal failure, especially in females with high titers (40,41). Additionally,patients with premature ovarian failure and a positive test for AA have a higher risk for the development of Addison's disease than those who do not test positive(42). As mentioned above, the major steroid cell autoantigens involved in the reactions of AA have now been identified as the p450 steroidogenic enzyme 21-hydroxylase (21-OH), alpha-hydroxylase (17-OH) and P450 side-chain cleavage enzyme (P450scc) (see table 3). The major antigen for SCA is 17-hydroxylase, a 55-kDa gonadal and adrenal steroid biosynthetic P450 microsomal enzyme (43). The

frequency of antibodies to 21-OH varies depending upon the techniques used, and are generally at higher titers for patients with Addison's disease in association with APS than in patients with an isolated Addison's disease (44, 45-47). The frequency of antibodies to 17-OH and P450scc were also higher in patients with APS-1 than in patients with isolated Addison's disease (44). The presence of antibodies to 17-OHase or P-450 side-chain cleavage in patients with isolated Addison's disease, therefore may indicate progression toward hypogonadism in an APS.The dominant epitopes on 21-OH recognized commonly by autoantibodies from patients with Addison's' disease as an isolated disease, or in association with APS, are located in the C-terminal end and in a central region of 21-OH (48,49). The recognized epitopes on 21-OH can either be conformational (50) or linear in nature (51).

Autoantibodies to 21-OHase or AAs are useful markers indicating risk for the development of Addison's disease. Occasionally, AA-positive individuals who do not have overt adrenocortical failure can be identified by screening patients with autoimmune diseases, especially autoimmune endocrine diseases, and their family members. About 20% of asymptomatic AA_positive relatives were reported to have elevated basal serum levels of adrenocorticotropic hormone (ACTH) and/or rennin or blunted adrenocortical responses to an intravenous infusion of ACTH- features that are indicative of subclinical glandular dysfunction. In two separate studies, autoantibodies to 21-OHase were found at frequencies of 2.3% (7/304) [9] and 1.7% (11.629) [52] in patients with T1DM. These results are similar to the frequencies of AA found a decade previously. [53] Furthermore, many of these patients had raised ACTH/rennin levels indicative of impending Addison's disease. [54]

Whereas cellular immune mechanisms are thought to cause the glandular destruction seen in autoimmune endocrinopathies, a pathogenic role for humoral autoreactivity in autoimmune oophoritis has been suggested by studies showing complement-mediated cytotoxicity of cultured granulosa cells in the presence of sera from affected patients, though not in the presence of sera from control patients (55). Binding of SCA to granulosa cells by indirect immunofluorescence, however, can only be demonstrated when autoantibodies are present in high titers. Antibodies of patients with Addison's disease have been shown to have inhibitory effects on recombinant 21-OH enzyme activity in-vitro (56), but such enzyme inhibitory effects are not so evident in-vivo (57), and are conceptually unlikely to account for the resultant disease, because such antibodies cannot penetrate adrenocortical cells to inhibit steroidogenesis.

Parathyroid Specific Autoantibodies

Autoantibodies to parathyroid gland were firstly reported in 38% of 74 patients with idiopathic hypoparathyroidism by indirect immunofluorescent assay (58). Subsequent investigations, however, have found that anti-parathyroid serological immunoreactivity is rare in patients with failed glands (59) and usually is not parathyroid-specific (60). Antibodies considered to be against parathyroid antigens have been confused with mitochondrial autoantibodies in previous reports, and humoral sensitivity to parathyroid tissue may have delineated a tissue-specific response to antigens within the endothelial component of the gland (61). However, in a recent study by Western blotting, **the** calcium sensing receptor was recognized in 32% (8/25) of

patients with hypoparathyroidism associated with APS-1 and in a few patients with hypothyroidism in whom hypocalcemia had been reported. The major epitope located on the external domain of the receptor (62). This finding suggests that such autoantibodies could have a pathogenic role involving down-regulation of parathyroid hormone secretion through signal transduction events in parathyroid cells. Such was actually demonstrated by Kifor and colleagues in two patients with autoimmune hypoparathyroidism. (190). More recently Kampe and colleagues identified antibodies against NALP5 (NACHT leucine-rich repeat protein 5 present in the cytoplasm of chief cells), which were highly specific for APS-1 patients with hypoparathyroidism. [63]

Pancreatic cell Autoantigens

The intensive studies of humoral autoimmunities against antigens expressed by pancreatic cells (Eg, islet gangliosides, insulin, proinsulin, glutamic acid decarboxylase (mainly GAD65, and tyrosine phosphatases (IA-2 and IA-2β)) highlight the complexity of disease-autoantibody relationships. The presence of islet cell autoantibodies (ICAs) detected by immunofluorescence together with insulin autoantibodies and/or anti-GAD or IA-2 has a high predictive value for the development of T1DM. However, islet cell autoantibodies as well as GADautoantibodies also occur in many patients with APS-1 (64,65), a syndrome with only a low likelihood of progression to clinically overt T1DM (65, 66), at least in U.S. patients. Recent studies indicate that autoantibodies to islet cell autoantigens in patients with APS-1 have different reactive characteristics from those of patients with T1DM as in APS-2a and 2b. For example, GAD65 autoantibodies from patients with APS-1 are readily detectable by Western blotting (65), similar to the GAD autoantibodies present in the sera of patients with stiff-man syndrome, an autoimmune neurologic disorder, [67] thus indicating that these autoantibodies recognize linear epitopes on denatured GAD65. GAD65 autoantibodies present in patients with T1DM, however, usuallyreact with conformational epitopes of native orundenatured proteins (68). This suggests that different immune regulations could have been involved in driving the production of these two sets of GAD65 autoantibodies. It also suggests that the presence of islet cell autoimmunity in APS-1 and stiff-man syndrome may not necessarily indicate the destruction of islet cells, at least with the same intensity as in T1DM.Here the islet cell autoimmunity presumably lacks other components of the pathogenic process (e.g., antigen-specific cytotoxic T lymphocytes) that are necessary to produce b-cell damage and thus overt hyperglycemia. ICA seen in APS-1 may also be directed against additional antigens that are not targeted in T1DM. Cystein sulfinic acid decarboxylase (CSAD) shows 50% amino acid sequence identity with GAD65. Recently, it has been shown that CSAD autoantibodies can cross-react with GAD65 (191). Despite close structural relation to GAD65, CSAD autoantibodies were negative in T1DM serum samples, and did not appear to be associated with any of the known autoimmune manifestations of APS-1 except mucocuatenous candidiasis, which was the only common manifestation of APS-1 patients studied. The cross-reactivity with GAD65 and their presence only in APS-1 patient sera, CSAD autoantibodies may reflect the tendency of APS-1 patients to develop anti-GAD antibodies directed against different epitopes than T1DM (192). Therefore, the islet cell non-destructive autoimmune response in patients with APS-1 may result from an impaired cellular immune regulatory mechanism, which differs from that in APS-2, albeit these speculations need to be systematically proven through specific studies. In our experience, some APS patients with

high titers of GAD autoantibodies may exhibit neurologic symptoms, such as cognitive loss, muscular spasms, and partial epilepsy. Whereas these associations need to be confirmed, the possibility that loss of neuronal GABA occurs by action of GAD autoantibodies as they pass the blood-brain barrier needs to be explored.

