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Autoimmune Type 1 Diabetes

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Keypoints

- The pathophysiologic mechanisms in type 1 diabetes (T1DM) involve loss of islet β-cell secretory function caused by selective killing of these cells primarily by aggressive autoimmune responses involving both cellular and humoral immune pathways.
- Inflammatory cells heavily infiltrate pancreatic islets leading to insulitis where $CD8^+$ T lymphocytes are thought to be responsible for selective and specific killing of β-cells.
- The complex etiology of T1DM involves a strong genetic predisposition, mainly human leukocyte antigen class II genes, and several putative environmental factors, which are thought to trigger autoimmunity or progression to clinical T1DM.
- A preclinical prodrome in T1DM may vary in duration in which one or more islet autoantibodies may precede insulitis and predict the disease at the early stages of pathologic insult.
- In genetically susceptible individuals with islet autoantibodies, metabolic indicators such as insulin release abnormalities and insulin resistance may best predict T1DM especially near clinical onset.
- Based on the improving understanding of the etiopathogenesis of T1DM, several clinical trials have been launched aiming at halting the autoimmunity responses, retarding disease progression or preserving remaining β-cell function after clinical onset.

Introduction

The differentiation between the two main forms of diabetes mellitus – type 1 (previously known as insulin dependent or juvenile onset) and type 2 diabetes (non-insulin dependent or adult onset) – has been possible for almost 50 years. In 1965, insulitis was rediscovered [1], supporting the view that autoimmune islet inflammation was associated with the etiopathology in type 1 diabetes mellitus (T1DM), a phenomenon absent in type 2 diabetes mellitus (T2DM) [2].The evidence for islet autoimmunity was further supported during the last decade by the identification of cellular reactivity with islet antigens [3] and the association between T1DM and other organ-specific autoimmune disorders [4]. More importantly, the long sought after antibodies against islet cells (ICA) were finally detected in sera of patients with concomitant T1DM and autoimmune polyendocrine syndrome [5]. At the same time, T1DM was found to be strongly associated

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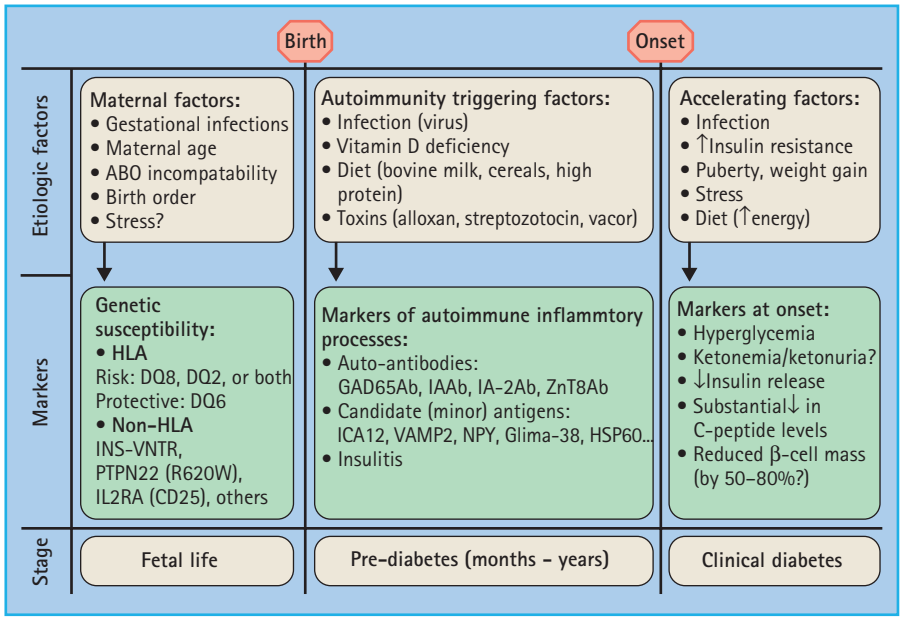

Figure 9.1 Schematic presentation of the natural history of type 1 diabetes (T1DM) showing possible etiopathologic factors and disease markers.

with the human leukocyte antigen (HLA) [6]. It was also noted, however, that around 10% of adult patients classified as having T2DM were positive for ICA, a group of patients now commonly known as having latent autoimmune diabetes of adults (LADA) [7]. Several genetic and autoimmune similarities are found between childhood T1DM and LADA; nevertheless, these two entities differ in other genetic and autoimmune processes [8].

T1DM results from an almost complete loss of insulin brought about by selective autoimmune destruction of the pancreatic islets β-cells, which manifests clinically as hyperglycemia-related symptoms and signs. Among non-Hispanic Caucasian populations, more than 90% of T1DM is immune-mediated (also known as T1ADM), in which HLA association is documented and one or more islet cell autoantibodies are detectable at time of diagnosis [2]. The remaining 10%, often termed "idiopathic" (also known as T1BDM as discussed later in this chapter), is highly inheritable but has neither HLA associations nor detectable islet cell autoantibodies [2]. The latter subgroup is found among non-Caucasian ethnic groups such as Asians [9], African-Americans and Hispanic-Americans and is thought to be related to viral infections [2,10]. Clinically, the two forms have similar clinical manifestations and diabetic ketoacidosis may develop in both.

The pathophysiologic mechanisms in T1DM include two distinct stages in genetically susceptible individuals:

1 Triggering of autoimmunity resulting in one or multiple islet cell autoantibodies associated with gradual β-cell killing; and

2 Loss of β-cell secretory function manifested by the loss of first-phase insulin release (FPIR), reduced C peptide levels, then glucose intolerance and finally hyperglycemia (Figure 9.1).

The autoimmune process with mononuclear infiltration of inflammatory cells (insulitis) including autoreactive $CD8^+$ T lymphocytes selectively destroys the β-cells. Both the humoral and cellular pathways of immunity are involved in the disease process; however, the role of B lymphocytes is evident in laboratory animals such as the non-obese diabetic (NOD) mice but not in humans [11]. Islet autoantibodies, however, may be present before insulitis [12] and therefore may not be a direct consequence of insulitis but rather markers of ensuing islet autoimmunity. The induction of islet autoimmunity in genetically susceptible individuals and the appearance of autoantibodies against specific islet cells autoantigens may precede the clinical syndrome by months to several years (Figure 9.2) [13]. During

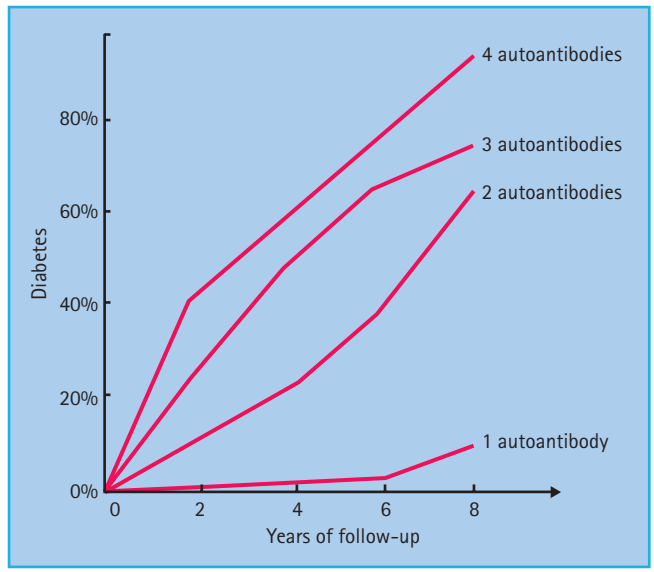

Figure 9.2 Diagrammatic presentation showing the effects of multiple islet autoantibodies on the risk of type 1 diabetes (T1DM) in the Diabetes Prevention Trial Type 1 (DTP-1). Courtesy of Jay Skyler.

this autoimmunity period the number of islet autoantibodies may reflect how β-cells are gradually destroyed. It is proposed that clinical manifestations became overt after loss of more than 80% of viable β-cell mass [14], although there may be variable degrees of both cellular regeneration and insulin sensitivity (Figure 9.3) [15].

