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Molecular Mechanisms in Autoimmune Type 1 Diabetes: a Critical Review

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Abstract Autoimmune type 1 diabetes is characterized by selective destruction of insulin-secreting beta cells in the pancreas of genetically susceptible individuals. The mechanisms underlying the development of type 1 diabetes are not fully understood. However, a widely accepted point is that type 1 diabetes is caused by a combination of genetic and environmental factors. Although most type 1 diabetes patients do not have a family history, genetic susceptibility does play a vital role in beta cell autoimmunity and destruction. Human leukocyte antigen (HLA) regions are the strongest genetic determinants, which can contribute 40–50 % of the genetic risk to type 1 diabetes. Other genes, including *INS* also contribute to disease risk. The mechanisms of the susceptible genes in type 1 diabetes may relate to their respective roles in antigen presentation, beta cell autoimmunity, immune tolerance, and autoreactive T cell response. Environmental susceptibility factors also contribute to the risk of developing type 1 diabetes. From an epigenetic standpoint, the pathologic mechanisms involved in the development of type 1 diabetes may include DNA methylation, histone modification, microRNA, and molecular mimicry. These mechanisms may act through regulating of gene expression, thereby affecting the immune system response toward islet beta cells. One of the characteristics of type 1 diabetes is the recognition of islet autoantigens by autoreactive $CD4^+$ and $CD8^+$ T cells and autoantibodies. Autoantibodies against islet autoantigens are involved in autoantigen processing and presentation by HLA molecules. This review will mainly focus on the molecular mechanism by which genetic, epigenetic, and environmental factors contribute to the risk of type 1 diabetes.

Keywords Type 1 diabetes · HLA · Epigenetics · Molecular mimicry · Autoantigen · Autoantibody

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Introduction

Diabetes is now known to be an organ-specific autoimmune disease, but the phenotype differs in children versus adults. The two major autoimmune diabetic conditions include type 1 diabetes, which generally but not exclusively affects children, and latent autoimmune diabetes of adults (LADA) [1].

Almost 40 years ago, islet cell antibodies against type 1 diabetes (T1D)-specific antigens were found in the serum of T1D patients, suggesting that the beta cell loss of T1D was autoimmune in nature [2]. T1D is generally thought to be characterized by autoimmune destruction of insulin-producing pancreatic beta cells mediated by an autoantibody to islet cell antigens [3, 4]. The resultant loss of insulin causes an overproduction of glucose and a decreased cellular uptake of glucose, resulting in hyperglycemia. Loss of insulin also leads to an increase in fat breakdown and fatty acid oxidation, which, in turn, causes overproduction of ketones [5]. Overproduction of ketones leads to diabetic ketoacidosis, and lifetime exogenous insulin treatment is required for the treatment of diabetes patients.

T1D can be present at any age. The incidence of T1D in children has been increasing over the past several decades [6]. It is considered one of the most common chronic childhood diseases [7]. Abundant research on T1D has historically originated out of Europe and North America, including countries such as Finland, Norway, Sweden, UK, Canada, and the USA

[8, 9]. The incidence and prevalence of T1D vary substantially worldwide [10, 11]. For example, the incidence of T1D is 60 cases in Finland and 40 cases in Sardinia per 100,000 people each year, while the incidence of T1D in China and Venezuela has been reported to be as low as 0.1/100,000 per year [1, 12]. The mechanisms underlying the increased incidence of T1D in selected countries are unknown but have been attributed to environmental influences [4].

LADA was first reported over 27 years ago [13]. LADA patients are defined as glutamic acid decarboxylase antibody (GADA)-positive, initially without insulin treatment for at least 6 months, diagnosed over the age of 30 years according to the criteria of Immunology of Diabetes Society (IDS) [14]. LADA is a slowly progressive form of autoimmune diabetes in adults. The progression of autoimmune beta cell loss is associated with the development of islet cell autoantibodies in a manner similar to T1D, but the clinical features are more consistent with type 2 diabetes (T2D) [15]. LADA patients do not require insulin treatment during the first 6 months after diagnosis [16, 17]. Many other names have been used to describe this condition, including diabetes mellitus type 1.5 [18], non-insulin requiring autoimmune diabetes (NIRAD) [19], slowly progressive T1D (SPT1D) [20], and autoimmune diabetes in adults (ADA) [16].