Thyroid Autoantibodies

Autoantibodies in patients with APS react with thyroid gland proteins, including thyroid peroxidase, thyroglobulin, and thyrotropin receptors. While immunoglobulins against the thyrotropin receptor may stimulate or inhibit both thyroid gland activity and growth, no consistently discernible effect on thyroid function has yet been attributed to autoantibodies that recognize thyroid peroxidase or thyroglobulin (Table 3). Nevertheless, immunization of susceptible strains of mice with thyroglobulin in complete Freund's adjuvant induces a thyroid-specific immune infiltrate in experimental allergic thyroiditis (69). Autoimmunity against the thyroid gland is distinctly unusual in APS-1, but is frequent in APS-2a and -2b. Children with GAD antibody positivity in particular have been demonstrated to have a higher risk for the development of anti-thyroid antibodies. [70]

Thyroid-associated ophthalmology (exopthalmos) is accepted as an autoimmune inflammatory disorder of the periorbital connective tissue (71). The presence of anti-TSH receptor antibodies, in particular TSAb (72), has been correlated with the presence of thyroid eye disease, even in the 10% of individuals who are biochemically euthyroid, although at levels lower than in hyperthyroid Graves' disease. Furthermore, anti-TSH receptor antibodies are not detected in 20% to 70% of euthyroid patients with Graves' ophthalmopathy (72) and any correlations with disease activity levels remain controversial (73,74), making these assays of limited value to the clinician. The presence of anti-thyroglobulin (9%) and anti-microsomal antibodies (17%) is even lower in patients with euthyroid eye disease (72) and indeed, anti-thyroid peroxidase negativity has been suggested as a risk factor for ophthalmopathy, emphasizing the need for alternative markers. More recently, various groups have reported reactivity to a 64-kD protein in human and porcine eye muscle in the serum of patients with ophthalmology (75,76) and this has been identified as a 67kD flavoprotein subunit of the mitochondrial enzyme, succinate dehyrogenase (77) besides three additional protein targets for anti-eye muscle antibodies (78-81). Antiflavoprotein and anti-G2 antibodies are most strongly, although not exclusively, associated with eye disease. Further studies using purified human antigens and confirmation of these results by other laboratories are required to verify these results. Currently, only anti-TSH Receptor antibodies can be recommended for routine clinical use.

Autoantibodies in Atrophic Gastritis and Pernicious Anemia

Achlorhydria and pernicious anemia occurring as part of the APS **s** are associated with the presence of circulating autoantibodies against gastric parietal cells (PCAs) and, less frequently, against intrinsic factor (IFA). Approximately 10% of patients with T1DM have co-existing-circulating PCAs, of whom many develop achlorhydria (12). The pathogenic importance of these immunoglobulins is suggested by their toxic effects on the gastric mucosas of frogs and rats (82,83). The parietal cell proton pump (H, K+-ATPase) represents at least one target of PCA

(84). Thus PCAs appear to be primarily associated with atrophic gastritis and achlorhydria, while IFAs may arise secondarily as a consequence of gastric cell damage and are associated with increasing likelihood of clinical pernicious anemia Gastric carcinoma is unfortunately an added risk for such patients. The author has experienced this in patients with APS-1 who have gastric autoimmunity.

Vitiligo Autoantibodies

Melanocyte autoantibodies have been demonstrated in a small number of individuals with APS-1 and vitiligo. The author has noted that chronic lymphocyte infiltrations in the margin of active vitiligo lesions are common when biopsies are performed. Furthermore, tyrosinase, the rate-limiting enzyme for melanin formation, is one target for autoantibodies in patients with vitiligo associated with endocrine diseases (51). However, controversy on the frequency of autoantibodies to tyrosinase in patients with vitiligo has been reported. [85,86] It appears that patients with vitiligo in association with APS tend to have higher frequencies of antibodies to tyrosinase than do patients with vitiligo only. Tyrosinase-reactive T cells are present in the normal immune system and are responsible for the stimulation of peptides derived from tyrosinase. [87] Immunization by tyrosinase-related protein-1 has been shown recently to induce destruction of melanocytes in mice, [88] whereas the Smyth chicken model of vitiligo is characterized by autoantibodies to tyrosine-related protein-1. [89]

Pituitary Autoantibodies

Antibodies detected by indirect immunofluorescent labeling of hypothalamic vasopressin-producing cells also have been reported in a small number of patients with central diabetes insipidus who had other autoimmune endocrinopathies (90). In a report of 19 patients with a variety of endocrine autoimmunities, autoantibodies against anterior pituitary lactotrophs were detected (91), and scattered reports of humoral responses against somatotrophs and perhaps even gonadotrophs also have been published, but not independently confirmed. However rarely, if ever, have these patients had symptomatic disease of the hypothalamic-pituitary axis. In contrast, among 30 reported patients with proven or presumed symptomatic lymphocytic hypophysitis, autoantibodies directed against the pituitary gland have been described (92). Using transformed rodent pituitary cell lines as a substrate in an indirect immunofluorescence assay, immunoglobulins that specifically bound the hypophyseal cells in culture were observed in the serum of humans with the empty sella syndrome (1). However, the empty sella syndrome has been explained increasingly by hypoplasia of the anterior hypophysis caused by genetic lesions in one of the required differentiation factors. Clearly, pituitary autoimmunity is an area of potential research that needs more attention, especially since it has been reported with considerable frequency in T1DM.

Celiac Disease Autoantibodies

The relative contributions of humoral and cellular immunity in celiac disease remain unclear. A study looking at pediatric celiac patients showed a relationship between gluten ingestion and over-activity of intraepithelial T-cells. These lymphocytes, in turn, were shown to over-produce

cytokines such as interleukin-10 and interferon-gamma. [197] Autoantibodies to tissue transglutaminase have also been detected in patients with celiac disease. [198,199,93] In this disease, ingestion of gliadin in wheat appears to provoke a reversible autoimmunity with transglutaminase-associated symptoms. Thus, detection of autoantibodies to tissue transglutaminase in patients with Addison's disease, regardless of its association with APS, would help to identify the existence of celiac disease in patients tested. We do not advise testing for these autoantibodies in patients without possible symptoms of relevance, because the finding of such autoantibodies in the absence of such symptoms leads to therapeutic uncertainty about if and when a rigorous wheat-free diet should be begun.

Other autoantibodies in APSs

In addition to the common features of APS, malabsorption, alopecia, chronic active hepatitis, autoimmune lung disease, and primaryhypogonadism may develop in some APS patients. Therefore screening for marker autoantibodies for these associated diseases facilitates early diagnosis and treatment of the corresponding diseases. For example, antibodies to tyrosine hydroxylase are found in patients with alopecia areata (116); autoantibodies to tryptophan hydroxylase are associated with malabsorption (117); autoantibodies to mitochondria, smooth muscle and/or liver kidney microsomes are associated with autoimmune liver disease and chronic active hepatitis (118), while hypogonadism is associated with steroid producing cell autoantibodies (119). In their 35 patients with APS-1, Betterle et al., reported that hypergonadotropic hypogonadism (61%) was the most frequently observed additional disease followed by alopecia (38%), vitiligo (22%), chronic hepatitis (19%), and malabsorption (15%) (120). Recently, a report indicates that 100% of APS-1 patients have high titer antibodies to (IFN-α and ώ). Whereas the finding may have diagnostic importance, such antibodies may provoke an immunological dysfunction and thus be a target for immunological intervention.

CELLULAR AUTOIMMUNITY

The pathologic observations and experimental investigations of cellular immunity in multi-organ autoimmunity have yielded results that are similar to those found in the more intensive studies of isolated thyroid and pancreatic islet diseases. In this section, therefore, information derived from research into APS and other autoimmune disorders will be combined to review the principles of autoimmunity that are important in APS pathogenesis. The gross and microscopic pathological changes in APS-1 and APS-2 are similar to those of the component-isolated endocrinopathies. Histological examinations of affected adrenal, thyroid, and parathyroid glands, ovaries, pancreatic islets, and gastric mucosa have all yielded similar results (94-101). A mononuclear leukocyte infiltrate that is comprised mainly of lymphocytes with some macrophages, natural killer (NK) cells, and plasma cells is typically seen. The infiltrating lymphocytes are of both B and T lineages, while the T-cell population includes both the CD4+ and CD8+ subsets which display activation markers (95,99). Sparing of adjacent non-targeted tissue is striking in all organs. As the disease approaches its final stages, atrophy and scarring predominate, mediated in part through target cell-induced apoptosis. Fibrosis eventually becomes a prominent finding in most affected glands and may highlight islands of surviving endocrine tissue that are both hyperplastic and hypertrophied, as illustrated by "regenerative

nodules" in the adrenalitis lesions of Addison's disease. Such attempts at regeneration are invariably accompanied by continued inflammation.