The continuing progress in the understanding of the natural history of T1DM will be dependent on several longitudinal studies aiming at detecting factors that predict the disease and to implement both prevention and intervention trials.

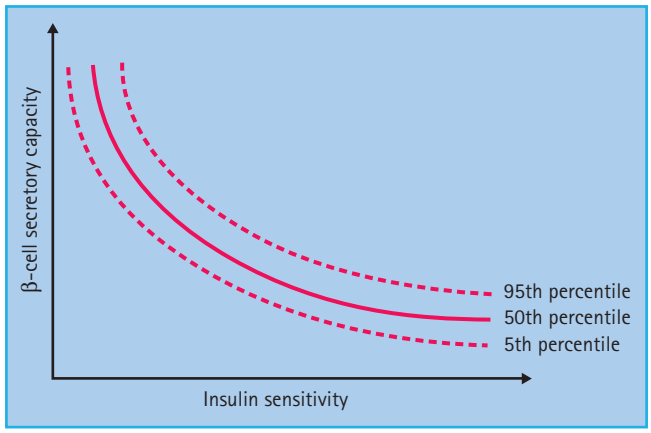

Figure 9.3 The relation between β-cell secretory capacity and insulin sensitivity. Courtesy of Carla Greenbaum, after Kahn *et al.* [15].

Etiology

The etiology of T1DM is multifaceted and may be divided into genetic and environmental etiology and possible gene–environment interactions. Genetic susceptibility increases predisposition for triggering islet autoimmune responses (Figure 9.1). Genetic factors may also help to accelerate the failure of β-cell secretion in response to exogenous environmental factors such as obesity.

Genetics

The concordance rate of T1DM among monozygotic twins ranges from 30–50% up to 70% [16], depending on follow-up duration, compared to only 10–19% among dizygotic twins [17]. This variability highlights the complexity of etiologic components of T1DM that involves the interaction of multiple genetic factors with a variety of environmental factors. Although more than 85% of T1DM occurs in individuals with no previous first-degree family history, the risk among first-degree relatives is about 15 times higher than the general population [18]. An affected father confers a 6–9% risk of T1DM to his offspring compared to 2–4% if the mother is affected and up to 30% risk if both parents are affected [18,19].

The genetics of T1DM has been studied extensively despite the fact that the mode of inheritance remains uncertain. Recent genome-wide association studies have confirmed the strong associations between T1DM and HLA; however, at least 47 non-HLA genetic factors are thought to contribute. The most prominent genetic factors are listed in Table 9.1.

Table 9.1 The most important genetic factors associated with the risk of type 1 diabetes mellitus (T1DM).

Genetic factor	Location	Description		Odds ratio
HLA genes*	Chr.6p21	Highest risk *Genotype*	*DR3-DQ2/DR4-DQ8*	18.7
HLA class II		Highest risk *Haplotypes*	*DR4-DQ8 (DRB1*04‡-DQA1*0301-B1*0302)*	2.0–11.4
			*DR3-DQ2 (DRB1*03-DQA1*0501-B1*0201)*	2.5–5.0
		Most protective *Haplotypes*	*DR15-DQ6 (DRB1*15-DQA1*0102-B1*0602)*	0.03–0.2
			*DR14-DQ5 (DRB1*14-DQA1*0101-B1*0503)*	0.02
Non-HLA genes†**				
INS-VNTR	Chr.11p15	Insulin II: Regulate central tolerance to insulin		2.25
PTPN22	Chr.1p13	PTPN8, LYP. Protein tyrosine phosphatase non-receptor type 22		1.95
IL2RA (CD25)	Chr.10p15	Interleukin-2 receptor, alfa chain		1.70
C12orf30	Chr.12q24	Shares similarity with KIAA0614 protein		1.33
ERBB3	Chr.12p13	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3		1.25
PTPN2	Chr.18p11	Protein tyrosine phosphatase non-receptor type 2		1.22
CLEC16A	Chr.16p13	KIAA0350: C-type lectine domain family 16, member A		1.22
CTLA-4	Chr.2q33	Cytotoxic T-lymphocyte-associated protein-4		1.20
IFH1 (MDA5)	Chr.2q24	Interferon induced with helicase C domain 1		1.15

HLA, human leukocyte antigen.
*The odds ratio (OR) varies and ranges shown represent high-risk populations. Data from [24,26].
†OR, odds ratio at 95% confidence interval. Data from [27].
‡Not *DRB1*0403*.

The major histocompatibility complex (MHC) of the short arm of chromosome 6 harbors the main loci involved in the genetic susceptibility of T1DM as well as many other autoimmune diseases [20]. The HLA genes represent almost 50% of the familial risk of T1DM. Certain alleles of the HLA region, such as the HLA class II DR and DQ alleles, are mainly present in specific association with each other, a phenomenon known as linkage disequilibrium. The HLA association of T1DM (Table 9.1) is therefore often described by haplotype or genotype of the individual [21].

The genotype that confers the highest risk of T1DM is the heterozygosity of the two high-risk HLA class II haplotypes: DR3-DQ2 (*DRB1*03-DQA1*0501-B1*0201*) and DR4-DQ8 (*DRB1*04-DQA1*0301-B1*0302*) (Table 9.1) [21,22]. One or both of these haplotypes were found in more than 95% of people with T1DM younger than 30 years but also in approximately 40–50% of the general population [23]. The concomitant inheritance of high-risk alleles and haplotypes appears to increase the risk of T1DM significantly through synergistic association of their single risks. For example, in patients with T1DM, DQ8 (*DQA1*0301-B1*0302*) is mostly inherited with certain variants of *DRB1*04* especially *DRB1*0401*, *DRB1*0404*, *DRB1*0402* but not *DRB1*0403* which has negative association (Table 9.1). DQ2 (*DQA1*0501-B1*0201*), however, is mostly inherited with *DRB1*03* [21,24]. While certain alleles confer higher risk, such as *DQB1*0302*, *DRB1*03* and *DRB1*0401*, which possesses an independent risk, others confer protection and may "neutralize" high-risk alleles when they are inherited together [25]. The most common protective haplotypes are DQ6 (*DQA1*0102-B1*0602* and *DQA1*0102-B1*0603*), also *DQA1*0101-B1*0503* and *DQA1*0202-B1*0303* [21,26]. Furthermore, other HLA class II (such as DPB1) and class I alleles have also been associated with T1DM risk and the search for new associations is continuing (for review see [24]).

Using a candidate gene approach, several other non-HLA genes were found to be associated with increased risk of T1DM, but their contribution is less than the HLA haplotype associations (for references see http://www.t1dbase.org/page/Welcome/display or the T1D Genome Consortium website: https://www.t1dgc.org/home.cfm). The most important genes are *INS-VNTR* on chromosome 11, *PTPN22* (*LYP*) on chromosome 1, *IL2RA* (*CD25*) on chromosome 10 (Table 9.1) [27].

Environmental factors

The concordance rate of 50–70% among identical twins [16], the seasonality of diabetes incidence and time of birth [28], the association of diabetes with viral infections [29] and the fact that only 10% of HLA-susceptible individuals develop T1DM [28] are among several observations that indicate a possible etiologic role of environmental factors (Figure 9.1).

Table 9.2 The main putative environmental risk factors associated with type 1 diabetes mellitus (T1DM).