The relationship between LADA, T1D, and T2D remains controversial [21, 22]. LADA was once considered a slowly progressing subtype of T1D. However, the clinical features more resemble T2D. It is suggested that LADA is different from both classic T1D and T2D [23]. Studies from our group [14] and others [24] have demonstrated that human leukocyte antigen (HLA) protective haplotypes are less frequent in LADA. However, other studies have shown that LADA share similar susceptibility genes to classic T1D [25–28] and T2D [29]. Some researchers believe that diabetes occurs on a continuum. Our results suggested that the susceptible haplotypes of the HLA-DQ gene present a continuous spectrum from T1D, through LADA, to T2D [30]. Autoimmune diabetes is not triggered by a single factor but results from a complex interaction between genetic and environmental factors. The molecular mechanisms involved in susceptibility and the development of autoimmune diabetes, T1D in particular, are complex and redundant immune pathways.

Genetics

TID is caused by both genetic and environmental factors. Genetic susceptibility plays a vital role in the pathogenesis of T1D. It was reported that the risk of diabetes in sibling is 6 %, which is 15 times higher than that in the general Caucasian population [31]. The concordance rate for monozygotic twins (30–40 %) is much higher than that for dizygotic twins (6–8 %) [32, 33]. These observations suggest that the genetics is a significant risk factor. Over 50 susceptibility regions have been identified to associate with T1D (Table 1). The major susceptibility genes to T1D are located in HLA region. The first reports regarding the association between HLA and T1D were published 40 years ago [70], which spawned extensive research from all regions of the world to determine which alleles of HLA are associated with T1D. In addition to HLA, other genes, such as *INS*, cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*), and *PTPN22*, also contribute to the risk of T1D.

HLA Association

HLA is located on human chromosome 6p21.3, spans about 4,000 kb, and contains over 200 genes (Fig. 1). Certain HLA genes are reported to have immune response functions to environmental pathogens and in autoimmune diseases [72, 73]. The genes that encode class I (A, B, and C) and class II (DP, DQ, and DR) molecules are important in self and non-self-immune recognition. Nine thousand five hundred and forty-six polymorphisms of the HLA region have been reported so far (Table 2). The extreme polymorphism of the HLA makes it an invaluable tool for T1D association studies. HLA class I molecules are widely expressed as single chain proteins that can present intracellular antigen to $CD8^+$ T cells.

HLA class II molecules are heterodimers expressed mainly on professional antigen-presenting cells. They are composed of α and β chains and are responsible for presenting extracellular antigen to $CD4^+$ T cells [2]. The strongest association with the development of T1D is in the HLA class II loci, which can contribute about 40–50 % risk to the T1D susceptibility [2, 75]. The precise mechanisms by which the HLA class II genes confer susceptibility to the loss of islet beta cells are largely unknown, but the binding properties of key peptides derived from proinsulin, insulinoma-associated antigen 2 (IA-2), glutamic acid decarboxylase (GAD), and zinc transporter 8 (ZnT8) to antigen-presenting cells may play a role [68].

Specific combinations of alleles, genotypes, and haplotypes of the class II genes may contribute to the risk of T1D. DRB1 and DQB1 are considered to be associated with T1D in people from almost all regions of the world [76]. It has been shown that both susceptible and protective alleles may be found at the DRB1, DQA1, and DQB1 loci, including DQB1*0602, DQB1*0302, DRB1*0301, DRB1*0401, and DRB1*0405 alleles [77, 78]. Specifically, DRB1*0401, DRB1*0402, and DRB1*0405 have been suggested to confer susceptibility to T1D, while DRB1*0403 and DRB1*0406 confer protection from T1D [79, 80]. However, the susceptible and protective alleles in Asians are different from the Caucasian population. Susceptibility and protective class II alleles in Japanese populations with T1D include DQB1*0301, DQB1*0602, DRB1*1501, and DRB1*1502 [81, 82]. In the Korean population, these alleles include DQB1*0301, DQB1*0503, DQB1*0601, DQB1*0602,