Effector Functions

The presence of circulating tissue-specific autoreactive leukocytes in APS patients was first demonstrated by the elaboration of migration inhibitory factors (MIF's) after incubation of target-organ homogenates with peripheral blood mononuclear cells (PBMC's) from affected individuals. Subsequently, increased levels of PBMC's expressing activation markers such as HLA class II antigens have been observed in patients with early but not end-stage T1DM, Graves' disease, thyroiditis, Addison's disease, and oophoritis (95,101). Since surface antigen phenotyping does not reliably distinguish lymphocytes with different functions, cytokine production profiles of PBMC's are coming under scrutiny. Diminished production of interleukin-4 and increased production of interferon-γ in response to mitogens has been observed in patients with new-onsetT1DM (102). In contrast, autologous thyroid cells elicited interferon- **γ** production by PBMC's harvested from patients with autoimmune thyroid disease, but not from those with nontoxic goiters or thyroid cancer (103). Examinations of affected end organs obtained early in the disease process will ultimately be more informative than those of circulating lymphocytes. Unfortunately, tissue specimens obtained at or after the time disease becomes clinically apparent contain infiltrates that represent a complex response against a multitude of antigens. Animal models of disease are now being used to follow the kinetics of leukocyte infiltration of autoimmune targets. In non-obese diabetic (NOD) mice, descriptions of early insulitis have described initial infiltration by macrophages and CD8+ T lymphocytes, followed by CD4+ T lymphocytes and B lymphocytes (104).

It appears unlikely that the action of a single T-lymphocyte clone can result in clinically important organ failure since adoptive transfer of either T1DM or thyroiditis requires transfusion of both CD4+ and CD8+ lymphocytes. Nonetheless, it is likely that autoimmunity against a single antigen initiates disease. One report, however, in which insulin gene promotor-linked GAD antisense DNA was used to reduce islet cell expression of the antigen suggested that islet cell autoimmune diabetes was abrogated promptly. [105] Target-organ invasion by restricted T-cell families, identified by their expression of T-cell receptor genes that contain uniquely rearranged variable (V) or complementarity-determining regions (e.g., CDR3), or monoclonal expansion of B lymphocytes has not been demonstrated convincingly in APSs. Preferential use of certain T-cell receptorfamilies may occur, however, in an antigen-specific fashion during the inductive events (106).

Despite the multitude of investigations, the sequence of effector events leading to eventual cell destruction has not been resolved with any certainty. It has been difficult to determine how the local effects of cytokines (released from either leukocytes, damaged endothelium, or possibly endocrine epithelium (94,107), aberrant targeted cell expression of class I and/or class II MHC (15,95), and adhesion molecules (ICAM-1) (108,109) on the endocrine epithelium surface contribute to the pathological process. In diabetic rodents, β-cell expression of class I MHC is observed early in the pathogenic sequence, perhapsenhancing the ability of CD8+ T cells to lyse these cellular targets. Later, there may be enhanced class-II MHC reactivity due to the

invasion of macrophages and perhaps some patchy aberrant expression of these antigens on pancreatic β-cells.

Recurrent mucocutaneous candidal infections in APS-1 that are often resistant to treatment most certainly reflect an abnormality of T-lymphocyte function. No specific T-cell defect to account for these findings has been consistently identified, albeit one must exist (101). The possible role of the elusive transfer factor remains unclear (110). Again, this area needs further investigation, especially regarding function of the recently identified AIRE gene.

IMMUNO-GENETICS

While the pathogenic processes of APS-1 and APS-2 appear to be similar, their genetics are distinct. APS-1 is a rare monogenic disorder associated with mutations of the AIRE (autoimmune regulator) gene. APS-2a and APS-2b have dominantly inherited features associated with HLA-class II genes, with distinctive associated HLA alleles for each of them. These two latter syndromes remain to be further defined genetically, especially with respect to their underlying non-HLA genes.

Genetic Studies in APS-1:

APS-1 is an autosomal recessive disease with a pattern of inheritance initially observed by analysis of patients with idiopathic Addison's disease and hypoparathyroidism (127), and later reported by others in different racial groups (111-113, 128). By allelic association and linkage analyses a candidate gene was initially mapped to the long arm of chromosome 21 (21q22.3) in 14 Finnish families of patients with APS-1. [26] Thisgene was later narrowed down to within 500 kb of a gene encoding phospho-fructokinase of liver type (PFKL), by linkage analyses and physical mapping in a relatively homogenous Finnish and European patients with APS-1 (129,130), and later in a heterogeneous US patients with APS-1 (131). Ultimately, two individual groups identified the responsible gene, AIRE, located proximal to the gene for PFKL on the long arm of chromosome 21. [33,34] The AIRE gene consists of 14 exons and encodes a protein with an estimated 545 amino acids that contains two plant homeodomain (PHD) zinc-finger motifs, three LXXLL motifs, and a proline-rich region, suggestive of its putative role as a nuclear transcriptional regulator (Figure 1 from old article). Multiple mutations have been detected in patients with APS-1 and different racial backgrounds, thus indicating that this gene is the disease gene responsible for APS-1.

In addition, 15 polymorphisms have been reported, six of which were found in the coding region, but only one resulted in amino acid substitution (121,122). The mutations are spread throughout the coding region of the gene, although there are two mutation hotspots, as shown by the presence of the same mutations present on many different haplotypes from several populations (121,122,123,124). Other mutations common in isolated populations include a single nucleotide substitution A374G, which leads to a substitution of a tyrosine residue in cysteine in the HSR domain in the Iranian Jewish population (125) and the predominant Sardinian APS-1 mutation R139X, responsible for 90% of Sardinian APS-1 alleles (120). Because the functions of the AIRE protein are still unresolved, the mechanisms by which different mutations disturb the

physiological functions of the protein are unknown. However, recent data indicates that mutations in different regions of the gene have different effects on the intracellular targeting and transcriptional regulation functions of the AIRE protein (125,126). The predicted outcomes for most of the AIRE mutations are truncated conceptual protein of AIRE due either to the introduction of a stop codon, or a frame-shift of the coding gene. However, there are missense mutations e.g. R15L, L28P, Y90C and K83E, which result in the substitution of a single amino acid in exon 1 or 2. It remains to be confirmed whether such missense mutations could disrupt the function of AIRE protein. Two of the most frequently detected mutations in various racial groups, one R257X is located at exon 6, and the other 1094del13 is located at exon 8 of AIRE gene. Other mutations are much less frequent, and some of them have only been detected in a single allele. R257X is due to a transition of C to T at amino-acid position 257. This results in the change of an Arg codon (CCA) to a stop codon (TGA) and would produce only a protein with about 256 amino acids (33, 34). R257X is a dominant mutation for Finnish patients with APS-1 and is also frequently present in patients with other ethnic backgrounds, such as in north Italians, Swiss, British, Germans, New Zealanders and American Caucasians (121, 124). 1094del13 is a 13 bp deletion at nucleotide position of 1094-1106, and result in a frame shift to produce a truncated 372 amino acid residue. 1094del13 occurs in APS-1 patients with various ethnic backgrounds (34, 132, 121, 124, 120). In addition, 1094del13 is a dominant AIRE gene lesion for British patients with APS-1, since 74% (17/23) of mutated AIRE gene alleles from British patients with APS-1 contains this deletion (132).