Factor	Proposed effect mechanisms	Examples
Maternal factors	Triggering autoimmune response	Gestational infections
	Unknown	Higher maternal age
	Unknown	Higher birth order
	Unknown	ABO blood group incompatibility
Virus infections	Direct β-cell killing (cytolysis)	Mumps virus
	Mimicry of β-cell autoantigens	Rubella virus
	Autoreactive T-cell activation and subsequent β-cell killing	Enterovirus/Coxsackie B virus
		Rotavirus
	Inhibition of insulin production through inducing expression of HLA genes and interferon	Cytomegalovirus
		Epstein–Barr virus
Dietary factors	Triggering autoimmune response	Bovine milk/short breastfeeding
	Triggering autoimmune response	Cereals
	Unknown	High protein content
	Lack of possible protective effect of vitamin D	Vitamin D deficiency
Factors related to insulin sensitivity and/or resistance	Stressing β-cells with excess demands "accelerator hypothesis"	Puberty
		High energy food
	Increase insulin resistance	Weight gain
Psychologic stress	Affect hypothalamic-pituitary-adrenal axis leading to disturbance in autonomic nervous system and autoimmune dysregulation	Stress during pregnancy
		Child–parent separation
		Behavioral deviances
		Difficult adaptation
Toxic substances	Direct damage to β-cells	Alloxan
		Streptozocin
		Vacor

The most prominent environmental factors (Table 9.2) include maternal factors [30], viral infections [29], dietary [31,32], high birth weight and growth rate [33], psychologic stress [34] and toxic substances [35]. The concurrent association of islet autoimmunity and factors increasing insulin resistance such as obesity and accelerated growth may boost the autoimmune destruction of β-cells [36]. The hygiene hypothesis proposes that better sanitation created a pathogen-free environment reducing the exposure to pathogens and their products. According to this hypothesis, the immune systems of children tend to be underdeveloped and therefore prone to autoimmune reactions. Additionally, it was also proposed that younger children received low-level antibodies from their mothers and, when exposed to infections such as enterovirus, it increased their T1DM risk [29].

Pathogenesis

Autoimmune T1DM results from loss of immunologic tolerance to β-cells and environmental factors are thought to be involved in initiation or promotion of autoimmunity or both [28]. The selective destruction of β-cells implies specific mechanisms targeting β-cells by autoimmune reactions, which involve infiltration of pancreatic islets by $CD4^+$ and $CD8^+$ T lymphocytes and macrophages leading to insulitis [1]. During the period preceding the clinical onset, autoantibodies targeting specific islet autoantigens such as insulin, glutamic acid decarboxylase (GAD65), islet antigen-2 (IA-2) and zinc transporter (ZnT8) may be detectable for months up to years before hyperglycemia becomes overt [11]. It has been assumed that the occurrence and number of islet autoantibodies such as GAD65Ab and IA-2Ab were associated with insulitis [37]. A recent study, however, detected islet autoantibodies among 62 (4%) individuals out of 1507 pancreatic donors aged 25–60 years [12]. Although those 62 individuals also had HLA susceptibility, only two of them showed insulitis, indicating that the presence of islet autoantibodies is not necessarily a marker of insulitis. The exact role of these autoantibodies is therefore not understood and it needs to be established to what extent they are markers of β-cell destruction by cellular autoimmunity [38].

The intensity and duration of β-cell destruction varies and seems to be related to the presence of high-risk HLA haplotypes especially DR3-DQ2, DR4-DQ8, or both [39]. The HLA class II family molecules are expressed on the surface of antigen-presenting cells (APC) such as dendritic cells and macrophages but also on activated B and T lymphocytes or even activated endothelial cells. High-risk HLA molecules on APC are likely to facilitate activation of $CD8^+$ T lymphocytes by $CD4^+$ T lymphocytes. This activation is indirectly exemplified in T1DM siblings who developed the disease by the age of 12 years because T1DM occurred in 55% of siblings sharing the high-risk HLA DR3-DQ2/DR4-DQ8 genotype compared with only 5% of those who shared zero or one haplotype [40].

It has been widely claimed, based on autopsy studies, that around 80–90% of β-cells are already lost at clinical onset [14,41]. Recent reanalysis of patients who died soon after diagnosis, however, revealed that the level of β-cell loss required for hyperglycemia was age-dependent, being about 40% in subjects aged 20 years [42]. Additionally, there are suggestions that β-cell regeneration may have taken place, contributing to the approximately 50% of viable β-cells present at diagnosis [43]. The progressive destruction of β-cells is likely to vary in intensity and duration depending on the age at diagnosis [44]. It is a major drawback, however, that direct and precise assessments of β-cell loss before and after diagnosis are not available in humans. Much of the current knowledge of β-cell function prior to the clinical onset has been derived from laboratory animals such as NOD mice and bio-breeding rats [45]. The β-cell destruction in humans may be estimated indirectly by assessing insulin secretion during intravenous glucose tolerance tests (IVGTT). In particular, FPIR measured by insulin or C peptide is thought to reflect loss of β-cells and to predict T1DM [46]. Data from the Diabetes Prevention Trial Type 1 showed that post-challenge C peptide levels were remarkably reduced 6 months before clinical onset [47].

Cellular autoimmunity

The genetic susceptibility of T1DM predisposing to loss of immunologic tolerance and eventual autoimmune killing of β-cells may be explained by a disordered antigen presenting mechanism [43]. The HLA class II molecules are heterodimers that regulate the immune response and are expressed on the surface of APC such as macrophages. The heterodimer binds peptides generated intracellularly either from self-proteins or from exogenous antigens taken up by phagocytosis. The resulting trimolecular complex represents the ligand for the T-cell receptor (TCR). The interaction between the trimolecular complex and the TCR activates the T lymphocyte. The HLA class II molecules on APC are responsible for antigen presentation to T-helper lymphocytes ($CD4^+$). Upon non-antigenic stimulation, macrophages from people with T1DM and the high-risk HLA DQB1*0201/*0302 genotype showed excessive secretion of proinflammatory cytokines and prostaglandin E_2 [48]. Cytokines may damage β-cells directly or indirectly by activating other cells such as T and B lymphocytes [49]. The APC presenting β-cell autoantigens may thus be actively involved in the anti-self autoimmune response that may result from failure to sustain self-recognition or from promoting an anti-self response. APC, $CD4^+$ and $CD8^+$ T lymphocytes were all detected in pancreatic autopsies of subjects who died shortly after onset [50], indicating their role in insulitis. Autoreactive $CD8^+$ T lymphocytes may have the most significant role in autoimmune destruction of β-cells [51]. Natural killer cells (NK) may also be found with abnormal activity and count [52]. The detection of autoreactive T lymphocytes in insulitis and in the circulation at the time of diagnosis, in addition to the notion that immunosuppressive drugs such as cyclosporine or anti-CD3 monoclonal antibodies can temporarily abort disease progression, are all considered to support the role of cellular immunity in β-cell destruction [53].

The mechanism involved in β-cell destruction is not yet fully clear. One possible scenario is that β-cells are first destroyed by an environmental factor such as virus. The dying or dead β-cell is next phagocytozed by local dendritic cells (APC), which are then activated and migrate through the lymphatics to a pancreatic draining lymph node. The antigen presentation to and activation of $CD4^+$ T lymphocytes takes place in the lymph node to include activation of $CD8^+$ T lymphocytes specific for islet autoantigens. These islet autoantigen-specific $CD8^+$ T lymphocytes return to the blood circulation, eventually ending up in islets to destroy β-cells. The β-cell killing will generate a new cycle of islet autoantigen presentation known as epitope spreading [49,54]. $CD4^+CD25^+$ regulatory T lymphocytes are also thought to have an important role in pathogenesis of T1DM as they may inhibit islet autoantigen specific $CD4^+$ T lymphocytes [54,55]. These cells express FOXp3 from the X chromosome and are important in development of peripheral tolerance.

