Egypt J Pediatr Allergy Immunol 2008; 6(1): 3-12.

Review article

Immunogenetics of type 1 diabetes mellitus

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Introduction

Type 1 diabetes mellitus (T1D) is one of the most common autoimmune diseases with several million people already affected around the globe. It can occur at any age, but is most commonly diagnosed from infancy to the late thirties. It is characterized by an absolute loss of insulin secretion, and results from an autoimmune process that destroys insulin-producing ß cells within the pancreatic islet. Similar to other autoimmune diseases, the etiology of T1D remains obscure but develops on a genetically susceptible background and also involves a variety of factors, ranging from immune dysregulation to environmental triggers. The outcome is the production of auto-antibodies as well as an expansion of auto-aggressive T cells.[1]

The incidence of T1D worldwide varies between as high as 30-40 per 100 000 children in Finland and Sardinia to values as low as 30-40 per 100 000 children in Japan and China [2] .In Egypt , the prevalence of T1D in school aged children varies between 1.12- 1.9 per 1000 in various studies [3-4]. Worldwide, the incidence of T1D is increasing, particularly in the under-5-years age group.[1] The standardized mortality ratio for T1D has been estimated as 4-fold for females and 2.7-fold for males in the Western countries. Even with tight glucose control, there is a significant risk of neuropathy, retinopathy and nephropathy, as well as a 3-fold increase in the risk of severe hypoglycaemia.[5]

Understanding the pathology of T1D may help improve prevention and management. This review will focus on the immunology of T1D, and how this understanding may influence the clinical management, and development of new treatments for this disease.

The immune nature of type 1 diabetes

There are over one million islets in a healthy adult pancreas. They make up 1% of the total pancreatic volume, weigh about 1 g, and contain about 1 mg of insulin. Histological analysis of the pancreas from patients with T1D shows immunological activity limited to insulin-containing islets, including infiltration by activated lymphocytes, antibodies and components of the complement system. These histological findings are consistent with TD1 being an immune-mediated disease. [6]

Further evidence comes from studies showing that T1D is characterized by the presence of antibody (humoral) and T-cell (cellular) responses to islet proteins (antigens) (Table 1). Immune responses to these antigens predate the clinical onset of diabetes, giving further support for an immune etiology to T1D (Table 2).

Table 1. The major ß cell autoantigens in type 1 diabetes [1,6]

Islet cell antigen (ICA)	The first islet 'autoantigen' to be described. Now known to be a complex of autoantigens. Antibodies to ICA are present in 90% of type 1 diabetes patients at the time of diagnosis.
Insulin and pro-insulin	Antibodies to insulin and pro-insulin, the biochemical precursor to insulin, are present at diagnosis in 23% and 34% of type 1 diabetes patients, respectively.
Glutamic acid decarboxylase (GAD)	A constituent of the ICA antigen complex. Present in the 65 kDa form in the human islet. Also present in the central nervous system. GAD antibodies are present in 73% of type 1 diabetes patients at diagnosis.
Protein tyrosine phosphatase (IA-2)	A transmembrane protein from the insulin secretory granule. Also present in central nervous tissue. IA-2 antibodies are present in 75% of type 1 diabetes patients at diagnosis.

Table 2. Evidence for an immune etiology to type 1 diabetes[1]

1. Pancreatic ß cells contain immune cell infiltrates.
2. Immunosuppressive drugs reduce disease incidence.
3. ß cell immunity predates disease onset.
4. HLA genes associate with disease risk or protection.
5. Established autoimmune diseases cluster with type 1 diabetes.

The natural history of type 1 diabetes

This is best demonstrated by looking at the prospective studies investigating the natural history of T1D.[7] The German BABYDIAB study commenced in 1989 to prospectively investigate islet autoantibody and diabetes development in newborn offspring of parents with T1D.[8] Currently, it represents the longest-running prospective study from birth examining the risks for islet autoimmunity and T1D. A total of 1,642 offspring have been recruited at birth and participated in the investigation. The Finnish type 1 Diabetes Prediction and Prevention (DIPP) project [9] started in 1994 and followed up newborn infants with increased genetic risk at close intervals for up to 10 years as well as relatives of patients with T1D. Similar prospective studies include the American (DAISY) study[10], the Australian BABYDIAB study [11], and the MIDIA study on Norwegian children.[12]