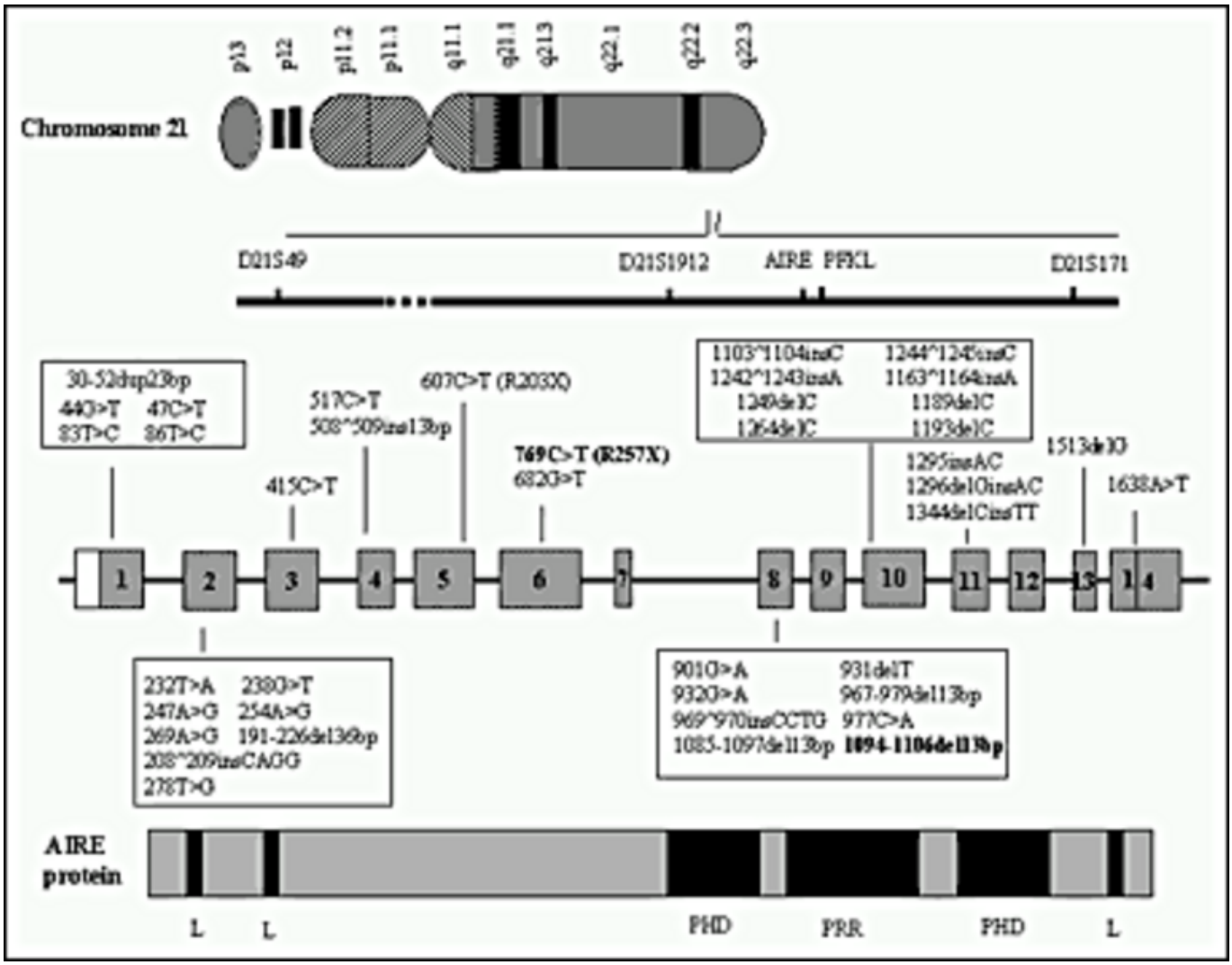

Figure 1. Chromosome localization of AIRE gene, AIRE gene mutations and AIRE protein. AIRE gene is located on chromosome 21 q22.3, close to gene encoding phosphor-fructokinase of liver type (PFKL). To date some 45 different mutations have been detected with R257X and 1094del13 to be the dominant mutations detectable in patients with different ethnic backgrounds. Shown in the figure are the mutations in various exons. AIRE protein contains two PHD zinc-finger motifs (PHD), three LXXLL motifs (L) and a proline-rich region (PRR), suggestive of its putative role as nuclear transcriptional regulator.

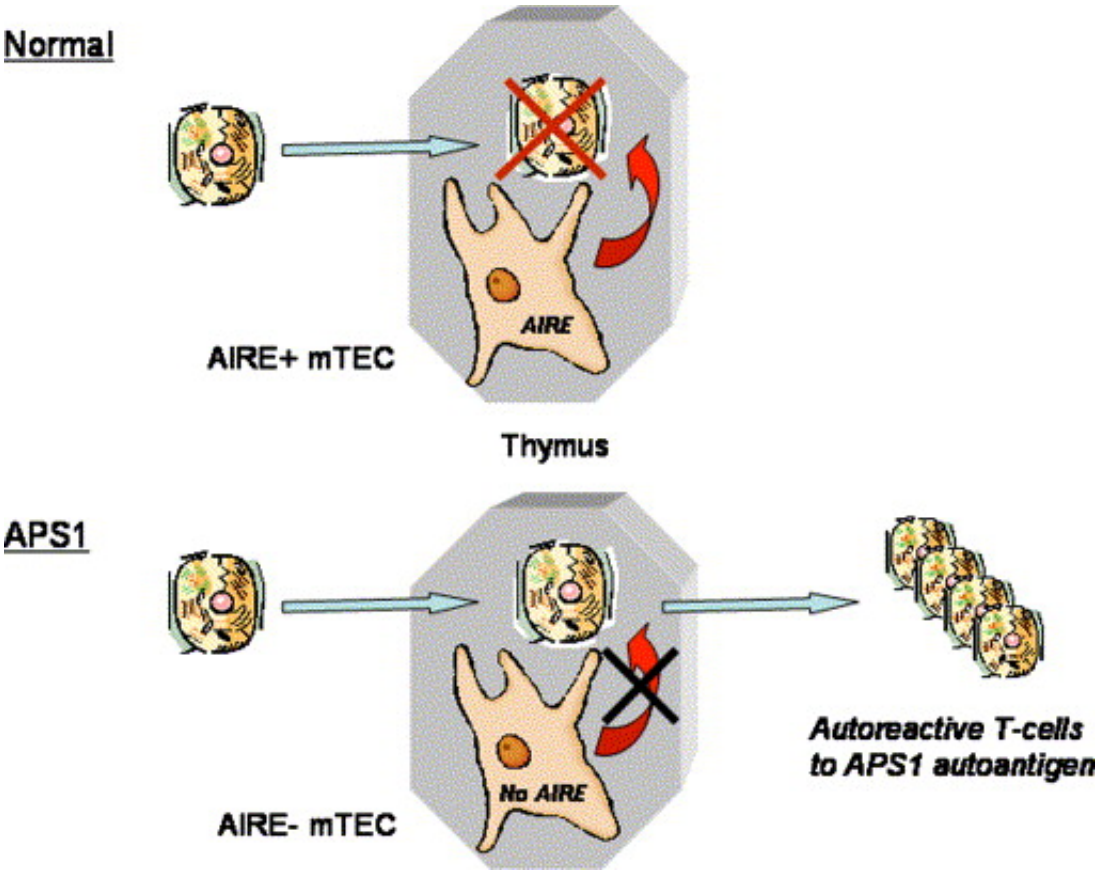

Figure 2. The function of AIRE. In normal thymus, AIRE regulates expression of self-antigens resulting in deletion of autoreactive T-cells. In APS1 thymus, the defect of AIRE expression causes insufficient presentation of self-antigens and autoreactive T cells escape from thymus. Abbreviations: mTEC, Medullary thymic epithelial cells; TEC: Thymic epithelial cells Reprinted with permission Ref:133

Genetic founder Effects

Despite its rarity, the prevalence of APS-1 is higher in certain ethnic groups, e.g., Iranian Jews (1/9,000) (40), Finns (1/25,000) (112), and Sardinians (1/14,000) (**193**). Since it is more prevalent in certain populations, it could be related to a founder gene effect. Founder effects exist for some genetically isolated populations according to the analyses on mutations and haplotype of polymorphic markers closely associated with AIRE gene locus. Recombination events are less common whentwo genomic markers are closer and linkage disequilibrium is thus stronger with polymorphic markers of closely located genes. Therefore, individuals are likely to have common ancestors if they share same haplotype for polymorphic markers that are in linkage disequilibrium, especially for those from genetically isolated populations. Haplotype analyses on polymorphic markers located closely to AIRE gene on Finnish patients have suggested that more than 85% of cases of APS-1 in Finnish patients are due to one major mutation that is commonly present in the ancestors of the Finnish population (130). This is in

concordance with the finding that the mutation R257X was present in up to 82% of Finnish patients with APS-1 and is accompanied by one predominant haplotype of closely linked polymorphic markers, such as D21S1912 and PFKL (33, 34). D21S1912 is located approximately 130 kb upstream of the AIRE gene, and PFKL is located 1.5 kb downstream of the AIRE gene (33, 34). This evidence suggests that R257X occurred as a single mutation event in the relatively homogeneous Finnish population. Studies of 12 British families with APS-1 for AIRE gene mutations found that 17 of the 24 possible mutant AIRE alleles tested had 1094del13 with a common haplotype spanning the AIRE gene locus, suggesting the presence of a founder effect in the British population (132). Mutation R139X has been found in 90% (18/20) of independent alleles with identical haplotypes for D21S1912 – PFKL in that ethnic group (120).