The identification of islet autoantigen-specific T lymphocytes has been challenging and the development of standardized assays of T lymphocytes specific to islet autoantigens (insulin, GAD65 and IA-2) is still difficult to achieve [53,55]. Soluble HLA class II tetramer assays to assess autoantigen-specific T lymphocytes [56] and ELISPOT (enzyme-linked immunospot) [57] assays to measure cytokines of each T lymphocytes are tests to assess anti-islet autoantigen T-lymphocyte reactivity. During islet autoimmunity prior to the clinical onset these autoantigen-specific T lymphocytes may not be found in peripheral circulation, rather they may accumulate in islets and are therefore hard to detect [58].

Humoral autoimmunity

Islet cell autoantibodies

The identification in 1974 of ICA was achieved using frozen pancreatic sections and indirect immunofluorescence [5]. Four years later, islet surface antibodies (ICSA) were identified [59] and complement-dependent antibody-mediated islet cell cytotoxicity was described in 1980 [60]. Because ICA assays showed wide variations among ICA-positive sera [61], assays specific to individual autoantigens were later developed to detect autoantibodies against GAD65, IA-2 [62], insulin [63] and recently ZnT8 (Table 9.3) [64]. Islet autoimmunity (single or multiple autoantibodies persistent for 3–6 months) proved to be useful in differentiating T1DM from other forms of diabetes [11]. Multiple islet autoantibodies (≥2) usually appear within 6–12 months following the appearance of the first autoantibody (Figure 9.2) [65]. Nevertheless, some individuals develop transient islet autoantibodies but they are usually solitary and associated with lower risk [66], possibly because of the presence of protective genes such as HLA DR15–DQ6 [25]. One or more of these autoantibodies can be detected months up to years before clinical onset in more than 95% of newly diagnosed patients with T1DM, even as early as in the perinatal period [67]. Moreover, the detection

Table 9.3 Characteristics of islet autoantigenes and autoantibodies.

	GAD65	IA-2	Insulin	ZnT8
Chromosome	10p11	IA-2: 2q35-36 IA-2β: 7q36	11p15	8q24
Molecular weight (kD)	64	IA-2: 40 IA-2β: 37	5.8	67
Tissue specificity	Pancreas, neuron, ovary, testis, kidney	Neuroendocrine cells (pancreas, brain, pituitary)	β-cell specific	β-cell specific
Function	GABA production (an inhibitory neurotransmitter)	Not clear (lack enzymatic activity)	Regulates glucose metabolism	Zn^{2+} transport and accumulation in β-cell vesicles
Genetic association	DR3-DQ2 DR4-DQ8	DRB1*0401	INS-VNTR, DR4	SLC30A8
Antibody abbreviation	GAD65Ab	IA-2Ab	IAA	ZnT8Ab
Standardized assay	RBA, ELISA	RBA	RBA	RBA
Sensitivity (%)	RBA: 80 ELISA: 89	RBA: 70 ELISA: 65	RBA: >60	RBA: 50 (C terminal)
Specificity (%)	RBA: 96 ELISA: 98	RBA: 99 ELISA: 99	RBA: 95	RBA: 98 (C terminal)
Variation with age	Higher detection with increase age	Less with increasing age	Higher predictivity in children	Increasing predictivity with age
Variation with gender	Female preference if onset <10 years	Male preference	None	None

ELISA, enzyme linked immunosorbent assay; RBA, radiobinding assay.
Workshop sensitivity and specificity for GAD65Ab and IA-2Ab were from the Diabetes Antibody Standardization Program [62].
Diagnostic sensitivity at 95% diagnostic specificity for insulin autoantibodies (IAA) were from [63].

of the four main autoantibodies may predict the disease by as much as 98% (Figure 9.2) [68].

Glutamic acid decarboxylase autoantibodies

The enzyme glutamic acid decarboxylase (GAD) is found in neurons and islet β-cells and produces γ-aminobutyric acid (GABA), which is a major inhibitory neurotransmitter. The 64K protein identified as GAD after immunoprecipitation of human islet [69] was found to represent GAD65, not the previously known GAD67 isoform, which shares 65% of the GAD65 amino acid sequence [70]. Unlike GAD67, GAD65, which is encoded by a gene on chromosome 10p11, is expressed mainly in pancreatic islets (Table 9.3) [70].

Autoantibodies against GAD are most commonly to the GAD65 isoform (GAD65Ab), which were found in 70–80% of children with new onset T1DM, 8% of T1DM first-degree relatives but also in about 1% of general population [71]. Unlike ICA, GAD65Ab remain detectable for many years even after considerable loss of β-cell function [72]. Additionally, GAD65Ab detection rate rises with age in new onset T1DM. If the onset was before 10 years of age, some gender differences with female preference is observed. Because GAD65Ab levels are persistent, more prevalent and correlated well with plasma levels of C peptide [38], they are currently considered as good markers for both prediction and follow-up of β-cell dysfunction among individuals at risk.

GAD65Ab were found to be associated with the high-risk HLA haplotypes DR4-DQ8 (*DRB1*04-DQA1*0301-B1*0302*) and DR3-DQ2 (*DRB1*03-DQA1*0501-B1*0201*) [73] but more often with the latter [72]. Recently, anti-idiotypic GAD65Ab were found to be markers that have lower frequency in T1DM and their absence was more predictive than the presence of GAD65Ab [74]. GAD65Ab can be detected with both radiobinding assays and enzyme-linked immunosorbent assay (ELISA) and these assays have been assessed and standardized in the latest Diabetes Antibody Standardization Program (DASP) [62]. The high and improved performance of these assays emphasizes the value of these autoantibodies in prediction and classification of T1DM and also their value as screening tools in individuals at risk (Table 9.3).

Islet antigen-2 autoantibodies IA-2Ab and IA-2βAb

This autoantigen is a member of the plasma membrane protein tyrosine phosphatase family [75]. Its composed of two isoforms: IA-2 (formerly known as ICA512) which is a 40K protein encoded on chromosome 2, and IA-2β (phogrin) which is a 37K protein encoded on chromosome 7 (Table 9.3) [76]. The two isoforms share many common epitopes and are present in several neuroendocrine tissues in addition to pancreatic islets with no clear function because they lack enzymatic activity.

The autoantibody reactivity of IA-2Ab is directed to the cytoplasmic portion of the autoantigen and the immuno-reactivity in T1DM is directed against the C-terminal region of IA-2 [77]. IA-2Ab is detected in about 60–70% of patients with new-onset T1DM [78] and in less than 1% of the general population [79]. IA-2Ab are often preceded by IAA, GAD65Ab and ICA, respectively [65], and the frequency decreases with increased age of onset [80]. This indicates that the predictive and screening abilities of IA-2Ab are more useful for younger children especially when combined with GAD65Ab and other markers.

Using radiobinding assays to determine epitope-specific IA-2Ab/IA-2βAb among healthy siblings of children with T1DM [81], it was found that progression to T1DM was more common with autoantibodies to the juxtamembrane region of IA-2 (IA-2-JM-Ab) while IgE-IA-2Ab conferred protection even when IA-2-JM-Ab were positive. Higher frequencies of IA-2Ab were found in association with *DRB1*0401* rather than with DQ8 [72]. Furthermore, patients with DQ2 [82] had less association with IA-2Ab indicating a role for additional mechanisms related to the HLA genetic component.

Assays to identify IA-2Ab were developed and standardized using radiobinding tests that can precipitate IA-2Ab, IA-2βAb along with GAD65Ab. These assays have high levels of sensitivity and specificity and were improved in subsequent DASP workshops [62]. Similarly, ELISA assays combining IA-2Ab and GAD65Ab using biotin-labeled preparations were also standardized and the latest evaluation showed progress in performance of these assays (Table 9.3) [62].