Table 1 SNP and genes associated with T1D

Chromosome	SNP	Candidate genes	References
1p31.3	rs2269241	*PGM1*	[34]
1p13.2	rs2476601	*PTPN22*	[34–38]
1q31.2	rs2816316	*RGS1*	[34, 35, 37, 38]
1q32.1	rs3024505	*IL10*	[34, 37, 38]
2p25.1	rs1534422	*IL18RAP*	[34]
2p23.3	rs478222	*EFR3B*	[37]
2q11.2	rs9653442	*AFF3*	[39]
2q24.2	rs1990760	*IFIH1*	[34, 35, 40]
2q32.3	rs6752770	*STAT4*	[34, 41]
2q33.2	rs3087243	*CTLA4*	[34, 35, 42, 43]
2q35	rs3731865	*SLC11A1*	[44]
3p21.31	rs11711054	*CCR5*	[34, 35]
4p15.2	rs10517086	(Gene desert)	[34, 37, 38]
4q27	rs4505848	*IL2*	[34, 35, 39]
5p13.2	rs6897932	*IL7R*	[39]
5p13.2	rs1445898	*CAPSL*	[39]
6p22.1	rs1592410	*LOC729653*	[45]
6p21.33	rs3094663	*HLA* region	[46]
6p21.32	rs9268645	*HLA*	[34, 47, 48]
6q15	rs11755527	*BACH2*	[34, 35, 43]
6q22.32	rs9388489	*C6orf173*	[34, 37, 38]
6q23.3	rs2327832	*TNFAIP3*	[34, 49]
6q25	rs237025	*SUMO4*	[50]
6q25.3	rs1738074	*TAGAP*	[34, 35]
7p15.2	rs7804356	*SKAP2*	[34]
7p12.1	rs4948088	*COBL*	[34]
7p12.2	rs10272724	*IKZF1*	[51]
9p24.2	rs7020673	*GLIS3*	[34, 52]
10p15.1	rs947474	*PRKCQ*	[34, 35, 43]
10p15.1	rs12251307	*IL2RA*	[34, 53]
10q22.3	rs1250558	*ZMIZ1*	[34]
10q23.31	rs10509540	*RNLS*	[37, 38, 40]
11p15.5	rs7111341	*INS*	[34, 35, 54, 55]
12p13.31	rs4763879	*CD69*	[34, 37]
12q13.2	rs2292239	*ERBB3*	[34, 39, 56]
12q13.3	rs703842	*CYP27B1*	[53, 57]
12q13	rs1265564	*CUX2*	[34, 58]
12q24.13	rs17696736	*SH2B3*	[34, 59]
13q22.2	rs539514	*LMO7*	[37]
13q32.3	rs9585056	*GPR183*	[34, 60]
14q24.1	rs1465788	*ZFP36L1*	[34, 37, 38]
14q32.2	rs4900384	*C14orf64*	[34]
14q32.2	rs941576	*MEG3*	[61]
15q25.1	rs3825932	*CTSH*	[34, 37, 38]
15q14	rs7171171	*RASGRP1*	[34, 62]
16p13.13	rs12927773	*PRM1*	[34, 39]
16p13.13	rs12708716	*CLEC16A*	[34, 37, 38]
16p12.3	rs12444268	*UMOD*	[34, 37]
16p11.2	rs4788084	*IL27*	[34, 63]
16q23.1	rs7202877	*CTRB1*	[34]
17p13.1	rs16956936	*DNAH2*	[34]
17q12	rs2290400	*GSDMB*	[34, 38]
17q21.2	rs7221109	*SMARCE1*	[34]
18p11.21	rs1893217	*PTPN2*	[34, 35, 39]
18q22.2	rs763361	*CD226*	[34, 35, 39]
19q13.32	rs425105	*SLC1A5*	[34]
19q13.42	rs602662	*FUT2*	[34, 64]
20p13	rs2281808	*SIRPG*	[34, 37, 38]
21q22.3	rs3788013	*UBASH3A*	[34, 35, 65]
21q22.3	rs760426	*AIRE*	[34, 66]
22q12.2	rs5753037	*HORMAD2*	[34]
22q13.1	rs229541	*C1QTNF6*	[34, 37]
Xp22.2	rs5979785	*TLR8*	[34, 67]
Xq28	rs2664170	*GAB3*	[34]