Independent Events in AIRE Mutations

Patients with same AIRE gene mutations often had different closely linked haplotypes, suggesting that either ancient mutational events or multiple independent events occurred to account for the AIRE mutations and haplotypes observed. For example, R257X is a major mutation present in patients from European countries other than Finland. However, non-Finnish patients who had R257X tend to have more diversified haplotypes of D21S1912 – PFKL (121). This is also the case for the other major mutation, 1094del13, since different haplotypes were present in patients with different ethnic origins. For example, 9 out of 15 alleles of 1094del13 detected in 13 patients from a group of American Caucasian patients with APS-1 had different haplotypes of D21S1912 – PFKL (124), suggesting multiple independent events led to the 1094del13 mutation. This should be expected since the patients were of heterogeneous origins typical of the North American population. Thus, the genetic data are now in hand for diagnosis and genetic counseling in families affected by APS-1.

Role of the AIRE Gene in the Pathogenesis of APS-I

An understanding of the biological role of the AIRE protein should provide needed insights into the mechanism of autoimmunity and to APS-1 in particular. The AIRE gene is expressed as mRNA most prevalently in the thymus, but is also expressed in other tissues such as lymph nodes, pancreas, adrenal cortex and PBMCs (33, 34). Recent studies in AIRE gene knockout mice indicate an important role of AIRE protein in eliminating autoreactive T cells through transcriptional control of tissue specific antigens at the levels of the thymus (Figure 2) (133). Attention has already been drawn to the putative nuclear localization and its role in transcriptional regulation of the encoded protein based on the analyses of the predicted amino-acid sequence (33, 34). (134). Mi-2 autoantigen is a 240-kDa human nuclear protein recognized by sera from patients with autoimmune dermatomyositis (135). The Mi-2 autoantigen is actually a partial fragment of a chromo-helicase-DNA (CHD) binding protein, CHD3, identified recently (136). The families of CHD proteins are known to play roles in gene expressions and regulations (136). Also, TIF1 is actively involved in the transcriptional control of the estrogen receptor (134, 137). Accordingly, AIRE gene is most likely participating in the regulation of the expression of another gene(s).

Whereas the number of AIRE gene mutations has grown since the last writing of this chapter [122,131,194], novel genotype/phenotype relationships have not been identified, suggesting that the outcome of the syndrome may be influenced by environmental factors or non-AIRE genes. However, one report has indicated that individual component diseases in APS-1 have the same HLA-genotype associations as they do in APS-1. [194] Identification of the AIRE gene underlying APS-1 has provided an area of major stimulation to research into the pathogenesis of autoimmune disorders. The AIRE transcript has the highest expression in adult thymus and fetal liver. [138] The AIRE protein is expressed mostly in thymic medullary epithelium, but is also seen in a rare subset of cells in lymph nodes, spleen, and fetal liver. [139] AIRE transcripts were reported to be expressed restrictively in peripheral CD14-positive monocytes, but not in polymorphonuclear neutrophils or T cells; AIRE protein was also found in differentiated dendritic cells. [140] Although AIRE gene knockout mice generally develop normally, they do develop multiorgan lymphocytic infiltrates, autoantibodies, and infertility, and when antigenically challenged, show enhanced T cell proliferation. [141] The absence of AIRE gene expression in knockout mice was associated with loss of expression of peripheral antigens in medullary cells of the thymus. [142] In transgenic AIRE gene knockout mice, in which the transgene results in CD4+ T cells against a pancreatic antigen, mice were deficient in eradicating these autoreactive T cells, [143] emphasizing the role of central tolerance associated with AIRE gene functioning. Others have reported that E3 ubiquitin ligase activity is mediated by the first plant homeodomain of the AIRE gene, suggesting a mechanism by which AIRE gene mutations mediate loss of central tolerance. [144] Murine AIRE gene transfectants have been shown to result in downregulation of IL-1 receptor antagonist (IL-1Ra) and class II molecules, as mediated by competition of the transcriptional coactivator (CREB-binding protein or CBP), perhaps explaining the autoimmune and immunodeficient nature of APS-1. [145]

The core phenotype of APS-1 includes the 3 diseases, mucocutaneous candidiasis, hypoparathyroidism and Addison's disease. Patients with APS-1 are also frequently accompanied by one or more other autoimmune diseases, such as chronic active hepatitis, alopecia, vitiligo, or by evidence for an immunodeficiency state, represented by chronic diarrhea/malabsorption, chronic mucocutaneous candidiasis and our observation of oro-pharyngeal carcinomas. Not all patients with APS-1 express all of the three core component diseases or the frequently accompanied diseases. Even patients of same ethnic origin with the same AIRE mutation often present with different component diseases or with different orders of the appearance of the component diseases of APS-1 (121,120,132). Different phenotypic expressions are also present in affected siblings. However, some ethnic groups of patients with APS-1 may develop specific component diseases, suggesting that the outcome of the syndrome is influenced by background genes within population. For example, there is a relative rarity of candidiasis among the Iranian Jewish patients with APS-1 (113). Also, T1DM is rarely seen in patients with APS-1 in the United States; however it does occur in some 15% of Finnish patients with APS-1, especially with increasing age. Finnish patients with APS-1 often have ectodermal and enamel hypoplasia (112). Calcium deficiency due to hypoparathyroidism should not be the primary cause for enamel dystrophy, since ectodermal and enamel hypoplasia occurs in APS-1 patients with or without hypoparathyroidism (112,146). In addition, those non-Finnish patients with APS-1 who had hypoparathyroidism are seldom seen with enamel hypoplasia (113,131). Presumably, different mutations in the responsible gene could be involved in these various phenotypes as shown in the presence of different haplotypes for genetic markers among

patients with different ethnic backgrounds (130). In other words, background genes or genes with epistatic effects may be responsible for variations in the expressed phenotype. Alternatively, more than one gene may be responsible for the development of APS-1, albeit, this is becoming increasingly unlikely. Thus, studies for the function of the identified AIRE gene could shed more light into the pathogenic mechanism of APS-1.

Genetic Studies in APS-2a and APS-2b

In contrast to the autosomal recessive pattern of APS-1, APS-2a and AP-2b express an autosomal dominance pattern with incomplete penetrance (4, 114). Addison's disease, as a component disease of APS-2a or as an individual disease, is reported to associated with HLA-DR3 and HLA-DR4 (147,148), however the HLA-DR4 haplotype, when seen in APS-1, might rather relate to coexisting β-cell autoimmunity (149). Thyroid autoimmunity in pedigrees with T1DM may segregate independently from the HLA complex. For example, DR3-DQB1*0201/DQB1*0302 are associated with APS-2a when there is T1DM (150). Such HLA associations suggest that particular molecules of HLA are required in the development of component autoimmune diseases, and the expression of a particular autoimmune phenotype depends on the involvement of other gene products, especially in a multicomponent autoimmune syndrome like APS-2a. Unlike APS-1, the order of the appearance of the component diseases of APS-2 varies greatly. Individual patients can present with Addison's disease and then develop T1DM and/or autoimmune thyroid disease, or any other sequence. This variability may indicate the presence of different pathogenic pathways during the development of APS-2. Thus, it might be expected that non-HLA genes plus particular alleles of HLA genes may influence the susceptibility of individual patients to a constellation of diseases. For example, the association of DQB1*0302 with APS-2a was abolished in one study when those patients with APS-2a, and overt clinical T1DM or positive autoantibodies to islet antigens, were excluded from the analysis (149). Also, the development of the same disease with different susceptible HLA alleles has been observed in inter-racial studies for HLA susceptibility (151). In addition, multi-genetic involvement in the development of the individual component diseases of APS-2a has been proven, such as linkage of T1DM to more than 10 loci in non-HLA genomic regions (152), or autoimmune thyroid disease which appears to be polygenic as well (153,154).