Insulin autoantibodies

The most highly specific autoantigens of β-cells are insulin and its precursor proinsulin because they are expressed only in β-cells. In 1983, using radioligand-binding assays, insulin autoantibodies (IAA) were first identified in 50% of patients with newly diagnosed diabetes before initiating treatment with exogenous insulin [83]. IAA, which are able to react with both insulin and proinsulin, tend to be the earliest marker of islet autoimmunity [65] but their levels are often fluctuating and present in low titers. The predictive value for T1DM using IAA alone appears to be related to age; it is higher among younger children, possibly related to a higher rate of β-cell destruction. IAA were detectable in 90% of children who progress to T1DM before the age of 5 years compared with only 40–50% of adolescents older than 15 years (Table 9.3) [84].

DR4 is associated with a higher frequency of IAA, which may be related to the linkage disequilibrium with the high-risk DQ8 haplotype [85]. IAA were also associated with the insulin gene on chromosome 11p15 [86] where the number of tandem repeats (VNTR) were found to be associated with T1DM whether IAA were present or not.

Antibodies against exogenous insulin showed no correlation with IAA levels detected at clinical onset of T1DM and appear to be independent of autoimmunity [87]; however, they do share some similar binding features [88]. Unlike IAA, antibodies against exogenous insulin shows higher specificity, therefore they may be detected using the ELISA test, which does not predict T1DM [89]. The IAA fluid-phase radioimmunoassay shows high sensitivity and specificity to detect T1DM and has been modified to use less serum volume (25 μL instead of 600 μL) in

a new assay known as "micro-IAA" (Table 9.3) [90]. Nevertheless, poor inter-laboratory concordance remains a problem that has delayed standardization of IAA [63].

ZnT8 Transporter (SLC30A8) autoantibodies ZnT8Ab

The zinc transporter (ZnT8 isoform-8 transporter) has recently been described as a second novel β-cell-specific autoantigen, in addition to insulin [64]. A polymorphism in the gene encoding this autoantigen, SLC30A8, is also associated with the risk of T2DM [91]. ZnT8 is important for zinc-insulin crystallization and insulin secretion. It facilitates transport and accumulation of cytoplasmic zinc into the secretory vesicles of β-cells. Inside these vesicles, insulin molecules are co-crystallized with two Zn^{2+} ions to form solid hexamers.

Nearly 60–80% of patients with new onset T1DM react positively to ZnT8Ab [91], which were detected in around 26% of patients who were negative for the conventional islet autoantibodies (GAD65Ab, IA-2Ab and IAA) [68]. By contrast, ZnT8Ab were detected in only 2% of controls and less than 3% of T2DM [68,91]. Additionally, ZnT8Ab were also detected in 30% of patients with other autoimmune diseases associated with T1DM [64]. Being a target of humoral immunity in T1DM, the high β-cell specificity of ZnT8 is seen as an advantage over other non-specific β-cell autoantigens such as GAD65 and IA-2. This high specificity and independence from other islet autoimmune markers, in addition to the fact that ZnT8Ab titers increase with age, all emphasize the value of ZnT8Ab in predicting T1DM, especially among older children.

The polymorphic *SLC30A8* gene located on chromosome 8 encodes the ZnT8 [91]. This locus and other chromosome 8 loci have not been associated with T1DM risk as such but were associated with the risk of ZnT8Ab [92]. ZnT8Ab were found to react with the C-terminal of the autoantigen and variation at amino acid position 325 determines two important susceptibility markers of ZnT8Ab, which can either be arginine (ZnT8-R) or tryptophan (ZnT8-W) [92]. Immunoprecipitation assays for ZnT8Ab were developed and fluid phase radioassays for the C-terminal of ZnT8Ab were standardized and validated in the DASP workshop (Table 9.3).

Candidate (minor) autoantigens

Several studies have reported associations of a group of molecules and substances with T1DM. This group included a wide variety of minor or candidate autoantigens that are thought to be associated with T1DM, autoimmunity or both; examples are, ICA12/SOX13 [93], glima-38 [94], vesicle-associated membrane protein-2 (VAMP2) [95], neuropeptide Y [95], carboxypeptidase H [96], GLUT-2 [97], heat shock protein 60 [98], imogen 38 [99], ICA69 [100] and others (Table 9.4).

Conclusions

Considerable progress has been made in the understanding of T1DM pathogenesis as it relates to the appearance of islet autoimmunity prior to the clinical onset of the disease. The development

Table 9.4 The main candidate (minor) islet autoantigens.

Autoantigen	Molecular weight (kD)	Description	Autoantibody frequency
ICA12 (SOX13)	–	SOX family protein present in pancreas, kidney and placenta. Anti-SOX-13-Ab found more in children	T1DM: 10–30% T2DM: 6–9% Healthy controls: 2–4%
Glima-38	38	Amphiphilic glycated β-cell membrane protein. Specific expression on islet and neuron cells	New onset T1DM: 19% Prediabetes phase: 14% Healthy controls: missing
VAMP2	12.6	β-cell secretory vesicles-related protein	T1DM: 21% Healthy controls: 4%
NPY	10.9	β-cell secretory vesicles-related protein	T1DM: 9% Healthy controls: 2%
CPH	43.4	Carboxypeptidase B-like glycoprotein present in islets and brain. Related to cleavage of insulin from proinsulin	ICA^+ relatives: 20% Healthy controls: missing
GLUT-2	55	Glucose transporter type 2 of β-cells	New onset T1DM: 32–80% Healthy controls: 6.6%
HSP 60	60	A "stress" protein which is thought to be produced and upregulated in response to cellular stress	T1DM: 15% Rheumatoid arthritis: 20% Healthy controls: 1.2%
Imogen 38	38	A protein found in β-cell mitochondria and to a lesser extent in α-cells	No antibodies found
ICA 69	69	A peptide mainly present in islets and neuroendocrine, but also brain, kidney and lung	New onset T1DM: 5–30% Healthy controls: 6% Rheumatoid arthritis 20%

of standardized islet autoantibody tests (GAD65Ab, IA-2Ab, ZnT8Ab) has made it possible to begin screening for subjects at risk to be included in clinical trials aimed at preserving residual β-cell function. Analyses of cell-mediated immunity need further development and standardization to be useful in clinical trials. HLA-DQ on chromosome 6 remains the most important genetic factor for T1DM risk. It is well-established that these HLA class II heterodimeric proteins are necessary but not sufficient for disease. Recent genome-wide association studies have provided a smorgasbord of candidate factors to be explored for T1DM risk. We are still at a loss as to which environmental factor(s) may be responsible for triggering islet autoimmunity and studies such as The Environmental Determinants of Diabetes in the Young (TEDDY) [101] are needed to test fully the multitude of candidate triggers.

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Other Disorders with Type 1 Phenotype

Alice P.S. Kong & Juliana C.N. Chan

Keypoints

- Patients with type 1 diabetes classically present early, require continuous insulin treatment and carry autoimmune markers such as antibodies to glutamic acid decarboxylase (GAD).
- There are other types of diabetes with type 1 phenotype secondary to heterogeneous etiologies including: maturity-onset diabetes of the young (MODY) and other forms of monogenic diabetes caused by mutations of mitochondria, amylin or pathways implicated in pancreatic β-cell function; latent autoimmune diabetes of adults (LADA); fulminant type 1 diabetes presenting with diabetic ketoacidosis after viral infections and another form which reverts to a clinical course resembling type 2 diabetes after the initial ketotic presentation.
- The correct diagnosis of these disorders with type 1 phenotype is clinically important because of their different clinical course, prognosis and management.

Introduction

Classic type 1 diabetes mellitus (T1DM) is considered an autoimmune disease with pancreatic β-cell destruction. Affected subjects typically have onset of their disease at a young age with an acute presentation including diabetic ketoacidosis requiring continuous insulin treatment [1]. With a better understanding of the epidemiology and molecular mechanism of diabetes, however, clinical features such as the younger age of onset (e.g. less than 35 years old) or dependence on insulin treatment cannot adequately define the etiology of patients presenting with hyperglycemia.