Early characteristics of islet autoimmunity

Children developing T1D in early childhood (<10 years of age) have the first signs of islet autoimmunity very early in life, with the majority by 2 years of age.[13] Around 4% of offspring of parents with T1D in the BABYDIAB study and around 6% of genetically at-risk infants from the general population in the Finnish DIPP study have developed islet autoantibodies by age 2 years.[13] Children who develop autoantibodies within the first 2 years of life are those who most often develop multiple islet autoantibodies and progress to T1D in childhood.[14] Autoantibodies do not exclusively develop before age 2 years, but children who develop autoantibodies later have a slower progression to multiple antibodies and T1D.[7]

Islet cell autoantibodies (IAAs) are usually the first autoantibodies detected.[15] Children who progress to T1D have IAAs of high affinity[15] and also develop GADAs concomitantly or soon after the first IAA response. Spreading of the response to IA-2 and IA-2ß often follows.[13,14] Overall, autoantibodies of the IgG1 subclass are the dominant component of the early humoral immune response against each islet antigen, and other subclasses are usually only detected during high-titer peak IgG1 responses.[6,15]

Once islet autoantibodies appear, they usually persist, although significant fluctuations in antibody titer can be observed during the pre-diabetic phase.[6] Of the three islet autoantibodies discussed, IAAs are reported to be the least persistent. One reason why IAAs, and indeed GADAs or IA-2As, may not persist is because they may be transferred from the mother with T1D during pregnancy.[7,12] Depending on the titer of antibodies in the mother, maternal insulin antibodies can persist in the circulation of the child for up to 1 year and maternal GADAs for up to 18 months.[7,12]

Autoantibody effects on antigen presentation

One potential important role played by autoantibodies in the T1D disease process is their effect on autoantigen processing and presentation by class II major histocompatibility complexes. Antigen-presenting cells can capture antigen for presentation to the immune system via both specific and nonspecific mechanisms[1]. Antigen-specific B-cell receptors and Fc receptors on monocytes, macrophages, and dendritic cells increase the efficiency of antigen capture by the antigen-presenting cells and thus lower the threshold for a T-cell response. Several sets of experiments have demonstrated that, in the presence of autoantibodies, the T-cell response to autoantigen is either enhanced or shifted in its focus.[6] This has led to the hypothesis that the process of antibody-mediated antigen internalization alters postendocytic transport and processing events, resulting in the presentation of different T-cell epitopes and potentially unmasking "cryptic" self-determinants, thus manipulating the T-cell response.[6,16,17]

Genetic factors influencing the development of islet autoimmunity

Islet autoimmunity and T1D develop in genetically susceptible individuals, and a major risk factor is a first-degree T1D family history.[18] Familial aggregation of T1D has been recognized for many years, and ~10–13% of newly diagnosed children have a first-degree relative affected with T1D.[19] With respect to family history, risk of developing islet autoimmunity varies depending on which relative(s) have T1D. In the Diabetes Prevention Trial 1 (DPT-1), siblings of type 1 diabetic patients developed islet autoantibodies more frequently than offspring or parents of type 1 diabetic patients.[20]

The MHC region on human chromosome 6p21 has been identified as a critical susceptibility locus for many human autoimmune diseases, including T1D. The major T1D susceptibility genes are found within the HLA class II region on chromosome 6p21 (*IDDM1*).[18] HLA genes are thought to contribute as much as 50% of the genetic risk for T1D. Remarkable with respect to HLA genotypes is that, whereas several genotypes confer increased risk, other genotypes confer protection (e.g., genotypes containing the HLA DQ6 haplotype).[21] In Caucasians, islet autoimmunity and T1D are

strongly associated with HLA DR3-DQ2 and DR4-DQ8 haplotypes), and recent studies from different European countries have confirmed that the HLA DR3-DQ2/DR4-DQ8 genotype is associated with the highest diabetes risk[21]. This genotype is found in 20–30% of type 1 diabetic patients and in almost 50% of patients diagnosed in early childhood.[22]

Islet autoantibodies differ in their association with HLA haplotypes. GADAs are more frequent in patients with HLA DR3-DQ2, whereas IAAs and IA-2As are more frequent in patients with HLA DR4-DQ8. Patients without these haplotypes are more frequently islet autoantibody negative.[6,7]

HLA haplotypes can also be used to identify children who are more likely to develop islet autoantibodies. Results from longitudinal studies of children carrying high-risk HLA genotypes revealed that they have a higher risk for early and more frequent development of islet autoantibodies in infancy.[14] Among BABYDIAB offspring, the risk of developing islet autoantibodies by age 2 years is 20% in individuals who have the high-risk DR3-DQ2/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotypes compared with 2.7% in offspring without these genotypes, and, overall, 50% of islet autoantibody–positive offspring have at least one of these genotypes.[20]