Patients with APS-2b lack Addison's disease and definitive genetic features except for their associations with its component diseases and their associated HLA alleles. For example, DQB1*0301 is increased in Hashimoto's thyroiditis, DRB1*03 in Addison's disease, DRB3 genes are increased in Graves' disease, and DRB1*13 in vitiligo. Non-HLA genes are however expected to be involved in the development of APS-2b also, and attempts have been made to map for non-HLA genes responsible for component disease of APS-2b. For example, Tomer and colleagues (155) linked a susceptible locus for Graves' disease to within 6 centimorgans at chromosome 20q11.2. Although this susceptible locus is waiting to be confirmed, the finding of a gene for the component disease of APS-2b would enhance our understanding of the pathogenesis of the syndrome. The susceptible locus at the chromosome 20q11.2 was linked to Graves' disease but not to Hashimoto's thyroiditis. This finding again suggests that the cause of pathogenic autoimmunity may involve different pathogenic processes, and that alternative pathways exist for the break down of self-tolerance of the immune system. Thus, the genetic

studies for autoimmune endocrine syndromes should facilitate understanding of the pathogenic process of these diseases, which may be applicable to the component autoimmunities.

THE CLINICAL SPECTRA OF APSs

An important responsibility for the clinician managing patients with single endocrine autoimmunity diseases is to determine those patients' risk for the occurrence of polyglandular disorders. Clues uncovered by a thorough history and physical examination may reveal the true multi-focal nature of a patient's condition. Subclinical or "compensated" deficiencies, identified by elevations of tropic hormones (e.g., normal thyroxine but elevated TSH in Hashimoto's disease), reflect early gland destruction that may be detected during the evaluation of more overt disease in other glands. Once recognized, each individual hormone deficiency should be treated and monitored using the same therapeutic replacement regimens as those used for patients who have isolated gland dysfunction. The authors urge the use of full diagnostic autoantibody panels followed by periodic monitoring of the function of any targeted organ for all Addison's disease probands and their immediate relatives and in patients with type 1 diabetes complicated by the presence of circulating thyroid gland autoantibodies.

APS-1

APS-1, also referred to as the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, is a rare childhood disease largely affecting males and females equally, but is more prominent in certain races such as in Finns (112), Sardinians (183) and Iranian Jews (113). APS-1 is diagnosed when a patient presents with at least two of its' three cardinal clinical features: hypoparathyroidism, chronic mucocutaneous candidiasis Any young person afflicted by troublesome moniliasis without the systemic infection which is generally associated with severe immune deficiency, should be assessed for APS-1. In one study, nearly 45% of pediatric patients with refractory monilial infections but no overt underlying T-cell defect had an autoimmune endocrinopathy (156). Of the 50 to 100% of APS-I patients who develop chronic mucocutaneous candidiasis, most have lesions that are restricted to the skin, nails, and oral and perianal mucosa (66). Common clinical features include severe oral thrush, fungal nail disease, candidal vaginitis, and chronic, crusted-appearing skin rashes. Gastrointestinal involvement in particular can become severe, especially when complicated by bacterial overgrowth, chronic diarrhea, or gastrointestinal hemorrhages. Whereas remissions of varying length occur, progressive courses are common and often necessitate chronic suppressive therapy. Furthermore, there is now evidence for the development of anti-fungal therapy resistance over time. [157] More than 75% of APS-1 patients develop hypoparathyroidism, usually presenting before age 15 years. Severe hypocalcemia manifested by carpopedal spasms, seizures, or laryngospasm can be the presenting feature of APS-1, especially in young children. These patients may not demonstrate positive antiparathyroid antibodies in their sera upon testing. [195] Patients with chronic mucocutaneous candidiasis and hypoparathyroidism have a high chance of developing Addison's disease, close to 60% (66). The latter rarely appears before the development of hypoparathyroidism (12). The symptoms of hypoparathyroidism, however, can be masked in the presence of untreated Addison's disease where it may manifest upon steroid replacement therapy (158). Adrenocortical failure typically develops between the

ages of 10 and 30 years. The detection of anti-adrenal antibodies in an asymptomatic patient is valuable for predicting the potential onset of future disease (PPV up to 92%). [40] Deficiencies of mineralocorticoids and glucocorticoids usually arise simultaneously, but their onsets can be dissociated by up to 5 years (112). Females suffer from gonadal insufficiency more often than males and usually present with maturational arrest after the onset of a normal pubarche and menarche. Autoimmune oophoritis also may present with failed pubertal development or with menstrual irregularities (159). Close to 50% of APS-1 patients will eventually develop autoimmune hypogonadism (66, 160). Hypothyroidism is another autoimmune disease seen with some frequency among APS-1 patients, though it occurs much less commonly than those just discussed. Autoimmune diseases such as hypopituitarism and T1DM rarely occur in association with APS-1.

Among the non-endocrine organ autoimmune diseases seen in association with APS-1, fat malabsorption is the most frequent. Malabsorption/chronic diarrhea has been associated with autoantibodies to tryptophan hydroxylase. Deficiencies of iron or vitamin B12 result from parietal cell autoimmunity with subsequent early appearance of achlorhydria followed by intrinsic factor deficiency and pernicious anemia. Typical atrophic gastritis arises in 15% of APS-1 cases with a mean age at onset of 16 years. Studies of Finnish patients have particularly emphasized manifestations of APS-1 in the teeth and integument. In decreasing order of frequency, enamel hypoplasia, ungual dystrophy (pitting), keratopathy, and tympanic membrane sclerosis have all been reported at rates from 33 to 77% (112). Vitiligo may be missed if not specifically sought using ultraviolet light (Woods lamp examination). Alopecia totalis or universalis is frequent, but all types occur. It has been suggested that hair loss may diminish after treatment of hypoparathyroidism is started (161), but this does not reflect the authors' experience. The appearance of hepatomegaly or jaundice with dark urine and clay-colored stools often heralds the onset of chronic active hepatitis. It occurs in up to 10% of patients and is not associated with persistent immunological hypersensitivity to hepatitis viruses. Sjőgren's syndrome (parotitis, arthritis, and sicca syndrome) is another non-endocrine autoimmune disease not infrequently found.

APS-2a and APS 2b

APS-2a, formerly known as Schmidt's Syndrome, is far more common than APS-1 and is diagnosed when a patient has adrenocortical deficiency with T1DM, chronic lymphocytic thyroiditis, or Graves' disease. Unlike APS-1, this syndrome can be more difficult to recognize before the onset of clinically significant multi-gland disease. The disease is markedly more common among females and commonly manifests in the third or fourth decade, but it is not uncommon before or after these ages. [162] It is heralded by adrenocortical failure in almost half the cases, although this estimate may be skewed by a selection bias in the literature. The remaining 50% either develops adrenocortical failure at the same time as thyroid dysfunction/ T1DM or afterwards, with the latter scenario being slightly more common. [163, 66] As many as 20 years can elapse before polyglandular involvement becomes evident. Furthermore, isolated thyroiditis and T1DM are common enough in these age groups that routine adrenal autoantibody screening of such affected patients is not justified unless adrenocortical insufficiency is clinically suspected. APS-2b, on the other hand is characterized by the presence

of autoimmune thyroid disease in association with one of the other organ-specific autoimmune diseases such as atrophic gastritis/pernicious anemia, vitiligo, primary hypogonadism (female>male) and/or T1DM, but in the absence of Addison's disease (12). Frequent associations common to APS-2b as centered around T1DM, myasthenia gravis (164), and vitiligo (51) have been well documented. Graves' disease and Hashimoto's thyroiditis are both frequent in APS-2, as are vitiligo and pernicious anemia. The authors recommend routine thyroid autoantibody screening of all T1DM diabetes patients and full endocrine autoantibody testing in those found to be positive. However, physicians should routinely elicit historical and physical features relevant to the diagnostic triad in all patients with T1DM and/or autoimmune thyroiditis. A family history of poly-glandular failure is often present in past generations that can serve as a flag for those patients who need extra monitoring. Close to half of cases are familial, though the patterns of heritability are variable. (66) The presence of non-endocrine autoimmune disease, such as alopecia or vitiligo, is less common than in APS-1. When such manifestations are present, however, they are important clinical indicators, especially if they are profound. The mortality risk of untreated adrenocortical failure in the 2% of patients with myasthenia gravis who develop associated endocrinopathies requires that all these patients under 40 years should be assessed closely for endocrinological disorders during their initial investigations.