Furthermore, there are major ethnic differences in disease pattern in terms of presentation and natural progression. In Caucasians, over 90% of patients with diabetes diagnosed before the age of 35 years have type 1 disease [1]. By contrast, autoimmune T1DM is uncommon in non-Caucasian populations [2–5]. Using Hong Kong as an example, which has a relatively homogenous southern Chinese population leading an affluent lifestyle, less than 10% of adults presenting with diabetic ketoacidosis have autoimmune markers. Similarly, only 10% of Hong Kong Chinese patients with young onset of disease had a type 1 presentation or antibodies to glutamic acid decarboxylase (GAD) [6]. Similar epidemiologic findings have also been reported in other Asian populations from India, Malaysia, Singapore and Mainland China [7]. In most case series, 60–80% of Caucasian people with T1DM have autoimmune markers such as autoantibodies to GAD and/or islet cell antigens (e.g. ICA-512). Conversely, 5–20% of young Asian patients with a non-ketotic presentation have autoimmune markers with a wide range of insulin reserve. These findings suggest that latent autoimmune diabetes in adults (LADA) is not uncommon in young patients with diabetes, especially those of Asian ethnicity, and there is considerable overlap between type 1 and 2 diabetes phenotypes (Figure 9.4) [6,8].

Our current understanding of the molecular pathways involved in the neogenesis, differentiation and maturation of pancreatic β-cells as well as the intracellular signaling mechanisms leading to insulin secretion are summarized in Figure 9.5. This large body of knowledge has provided the basis for the discovery and description of subtypes of diabetes with predominant β-cell failure from causes other than autoimmunity, such as monogenic diabetes. Patients with monogenic diabetes often have young onset of disease and lean body mass (see Chapter 15). They may also have delayed presentation with complications brought about by the insidious nature of their symptoms [9,10]. With the rising prevalence of young onset diabetes especially in low and middle income countries [11], there is a need for health care providers to be aware of these non-classic presentations of T1DM, because they have important implications on clinical management and family screening.

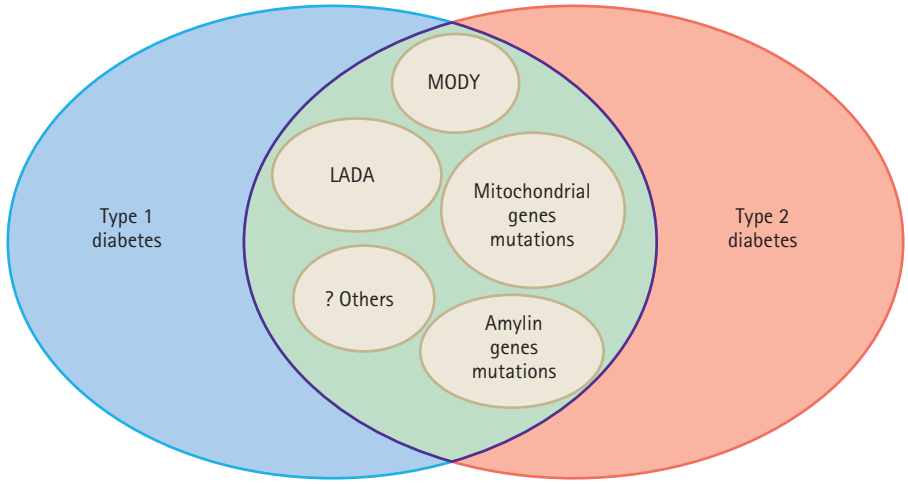


Figure 9.4 There are considerable overlaps between the phenotypes of type 1 and type 2 diabetes because of the heterogeneous genetic and autoimmune etiologies. These include maturity-onset diabetes of the young (MODY), latent autoimmune diabetes in adult (LADA) and genetic variants affecting the insulin, amylin and mitochondrial pathways. Other rare causes include fibrocalculous pancreatic diabetes and fulminant type 1 diabetes.

Atypical diabetes: heterogeneous etiologies of young-onset diabetes

In 1980, Winter *et al.* [12] first described a cohort of 129 African-American youths with acute ketotic presentation, of whom 12 patients subsequently did not require insulin and followed a clinical course resembling type 2 diabetes (T2DM). Since then, a number of reports have shown that there is a poor correlation between the mode of presentation of hyperglycemia, clinical course, need for insulin treatment and autoimmune status in different ethnic groups including Asians [13–15]. Many of these patients did not have HLA genotypes or autoantibodies typical of autoimmune T1DM. Some patients were obese, with insulin resistance and glucotoxicity contributing to the initial ketotic presentation [16].

Monogenic diabetes caused by genetic mutations encoding pathways implicated in insulin synthesis and secretion may lead to young-onset diabetes with atypical presentation. In young Chinese patients with diabetes, there is a higher prevalence of parental history of diabetes (32–47%), particularly maternal history, compared with those having a late onset of disease (12–19%) [10,17,18]. A progressively earlier age of onset of disease in successive generations has also been reported in some of these affected families [19]. These patients may exhibit a mixed phenotype, including young age of diagnosis, insulinopenia and normal body weight (as in type 1), with a non-ketotic state typical of T2DM, despite lack of insulin resistance and metabolic syndrome [20].