A second genetic susceptibility locus has been mapped by a variable number of tandem repeat (VNTR) in the insulin gene (*INS*) promoter region on chromosome 11p15 (*IDDM2*). Risk has been suggested to be conferred by different expression of the insulin protein in the thymus, leading to defective central tolerance to the insulin molecule.[23] In accordance with this, IAAs are less frequent in patients or relatives who have the T1D protective *INS* VNTR class I/III or III/III genotypes. Combining HLA and *INS* genotyping, therefore, will improve T1D risk stratification.[7]

Over the last decade, whole genome screens have indicated that there are at least 15 other loci associated with T1D,[14,18] and of those, another 2 genes intimately associated with T-cell activation have been identified recently. An allele of the gene for a negative regulator of T-cell activation, cytotoxic T lymphocyte antigen 4 (*CTLA-4*), found on chromosome 2q33, is considered to be the third susceptibility locus for T1D and has been associated with increased levels of soluble *CTLA-4* and the frequency of regulatory T cells.[23,19] A variant of *PTPN22*, the gene encoding LYP, also a suppressor of T-cell activation, has been deemed the fourth susceptibility factor.[22,24] The observation that the 4 most important susceptibility genes for T1D can all be represented on a single diagram of antigen presentation to T cells (Figure 1) emphasizes the potential importance of current therapeutic strategies targeting this interaction. It is also worth noting that the HLA, *CTLA-4* and *PTPN22* have all been implicated in autoimmune thyroid disease and other autoimmune diseases, which supports the premise that similar or overlapping biological pathways contribute to different autoimmune diseases.[25] Modification of risk for islet autoimmunity and T1D by the environment is also likely to be genotype specific, as shown for early exposure to cereals.[7,26]

Environmental factors influencing the development of islet autoimmunity

Studies in monozygotic twins have demonstrated a concordance rate of ~50%, suggesting that environmental factors play a role in the development of diabetes. Epidemiologic studies suggest that viruses, nutrition, toxic agents, or psychosocial factors may contribute to the etiology alone or in combination. As indicated in Fig.2, the long interval between exposure and clinical diagnosis, as well as the interaction of multiple genes, insults, or both, complicate the identification of triggers.[6,25]

Dietary factors and islet autoimmunity

Early feeding may influence the risk of type 1 (T1D) later in life. The information generated so far is, however, controversial. Long breastfeeding, exclusive breastfeeding in particular, and supplementation with vitamin D in infancy have been reported to confer partial protection against beta-cell autoimmunity and TID. In contrast, early exposure to cow's milk proteins and cereals and heavy weight in infancy have been implicated as risk factors for T1D.[27-29]

A recent study [30] investigated the relation between duration of breast-feeding and beta-cell autoantibodies in 5-year-old non-diabetic children in a prospective population-based follow-up; the results revealed positive association between a short duration of both total and exclusive breast-feeding, as well as an early introduction of formula, and positivity for beta-cell autoantibodies in children from the general population suggesting that breast-feeding modifies the risk of beta-cell autoimmunity, even years after finishing breast-feeding.

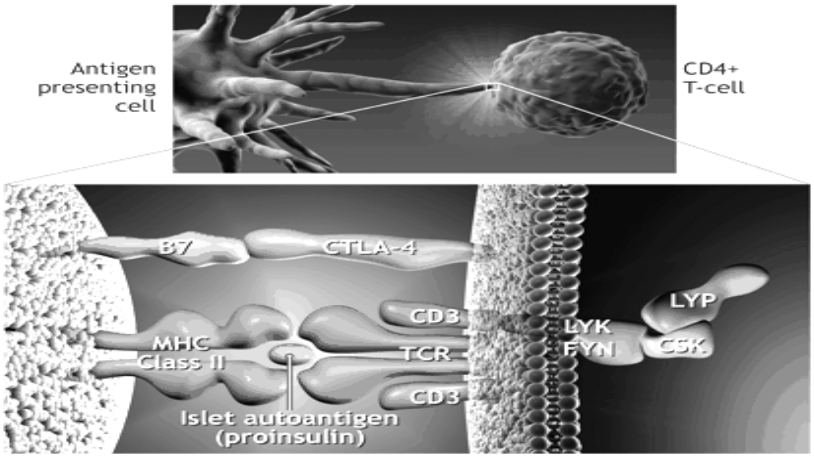

Figure 1. Representation of the process whereby antigen (in this case peptides of proinsulin) is presented to CD4 T cells by human leukocyte antigen (HLA) class II molecules on the antigen presenting cell. This results in T-cell activation. In this diagram the 4 major genes associated with type 1 diabetes are present. *CTLA-4* is an inhibitor of T-cell activation, as is lymphoid tyrosine phosphatase (LYP), which is encoded by the gene *PTPN22*. The complex of LYP–C-terminal Src kinase (CSK) inhibits Lck signaling after engagement of the T-cell antigen receptor (TCR). Quoted from Gillespie[25]