Other Associations with APS

Rare diagnoses have on occasion been reported in association with an APS. The authors and others have followed patients with APS-1 who developed severe, idiopathic, noninflammatory myopathy with eventual respiratory failure. Separate reports suggested that pure red cell hypoplasia and male infertility in patients with APS-1 responded well to glucocorticoid therapy (165, 166). In rare cases, neo-osseous porosis and sarcoidosis have been linked to APS-1 and APS-2 respectively (167, 168). Asplenia/hyposplenism may not be uncommon and it can be suspected when Howell-Jolly bodies are found in a peripheral blood smear (196). We recommend that all patients with APS-1 be vaccinated against pneumococcus since they are prone to septicemias and even sudden death from such infection. Lastly, diabetes insipidus, immune thrombocytopenia purpura, Sjogren's syndrome and rheumatoid arthritis are all autoimmune diseases rarely associated with APS-2.(66, 160).

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of APS at the time of initial presentation varies according to the disease manifested. When evidence of a second autoimmunity is present, consideration should be given to whether the patient has APS-1 or APS-2, since future monitoring and prognosis are different for these two syndromes. Chromosomal disorders such as trisomy 21 and Turner's syndrome (45X,O and its genetic variants) are associated with an increased risk for endocrine autoimmunities, especially Hashimoto's thyroiditis (up to 30 per cent) and T1DM (some 5%) (169). The primary hypogonadism of Turner syndrome, however, is not of autoimmune origin, and apparent growth hormone deficiencies in some of these females may resolve after estrogen priming. The DiGeorge syndrome is a developmental disorder of the branchial arches that results in facial deformities, aortic arch anomalies, and thymic and parathyroid gland agenesis. These patients develop hypoparathyroidism and mucocutaneous candidiasis, which are usually

diagnosed in infancy, but have few to no circulating T-lymphocytes and produce no autoantibodies. Congenital rubella is associated with the later onset of T1DM and hypothyroidism. Hemochromatosis usually presents with lethargy, malaise, abdominal pain, and hypermelanotic skin lesions. The similarity to Addison's disease can become confusing in patients with either T1DM or secondary hypogonadism induced by pancreatic or hypophyseal iron deposition. Rarely, thyroid, parathyroid, or adrenocortical insufficiencies have been reported in hemochromatosis. Myotonic dystrophy is associated with primary testicular atrophy, alopecia, and less frequently, diabetes mellitus (usually related to insulin resistance). Other more rare diseases which should be considered in the differential diagnosis of APS are listed in Table 4.

Table 4. Rare Polyendocrine Disorders

Disorder	Clinical Features	Cause
Hirata's Disease	Hypoglycemia +/- Graves Disease	Insulin autoantibodies, associated with methimazole use
IPEX	T1DM, Enteropathy	Mutations of *FOXp3* gene, impaired suppression of effector T-cells
Kearns-Sayre Syndrome	Hypoparathyroidism, 1° gonadal failure, non-autoimmune insulopenic DM, hypopituitarism	Myopathic disease, deletions of mitochondrial DNA
POEMS	Polyneuropathy, DM, Organomegaly, 1° gonadal failure. Seen mainly in Japanese population	Plasma cell dyscrasia with elevated M protein and cytokines
Thymic Tumors	Myasthenia gravis, RBC hypoglobulinemia, Graves disease, Autoimmune thyroid disease, Adrenal insufficiency	Thymomas, malignant more than benign
Type B Insulin Resistance	Severe insulin resistance	Insulin receptor autoantibodies
Wolfram's Syndrome	Diabetes insipidus, Non-autoimmune DM, Bilateral optic atrophy, Sensorineural deafness	Mutations of *WSF1* gene, which encodes wolframin gene

IPEX: immunodysregulation polyendocrinopathy enteropathy X-linked syndrome

POEMS : plasma cell dyscrasia with *p* olyneuropathy, *o* rganomegaly, *e* ndocrinopathy, *M* protein, and *s* kin changes.

DIAGNOSTIC PROTOCOLS

Two major laboratory approaches are used to diagnose an APS. First, serum screening for

autoantibodies is used to (1) verify the autoimmune nature of disease in patients with polyglandular insufficiencies, (2) identify patients affected by an isolated endocrinopathy who are likely to develop multi-organ autoimmunities, and (3) screen family members of APS patients, even if those relatives are currently asymptomatic. A complete screening panel includes assessments of adrenal (21-hydroxylase), steroidal cell (17-hydroxylase and P450 ssc enzymes), thyroid (peroxidase and thyroglobulin), islet cell (GAD65 and non GAD65), and parietal cell (H+, K+ATPase) autoantibodies (see Table 3). Thyroid-stimulating immunoglobulins may be required in select patients. A single negative examination does not rule out the possibility of future disease, and annual follow-up tests are optimal. The predictive value of a positive result has already been outlined above.

Second, assessments of end-organ function in autoantibody-positive individuals are required. Serum levels of thyrotropin, calcium, phosphorus, and fasting glucose performed annually can effectively assess thyroid, parathyroid, and pancreatic islet function of asymptomatic patients. Suspicion of subclinical gland dysfunction should prompt a complete functional evaluation of the suspect gland before determining a final diagnosis. Gonadal dysfunction is diagnosed when random serum gonadotropin levels (FSH) are elevated **,** typically in the face of low sex steroid levels.

While depression of early morning serum cortisol levels and electrolyte disturbances represent changes which occur at or just before the clinical onset of adrenocortical failure, it is best to follow high-risk, antibody positive individuals annually with basal serum ACTH (mid-afternoon or later) and supine plasma renin activity (PRA), performed after 1 hour in a supine position. To date, the authors have determined no clinically relevant advantage to screening for adrenal gland dysfunction by formal ACTH stimulation testing or by preceding PRA assessments with salt deprivation. In our studies, serum ACTH levels above 75 and 55 pg/ml at 6:00 and 20:00 hours, respectively, indicated that the anterior hypophysis was responding to adrenocortical insufficiency and thus warranted follow-up with a complete adrenocortical function assessment (170).

Annual hemoglobin or hematocrit determinations are essential, with accompanying examinations for erythrocyte and polymorph morphology. When nutritional deficiencies are suspected, serum levels of ferritin and/or vitamin B12 and red cell folate determinations are indicated.

Fat malabsorption in APS may occur for many reasons, some of which are reversed with proper treatment. It is therefore mandatory that it be completely investigated. Stool examinations for ova and parasites are helpful for diagnosing Giardia lamblia infections, but it may be necessary to obtain duodenal fluid or a jejunal biopsy for direct examination and culture. Bacterial overgrowth can be diagnosed with duodenal aspirate, and a small bowel biopsy is required to diagnose villous morphology. Serum IgA levels also should be assessed.

Patients with suspected recurrent mucocutaneous candidal infections which have been refractory to topical medication should have the diagnosis confirmed at least initially by culturing scrapings from the periphery of an affected area. In such patients, the method of choice is AIRE gene mutational analysis which allows for the diagnosis of 90% of APS-1. In a recent study,

combined analysis of 21-hydroxylase, SCA and AADC antibodies identified 89% of APS patients and this may be a faster and less expensive approach for diagnosis of APS-1 (195).

THERAPY OF AUTOIMMUNE ENDOCRINOPATHIES

The key to successfully managing patients with an autoimmune endocrinopathy is to identify and treat their autoimmunities before they cause significant morbidity and mortality. The treatment of organ insufficiencies is identical whether it occurs in isolation or as part of an APS. Endocrine replacement therapy remains the cornerstone of their clinical management. Patient education about the nature of the disease is often critical to the early recognition of additional new autoimmunities, and as with any chronic diseases, individualized needs for psychosocial support must be assessed. Genetic counseling is also warranted and family members should be screened with the use of specific tests. Given the potential risk for adrenal failure, emergency identification should be worn at all times by APS patients. The use of increased corticosteroid doses at times of acute stress usually averts adrenal crises in those with overt Addison's disease as well as those with adrenal autoantibodies and high risk of adrenal failure. The authors believe that exogenous glucocorticoid supplements given at times of acute stress are well advised in those asymptomatic individuals who have biochemical evidence of asymptomatic adrenocortical insufficiency.