Monogenic diabetes

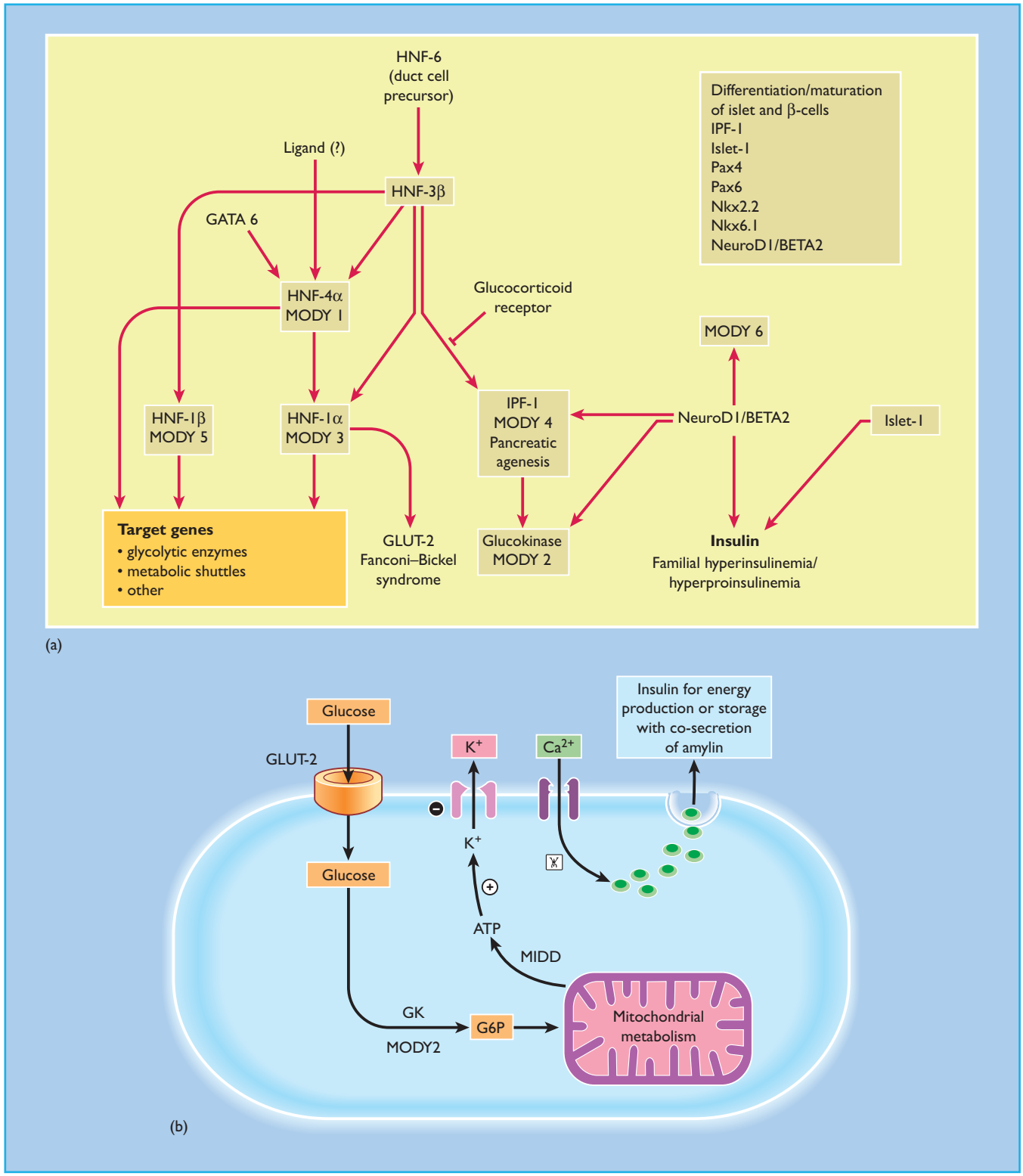
Maturity-onset diabetes of the young

Patients with maturity-onset diabetes of the young (MODY) typically present before the age of 25 years with a strong family history suggestive of autosomal dominant inheritance (see Chapter 15). Despite a non-ketotic mode of presentation, these patients often have features of abnormal pancreatic β-cell function. Some patients with MODY have a rapid deterioration in glycemic control after initial presentation while others experience mild hyperglycemia and do not require insulin despite having a long duration of disease.

To date, several subtypes of MODY have been reported. These include mutations of transcription factors: *MODY 1*: hepatic nuclear factor 4α (HNF-4α); *MODY 3*: HNF-1α or transcription factor 1 (TCF1); *MODY 4*: insulin promotion factor 1 (IPF-1); *MODY 5*: HNF-1β or transcription factor 2 (TCF-2); *MODY 6*: neurogenic differentiation 1 (NeuroD1) and *MODY 7*: carboxyl ester lipase (CEL) and glucokinase which is the glucose-sensor of the pancreatic β-cells (*MODY 2*). Transcription factors have key roles in pancreatic development including differentiation and proliferation of β-cells (Figure 9.4b). While genetic mutations in transcription factors typically cause significant insulin insufficiency and hyperglycemia, common polymorphisms of some of these transcription factors (e.g. HNF-1α [21], HNF-4α [22] and HNF-1β [23]) have also been shown to be associated with increased risk of diabetes or metabolic traits which may interact with other genetic or environmental and/or lifestyle factors to give rise to overt diabetes.

Over 80% of Caucasian patients with classic MODY (i.e. age of onset less than 25 years with autosomal pattern of inheritance) have been reported to have mutations in HNF-1α or glucokinase, while other MODY subtypes (HNF-4α, HNF-1β and IPF-1 mutations) were less common [24]. The frequencies of HNF-1α mutations ranged 25–50% in French [25], 36% in German [26], 13–18% in British [27], 8% in Japanese [28] and 5% in Chinese patients [9]. In unrelated young Chinese patients with diabetes, 5–10% were found to have glucokinase or HNF-1α mutations [13,29,30].

Although patients with some forms of MODY (e.g. *MODY 2*) have mild clinical course and rarely develop complications, other forms of MODY may be associated with severe insulin insufficiency and complications often brought about by the late pres-


HNF-6
(duct cell
precursor)
Ligand (?)
HNF-3β
GATA 6
HNF-4α
MODY 1
Glucocorticoid
receptor
Differentiation/maturation
of islet and β-cells
IPF-1
Islet-1
Pax4
Pax6
Nkx2.2
Nkx6.1
NeuroD1/BETA2
MODY 6
HNF-1β
MODY 5
HNF-1α
MODY 3
IPF-1
MODY 4
Pancreatic
agenesis
NeuroD1/BETA2
Islet-1
Target genes
• glycolytic enzymes
• metabolic shuttles
• other
GLUT-2
Fanconi–Bickel
syndrome
Glucokinase
MODY 2
Insulin
Familial hyperinsulinemia/
hyperproinsulinemia
(a)
Insulin for energy
production or storage
with co-secretion
of amylin
Glucose
GLUT-2
K+
Ca2+
K+
Glucose
ATP
MIDD
GK
MODY2
G6P
Mitochondrial
metabolism
(b)

entation and/or delayed use of insulin [10]. In light of the potential long duration of disease, young patients with diabetes are more prone to develop microvascular complications than those with late onset of disease [31], thus emphasizing the importance of family screening.

Furthermore, heterogeneous mutations in these transcription factors can be expressed sequentially in renal tubules with possible roles in various stages of the development of renal tubules [32]. Thus, patients with MODY may have heterogeneous phenotypes with metabolic and renal manifestations. Patients with *MODY 3* from mutations in HNF-1α were found to have a low renal threshold for glucose [33] while patients with *MODY 5* from mutations in HNF-1β may have mild diabetes but increased susceptibility to severe renal disease and other urogenital malformations [34]. In a consecutive unrelated cohort consisting of 74 young Chinese patients with T2DM and nephropathy, a novel missense genetic variant in exon 3 (E260D, GAG→GAC) of the HNF-1β gene was identified. Extended family analysis revealed four other siblings carrying this variant with heterogeneity in clinical presentation that included one member with uncomplicated diabetes, one with impaired glucose tolerance and one with microalbuminuria with normal glucose tolerance. A silent polymorphism Q378Q was identified in another unrelated subject in this study. While these findings will need replication in independent and larger cohorts, the phenotypic heterogeneity associated with these genetic variants is noteworthy [35].

Mitochondrial gene mutations

Mitochondria are important intracellular organelles in the maintenance of glucose homeostasis and energy balance. Mitochondria have their own genome and unlike nuclear DNA which is protected by histones, mitochondrial DNA is more vulnerable to oxidative stress and environmental toxins. Superoxide radicals generated by the mitochondrial respiratory chain are a major source of damage to mitochondrial DNA. Aged patients with a positive family history of diabetes have a high frequency of mitochondrial mutations [36]. Because of its maternal inheritance, mitochondrial DNA is a well-known cause of a subtype of maternally inherited diabetes mellitus [37].

In 1992, an A3243G mutation in the mitochondrial DNA coding for $tRNA^{Leu(UUR)}$ (mt3243) was first reported. This form of diabetes was found in patients with both type 1 and type 2 diabetes and was characterized by maternal inheritance and deafness [38]. In a random cohort of Chinese patients with diabetes, 1–3% had this mutation with either type 1 or type 2 clinical course [39–41]. Other point mutations associated with increased risk of diabetes include sites at 3316, 3394 and 14577 as well as deletion and rearrangement in mitochondrial DNA [36].

In keeping with its candidacy as a "thrifty gene," the frequency of a common polymorphism of the mitochondrial DNA (T16189C) is higher in Chinese subjects with metabolic syndrome than those without (44% vs 33%), after adjustment for age and body mass index (BMI) [42]. In a meta-analysis, Asian subjects without diabetes had a higher frequency of the 16189C variant than their European counterparts (31.0% vs 9.2%) [43]. Despite negative reports in European populations [43], there are consistent data showing the risk association of the 16189C variant with T2DM in Asians [44,45].