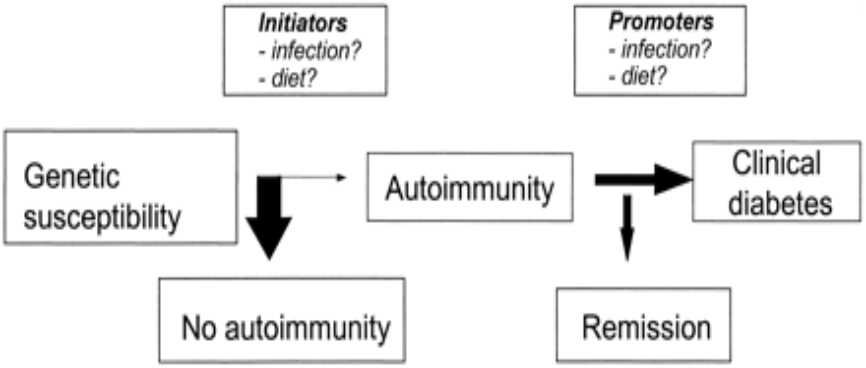

Figure 2. Two-stage development of type 1 diabetes. Step one is the development of persistent islet autoimmunity measured by the presence of GAD65, IA-2, or insulin autoantibodies, alone or in combination. The second step is the progression from islet autoimmunity to type 1 diabetes. Quoted from Pihoker, et al[6].

Viral infections and islet autoimmunity
Viruses may induce islet autoimmunity by molecular mimicry between amino acid sequences of viral peptides and islet antigens that can activate autoreactive T-cells, by bystander activation and expansion of previously activated autoreactive T-cells at a local inflammatory site (including the spreading of T-cell responses to other epitopes on islet antigens), and by nonspecific activation of autoreactive T-cells through viral superantigens.[7,25,31] It is possible that many common RNA viral infections may induce islet autoimmunity in genetically susceptible patients. Prenatal rubella infection in the first trimester is the best-established example of viral initiation of T1D. Twenty percent of patients with congenital rubella develop the disease.[37] Acute enterovirus infections, Coxsackie virus B4 infection and recently rotavirus

have also been associated with the appearance of serum autoantibodies to islet antigens by some investigators[10,31,32] but others could not confirm such observations.[33,34] Infection with other viruses, such as mumps and human cytomegalovirus (CMV) and rotaviruses have also been suggested to be diabetogenic in susceptible individuals.[37]

Progression from islet autoantibody positivity to type 1 diabetes

Once islet autoantibodies (one or several) have developed, the next question is what factors determine ß-cell killing to the extent that diabetes develops.

Factors affecting progression to disease

Genes

Genes clearly determine the likelihood of developing islet autoantibodies and progression to multiple islet autoantibodies. It remains controversial. However, if progression from multiple islet autoantibodies to T1D is influenced by genetic factors.[7] Most studies have stratified progression to T1D using the HLA genotype in autoantibody-positive relatives without distinguishing multiple- from single-antibody positivity. In these studies, the presence of the protective HLA alleles DQB1*0602 allele , or DPB1*0402 or, HLA DR2 were associated with decreased progression to T1D,[35-37] and the high-risk HLA DR3/4 genotype was associated with increased progression to T1D.[20,23,38]

Environment

Like genes, the same environmental factors discussed as triggers of islet autoimmunity are also claimed as possible accelerators or protectors of progression to T1D. Few hard data are available, however.[6,37]

Age

Age affects the risk of progression to T1D.[39] According to the BABYDIAB study, the risk for developing T1D can also be stratified on the basis of how early islet autoantibodies develop. Progression to diabetes is significantly faster in individuals who have multiple islet autoantibodies already within the first year of life than in individuals who develop multiple islet autoantibodies at age 2 or 5 years.[7,39] Thus, delaying progression to multiple antibodies may be effective in markedly delaying diabetes onset.