Patients with APS-1 who are diagnosed with Addison's disease and treated with steroids for the first time may have an underlying hypoparathyroidism that is unmasked at this time. Similarly, the introduction of steroid replacement therapy for Addison's disease in patients who already have hypoparathyroidism will induce falls in serum calcium. Malabsorption/steatorrhea can complicate the treatment of hypoparathyroidism with fat soluble vitamin D analogues (1, 25 dihydroxyvitamin D) as well as Addison's disease with cortef replacement because of their malabsorption. Calcium supplementation in hypoparathyroidism (20mg/kg/day up to 1gram/day) is best achieved using effervescent tablets in 3-4 divided doses daily, plus 1,25DOH-vitamin D at 0.5-2.0mcg/day (average 0.03mcg/kg/day). Magnesium levels need to be closely watched if not supplemented at 50-200mgs daily. Otherwise, replacement therapy in the APSs is not different from those with single endocrine organ failures. Addison's disease requires replacement by hydrocortisone at 15-25 mgs/M2 in three divided daily doses plus fludrocortisone at 0.05-0.150mg daily. In times of stress the glucocorticoid dose should be increased 2-3 fold. Of all the endocrine components of an APS, only T1DM does not carry a satisfactory prognosis when managed with well-monitored hormone replacement therapies. The long-term vascular complications have thus made T1DM a candidate for aggressive experimental approaches. Results of controlled trials using cyclosporin A and azathioprine for treating newly diagnosed T1DM have indicated that some metabolic benefits are provided, albeit they are not long-lived, even with continued immunotherapy (171, 172). A case study of a 13-year old girl with APS-1 and numerous severe autoimmune diseases evaluated the use of cyclosporine over an 8 month period. Higly promising results were found in several of her disease processes. [173] Exocrine pancreatic function, ectodermal dysplasia, keratoconjunctivitis, and alopecia all showed marked improvement. Insulin requirements and adrenal insufficiency, however, continued to progress. Anecdotal reports of improved orchitis (165), oophoritis (174), and hypophysitis (175) after immunosuppressive corticosteroid treatments are provocative but

require systematic evaluation. For now, all immunomodulating therapies must be considered experimental and should be prescribed only in the setting of a controlled clinical trial. As more autoantigens are identified and the disease pathogenesis becomes better understood, selective therapies that do not cause generalized immunosuppression may be developed. One possible avenue for this may follow the discovery of IFN antibodies in APS-1. Still further in the future lies the prospect of curative organ transplantation. Pancreatic and, to a lesser extent, islet transplants are currently used in kidney graft recipients with type 1 diabetes (176). Another potential area of growth is the use of non-ablative stem-cell transplantation in patients with autoimmune diseases, most notably in those with early T1DM. In a very recent clinical trial, fifteen patients with new diagnoses of insulin-dependent, GAD-65 antibody-positive diabetes underwent the procedure. [177] One patient was removed from the study due to progressive insulin requirements. The remaining fourteen, however, all became insulin-free over time. Three patients were studied for three years after transplantation and remained off of insulin therapy with hemoglobin A1C levels in the normal range. The remaining eleven were not followed beyond 9 to 24 months for various reasons. More clinical studies are needed in this potentially promising field. The introduction of ketoconazole and its' progeny anti-fungicide has helped greatly with the treatment of chronic mucocutaneous candidiasis, which is commonly resistant to topical antimicrobials. The drug frequently causes gastrointestinal upset and can interfere with glucocorticoid and sex steroid biosynthesis. Elevations of hepatic transaminases are usually transient, but fatal hepatic necrosis can rarely be caused by ketoconazole. The management of fat malabsorption should be first aimed at diagnosing and treating reversible causes. Bacterial overgrowth often responds to broad-spectrum oral antibiotics. Giardia lamblia infestations of the jejunum in APS-1 are best treated with metronidazole, while villous atrophy seen especially in APS-2a typically responds to dietary gluten withdrawal. If no specific cause for fat malabsorption is found, then nutritional support with fat-soluble vitamin and medium-chain fatty acid supplements may be required. This is best done in consultation with a nutrition or gastroenterology specialist. Improved survival for patients with chronic active hepatitis has been achieved with regimens of immunomodulating agents such as prednisone, cyclosporin A, and azathioprine (167). We have had success with intractable diarrhea/malabsorption using IVIG. Tertiary hepatic care is indicated for patients who develop this illness.

PROGNOSIS

The impact of an APS on a given patient's lifestyle varies considerably due to differences in the types of disease present, patient, family and physician-dependent factors. All patients with APS-1 or APS-2a/2b are committed to a regimen of lifelong hormone, mineral, and/or vitamin replacements. While it is usually best to counsel patients to continue participating in all their regular activities, health care providers must be mindful that an APS disease can dramatically alter a patient's life (e.g., an airline pilot who develops T1DM).

Systematic studies of the long-term prognosis in APS patients are lacking, but clinical impressions are that the APS-2 patients have rates of morbidity and mortality that are identical to those of the component diseases when they occur in isolation. Adrenal crises are still a significant cause of preventable mortality, and uncontrolled thyroid hormone imbalances can rarely present as emergencies, especially in the elderly. The complications of T1DM, both acute

and chronic, are as important in the APS setting as in isolated pancreatic disease.

While many patients diagnosed with APS-1 lead a full and vigorous life (112), poorer outcomes are common. Some develop a course of recurrent illnesses starting in their second decade of life. Problems include asthenia that is often of uncertain etiology, recurrent opportunistic infections that presumably arise because of an underlying T-lymphocyte deficiency, chronic active hepatitis that continues to be one of the most common causes of mortality in APS-1 and oropharyngeal or gastric carcinoma, which can be fatal if not diagnosed early. Mortality near the end of the second or during the third decade is unfortunately not uncommon among patients with APS-1.

SUGGESTED SCREENING PROTOCOL

Table 5. Screening Schedule in APS 2a and 2b

Component Disease	Evaluation	Frequency
Addison's Disease	ACTH, electrolytes, morning cortisol, aldosterone level, DHEA-S. Adrenocortical, steroidal cell or 21-hydroxylase autoantibodies	Annual, antibody levels at time of diagnosis
Alopecia	Physical Exam	Annual
Autoimmune Thyroid Disease	Thyroid function tests and antibody levels	Annual
Celiac Disease	Tissue Transglutaminase (tTG) – IgA levels, biopsies as needed	Annual
Pernicious Anemia	Antiparietal cell antibody levels, CBC, Vitamin B12	Every 5 years
Primary Hypogonadism	Luteinizing and follicular stimulating hormone levels, estradiol or testosterone	Annual
Type 1 Diabetes	Hemoglobin A1C, fasting glucose, autoantibodies (Eg, GAD-65, IA-2, and IAA)	Annual, antibody levels at time of diagnosis and every 3 to 5 years

Family members of a proband with T1DM or APS 2a/2b should undergo focused history, exam and screening every three to five years with T1DM autoantibody levels, TSH, serum B12, and anti-adrenal antibody levels.

If a patient is found to have thyroiditis, family members should **also** be screened for thyroid autoantibodies and goiter also.

CONCLUSIONS

As a group, the endocrine organs are commonly targeted by autoimmunity. The genetics of these diseases and studies in animal models have revealed common pathways through which susceptibility occurs. The discovery of AIRE gene and recent findings that suggest it influences the expression of the peripheral self antigens in the thymus has now brought the role of central tolerance back into spotlight and this may also help to explain why multiple organs are targeted for autoimmunity in the same patient. However the restrictive nature of the organs affected and their component antigens still require explanation, albeit HLA genotype influences disease phenotype. For APS-2, the CTLA-4 gene is emerging as a candidate disease gene as heavily influenced by HLA-DR/DQ phenotype (180).

In conclusion, there seems to be an inherent tendency to develop an autoimmune response against specific molecules of target organs in the majority of immunologically mediated endocrine diseases. The inheritance pattern of these diseases aids in the diagnosis of autoimmune disorders such as APS-1, T1DM and Addison's disease before progression to clinical onset in first-degree relatives of affected individuals. The diagnostic approach for these autoimmune mediated endocrine diseases is then based on genetic ascertainments, but can be complemented by the detection of a combination of immunologic markers with high predictive value. Meager et al showed high titers of neutralizing autoantibodies against type 1 interferons (IFN) (179). IFN antibodies to IFNs provide a new marker for APS-1 which is not seen in APS-2.

Given the rapid progress in the genetics and animal models of these diseases, there is hope that many of these questions will be answered soon.

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