Amylin gene mutations

Amylin, a 37 amino acid polypeptide, is co-secreted with insulin by pancreatic β-cells. It is the principal constituent of the amyloid deposits in the islets of Langerhans in T2DM [46,47]. In autopsy series, pancreatic amyloidosis was associated with β-cell loss in both Caucasian and Chinese subjects [48–50]. It is now evident that formation of intracellular islet amyloid polypeptide (IAPP) oligomers may contribute to pancreatic β-cell loss and progressive hyperglycemia [47]. Changes in metabolic milieu or genetic variants encoding proteins involved in amylin metabolism may lead to structural changes of amylin and increased oligomerization with β-cell death [51].

A S20G variant of the amylin gene has been shown to enhance cytotoxicity in transfected COS-1 cells and amyloidogenicity *in vitro* [52,53]. This genetic variant is found in 2–3% of Japanese, Chinese and Pacific Islanders with diabetes [9,52,54–57]. In Taiwanese Chinese, normoglycemic carriers of the S20G variant had reduced early phase insulin secretion [58]. Co-segregation findings in family studies of S20G variant, however, are incon-

Figure 9.5 (a) The cascade of transcription factors involved in pancreatic development as well as neogenesis, differentiation and maturation of pancreatic β-cells. Maturity-onset diabetes of the young (MODY) includes subtypes with mutations in transcription factors, namely MODY 1 with mutations of hepatic nuclear factor (HNF-4α); MODY 3: HNF-1α; MODY 4: insulin promotion factor (IPF-1); MODY 5: HNF-1β; MODY 6: NeuroD1: neurogenic differentiation 1 and MODY 7: carboxyl ester lipase (CEL) as well as glucokinase (GK) which is the glucose-sensor of the pancreatic β-cells (MODY 2). There are also interactions of glucose transporter 2 (GLUT 2) and endodermal factor, including GATA and various important genes and transcription factors governing the differentiation and maturation of pancreatic islet and beta cells. These include Pax genes family and genes encoding the homeodomain transcription factor Nkx 2.2 and Nkx 6.1. In South Asian Indian population, interaction of the NeuroD1, neurogenin-3 (NEUROG3) and HNF-1α genes has been observed to have combined effect in controlling islet cell development and insulin secretion, thus contributing to the overall glucose tolerance [93]. (b) The multiple steps involved in regulation of insulin secretion commencing with sensing of ambient blood glucose level by GLUT-2, glycolysis by GK, and ATP production by mitochondria. The generated ATP particles then close the potassium channel leading to membrane depolarization and opening of calcium channels. The intracellular calcium influx is associated with translocation of insulin and amylin containing vesicles to the cellular surface for extracytosis. During these processes, transcription factors are also activated resulting in insulin gene transcription and production to replenish the insulin containing vesicles and maintain continuous insulin secretion. GLUT, glucose transporter; MIDD, maternal inherited diabetes and deafness; MODY, maturity-onset diabetes of the young.

clusive, suggesting that it is likely to be a risk-modifying factor rather than a major diabetes gene [9,52,58].

Other genetic mutations affecting pancreatic β-cell function

Genetic variants of transcription factors implicated in pancreatic β-cell development, structure and function such as Pax6, Nkx2-2, Nkx6-1, NEUROG3 have also been reported in patients with type 1 or type 2 diabetes [59]. The pancreatic β-cell ATP-sensitive K^+ channels (K_{ATP} channels) comprises of two subunits, the inwardly rectifying potassium channel Kir6.2 and the sulfonylurea receptor SUR1. This transmembrane channel has an important regulatory role in insulin secretion (Figure 9.5b). Genetic variants encoding the K_{ATP} channels subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) are associated with reduced insulin secretion and diabetes in different ethnic populations [60–65] including Asians [59]. In the recent genome-wide association studies, polymorphisms encoding components of these K_{ATP} channels such as KCNJ11 and KCNQ1 have also been found to be associated with 20–30% increased risk of diabetes in Caucasian and Asian populations [66,67].

Autoimmune diabetes in adults

Autoantibodies to GAD are suggested to be sensitive markers of T1DM in Caucasians [13] although they can also be detected in patients with T2DM such as those with autoimmune diabetes in adults (LADA). In the UK Prospective Diabetes Study (UKPDS), approximately 10% of patients with T2DM had anti-GAD antibodies, the majority of whom eventually progressed to insulin dependency [68,69]. Reports from different ethnic groups suggest an estimated 10% prevalence of LADA in diabetic populations [68–70]. In Asians, 10–50% of patients with diabetes and acute or early onset of disease had anti-GAD antibodies depending on selection criteria and assay methodologies [2,6,13,71].

LADA is a slowly progressive form of autoimmune disease causing diabetes and is characterized by the presence of serum autoantibodies to pancreatic antigens [72,73]. Similar to T1DM, patients with LADA often carry other autoantibodies associated with celiac disease, adrenal and thyroid disorders [74,75]. Thus, it is plausible that LADA represents one end of a continuum of autoimmune diabetes with classic T1DM occupying the other end of the spectrum [76].

The nomenclature for this subtype of diabetes have been confusing, including T2DM with islet autoantibodies, slowly progressive insulin-dependent diabetes mellitus [77], type one-and-a-half diabetes [78,79], latent autoimmune diabetes in children (LADC) [80], latent autoimmune diabetes in the young (LADY) [81], autoimmune diabetes [82] and autoimmune diabetes in adults with slowly progressive β-cell failure (ADASP) [70], although LADA remains the most commonly used term. The World Health Organization (WHO) and American Diabetes Association (ADA) acknowledged LADA as a slowly progressive form of T1DM [83,84].

The correct diagnosis of LADA is clinically important because early use of insulin instead of sulphonylurea may prevent or reduce the rate of deterioration of β-cell function in these young patients with diabetes [85]. In patients with LADA, impaired β-cell response is evident at diagnosis and early use of insulin may reduce the adverse effects of glucotoxicity on β-cells [70,82]. Apart from high clinical suspicion, HLA studies may distinguish LADA from classic T1DM. In Caucasian populations, LADA is associated with HLA DQA1-DQB1*0102(3)-*0602(3)/X which is uncommon in patients with typical T1DM [86].

Other subtypes of diabetes with type 1 phenotype

In Japan and Korea, uncommon cases of fulminant T1DM presenting with diabetic ketoacidosis (often after a viral infection) have been reported in both young and old patients. These cases were characterized by absence of insulitis, negative autoantibodies, low C peptide levels, elevated pancreatic enzyme concentrations and association with HLA haplotypes [87,88].

In India, T2DM in youth often overlaps with monogenic forms of diabetes, fibrocalculous pancreatic diabetes and diabetes associated with malnourishment, all of which are ketosis-resistant forms of youth-onset diabetes [89]. In Indian patients with tropical calcific pancreatitis, the loss of endocrine function accompanying the exocrine damage may be an additional factor contributing to the clinical manifestation of diabetes in the presence of other stressors [90]. Using pancreatic specimens, Asian researchers have reported significant correlations between BMI and relatively low volume of β-cells [91] with amyloidosis, inflammation and fibrosis as common pathologic features [92].

Conclusions

Until recently, autoimmune T1DM was considered to be the predominant form of diabetes in children and young adults. With a better understanding of the pathogenesis of diabetes, it is now recognized that genetic or acquired factors that affect the pancreatic β-cell structure and function as well as associated mechanisms such as amylin deposition and mitochondrial damage may give rise to a broad range of clinical manifestations with considerable overlap between type 1 and type 2 phenotypes. Detailed medical history-taking (e.g. family history of diabetes, mode of presentation and exposure to infection), a complete physical examination (e.g. body leanness, microvascular complications, metabolic syndrome and associated cardiovascular risk factors) and the use of appropriate laboratory testing (e.g. autoantibodies against pancreatic antigens and genetic markers) may help clinicians refine the diagnosis. This will help to guide the treatment of these patients who often present at a young and have a long duration of diabetes ahead of them, this makes them eopecially at risk for the long-term chronic complications of diabetes.

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