Characteristics of autoantibodies in progressors versus nonprogressors

It is conceivable that the development of diabetes is accompanied by a maturation of the autoimmune response. It is now well established that subjects with multiple islet autoantibodies have considerably increased rates of progression to T1D than subjects with only one islet autoantibody.[12,40] Substantial effort has been made to identify other disease-specific characteristics of autoantibodies that will help distinguish who will and who won't develop T1D and who will develop T1D early and late.[15,41]

Autoantibody affinity determines progression

The affinity of IAAs and GAD antibodies (GADA) has been found to vary considerably among IAA-positive children.[42,43] IAAs range from high-affinity IgG in most individuals through to low-affinity cold-reactive IgM antibodies in others [15]. Children who developed high-affinity IAAs (K_d >10^9 l/mol) have persistent IAAs, develop multiple islet autoantibodies, and have a 50% risk for developing T1D within 6 years. In contrast, children who have IAAs of lower affinity infrequently progress to multiple islet autoantibodies or T1D. High-affinity IAAs differ from lower-affinity IAAs in their insulin-binding characteristics in a manner consistent with distinct epitope recognition and in contrast to the lower-affinity IAAs (which often do not bind proinsulin). In a recent study[42] it was found that children develop discrete, heterogeneous antibody responses to GAD, only some of which are diabetes relevant. Subtyping the GADA responses using affinity measurement improves T1D risk assessment.

Progressors have broader autoantibody responses

The breadth of the autoantibody response can be measured by the number of autoantibody epitopes it is directed against, and probably by the subclass usage.[6,41]

It is hypothesized that the initial autoantibody response in pre-diabetic subjects, as well as healthy subjects not at risk for T1D, is IgM specific. In progressors, the autoantibody response in pre–type 1 diabetic individuals switches to an IgG-dominated response. Isotype switching reflective of Th1 activity is observed in individuals who progress to T1D more often than nonprogressors or individuals with slow progression.[6]

In a recent analysis of autoantibody-positive relatives followed longitudinally, the highest risks for T1D were associated with high titer IAA and IA-2A responses, with the appearance of antibody subclasses IgG2, IgG3, and/or IgG4 of IAA and IA-2A and antibodies to the IA-2–related molecule IA-2ß.[40,43]

Association of type 1 diabetes with other autoimmune diseases

Patients and relatives with T1D are at increased risk of other immune mediated diseases. These most commonly include thyroid disease, celiac disease, autoimmune gastritis and Addison's disease.[1]

Thyroid disease

Prevalence of thyroiditis in the diabetic population is considerably higher than in the general population. Up to 25% of patients with T1D will have evidence of thyroid disease (the commonest autoimmune disease associated with T1D).[44] Most of these T1D patients will have subclinical disease, although about 5% will be clinically hypothyroid. Undiagnosed hypothyroidism can affect glycemic and lipid control in patients with T1D, making a good case for regular routine screening for this condition.[45]

Annual laboratory determinations of anti-thyroid peroxidase (TPO) antibodies and TSH should be part of routine tests in the diabetic population, especially in girls, children with T1D for > 9 years, patients above 12 years of age, and those in whom T1D is associated with another autoimmune disease. Anti-thyroid antibody positivity may indicate the necessity for thyroid function testing at shorter intervals.[46]

Celiac disease

Celiac disease is a gluten-sensitive enteropathy, prevalent at up to 1% of the Caucasian population, and treated by strict avoidance of gluten-containing products. The prevalence of celiac disease is increased at 7% in subjects with T1D.[47] Many patients with celiac disease do not describe the symptoms of tiredness, diarrhea, steatorrhea or weight loss traditionally associated with this disease, and up to 40% can be asymptomatic.[1] The risks of T1D and celiac disease being present together are higher at a younger age of onset and in the female sex. Screening is through measurement of serum anti-endomysial and anti-transglutaminase antibodies, with confirmatory biopsy of the duodenum through upper gastro-intestinal endoscopy. Antibody levels can fluctuate and screening should be conducted at regular intervals, particularly where clinical suspicion exists.[48]

Autoimmune gastritis

The gastric sodium-potassium ATPase appears to be the major autoantigen in autoimmune gastritis, manifesting achlorhydria and iron deficiency anemia. Parietal cell antibodies are a marker of pernicious anemia, and are present in up to 20% of patients with T1D. This necessitates regular screening for these conditions, at least with a full blood count.[44]

Addison's disease

The earliest descriptions of islet cell antibody were in patients with Addison's disease, and the prevalence of islet cell antibody has been reported to be as high as 6% in patients with Addison's disease.[47] Conversely, the prevalence of antibodies to 21 hydroxylase, a marker of autoimmune Addison's disease, in those with T1D is about 2%. There is little evidence as yet that T1D patients should be routinely screened for this condition.[1,44]

Multiple autoimmune endocrinopathies

Multiple autoimmune endocrinopathies tend to segregate in definable groups; are termed autoimmune polyglandular syndromes (APS). Two broad groups were originally defined. T1D can occur in all of these, but is most frequent in APS 2 (Table 3). APS 2 (Schmidt's Syndrome) is the commonest of the syndromes and is characterized by the presence of Addison's disease with T1D and/or autoimmune thyroiditis. APS 1 is characterized by the presence of at least two of Addison's disease, hypoparathyroidism and recurrent mucocutaneous candidiasis.[1,49]

The future

The greater understanding of the immunology of T1D led to several large studies conducted for diabetes prevention, some with promising results. Two strategies are open to physicians who have patients with T1D: the first is to prevent initiation of autoimmunity; the second is to reverse the effects of ongoing autoimmunity coupled with ß-cell regeneration. Although highly ambitious, the prevention of T1D could be possible by identifying and eliminating environmental risk factors. The next line of defense would be to re-educate the immune system through exposure to ß -cell antigens with the use of oral or nasal tolerance strategies. The observation that insulin may be the primary autoantigen provides support for therapies using insulin to induce tolerance.[1,6,20,25,29,50] In the meantime, immunological studies proved the association with other autoimmune diseases, that alter the morbidity of type1 diabetes. Clinically implemented, this understanding can help improve the quality of life and prognosis of patients with T1D.[1,25]

Table 3. Autoimmune diseases associated with type 1diabetes[44]

Disease	Autoantigens	Antibody: T1D	Disease: T1D	Antibody: general population	Disease: general population
Hypothyroidism (AIT)	TPO	17–27%	28%	13%	<1% overt
	TG	8–16%		11%	5% subclinical
Coeliac disease (CD)	EMA	10%	4–9%	<1%	0.9–1%
	TTG	12%		1.5%	
Addison's Disease (AD)	21-OH	1.5%	<0.5%	Rare	0.005%

Listed are autoimmune diseases associated with T1D, the prevalence of the autoantibodies, and disease in the general population and population with T1D.
TPO, thyroid peroxidase; TG, thyroglobulin; EMA, endomysial antigen; TTG, transglutaminase; 21-OH, 21-hydroxylase

Table 4. Selected genes associated with type 1 diabetes and related autoimmune diseases[44]

Gene	Associated diseases
HLA	
DR3-DQ2, DR4-DQ8	T1D
DR3; DR5	AIT
DR3-DQ2	CD
DR3-DQ2, DR4-DQ8 (DRB1*0404)	AD
MIC-A	T1D , CD, AD
PTPN22	T1D , AIT , AD
CTLA-4	T1D , AIT

T1D, type 1 diabetes; AIT, autoimmune thyroiditis; CD, celiac disease; AD, Addison's disease

Table 5. Characteristics of the autoimmune polyglandular syndromes[49]

	Type I	Type II
Incidence	<1:100,000/yr	1–2:100,000/yr
Male: female ratio	3:4	1:3
Age at onset	Childhood	Adulthood
Inheritance	Monogenic autosomal-recessive	Polygenic dominant
HLA associations	HLA-DRB1*03 (Addison's disease)	HLA DR3-DQB1*0201 (multiple diseases)
	HLA-DRB1*04-DQB1*0302 (alopecia)	DR4-DQB1*0302 (T1D)
	HLA-DRB1*15-DQB1*0602 (T1D)	HLA-B8 (Graves' disease)
		HLA-B8/DRw3
Candidiasis	Mucocutaneous (70–80%)	None
Endocrinopathies	Hypoparathyroidism (80–85%)	Thyroid disease (70–75%)
	Addison's disease (60–70%)	Type 1 diabetes (50–60%)
	Type 1 diabetes (<20%)	Addison's disease (40–50%)
	Hypogonadism (12%)	Hypoparathyroidism (0–5%)
	Thyroid disease (10%)	
	Hypopituitarism (0–2%)	
Nonendocrine autoimmune disorders	Vitiligo, alopecia areata, autoimmune gastritis, pernicious anemia, others	

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