Based from T1DBase and Pociot [68] and Morahan [69]

DRB1*0803, DRB1*1202, and DRB1*1405 [83, 84]. In the Chinese populations, susceptibility and protective alleles include DQB1*0301, DQB1*0402, DQB1*0501, DQB1*0503, DQB1*0601, DQB1*0602, DRB1*0403, and DRB1*0406 [85, 86]. Independent effects of HLA-A and HLA-B may also increase the risk of T1D independent of HLA class II genes [47].

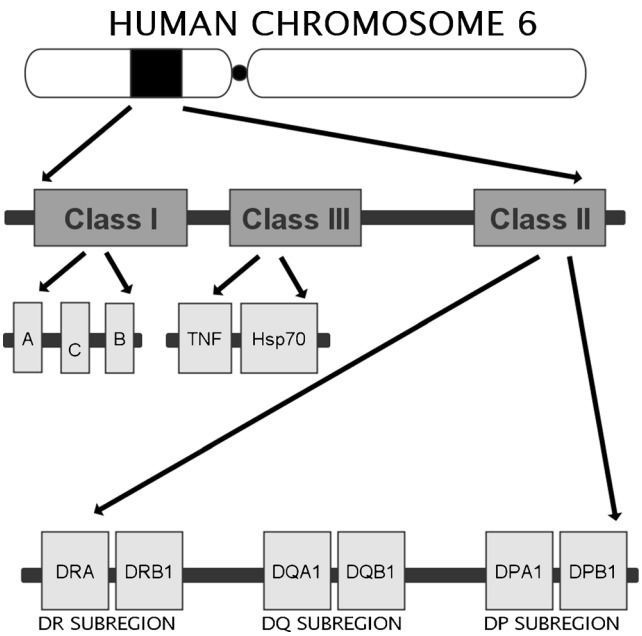

Fig. 1 Representation map of the HLA region on human chromosome 6p21. HLA genes confer ~50 % risk to T1D. HLA genes are arranged in three classes, *class I*, *class III*, and *class II*. The *class I* (*A*, *B*, and *C*) and *class II* (*DR*, *DQ*, and *DP*) genes are reported to be associated with T1D. Adapted from Mehers 2008 [71] and Kelly 2003 [5]

Table 2 Number of HLA alleles to date

Class	Locus	Number of alleles
HLA class I	A	2,365
	B	3,004
	C	1,848
	E	13
	F	22
	G	50
HLA class II	DRA	7
	DRB	1,456
	DQA1	51
	DQB1	416
	DPA1	37
	DPB1	190
	DMA	7
	DMB	13
	DOA	12
	DOB	13
Total		9,564

Number of alleles for HLA loci, data comes from IMGT/HLA Database and according to Noble and Erlich [74]; additional information can be found at www.ebi.ac.uk/imgt/hla/stats.html

With regard to genotypes and haplotypes, specific combinations of alleles at the DRB1, DQA1, and DQB1 loci contribute to the risk of developing T1D. DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 encode the HLA-DQ2 and HLA-DQ8 molecules, respectively. HLA-DRB1*03 and HLA-DRB1*04, which encode DR3 and DR4 molecules, are in linkage disequilibrium with DQ2 and DQ8, respectively. These alleles form the DR3-DQ2 and DR4-DQ8 haplotypes, respectively [5]. The highest risk of DR-DQ haplotypes for T1D are DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*0401-DQA1*0301-DQB1*0302 [77, 87]. Studies from different countries have shown that association of class II allele at the DRB1, DQA1, and DQB1 loci may vary among countries and ethnic origins [79]. For example, the DRB1*0301-DQB1*0201 and DRB1*0401-DQB1*0302 haplotypes are consistently associated with T1D in Caucasian individuals, while the DRB1*0405-DQB1*0401 and DRB1*0901-DQB1*0303 haplotypes are associated with Japanese individuals and East Asian populations [76, 81, 88]. Other haplotypes that are associated with T1D, including DRB1*0801-DQA1*040-DQB1*0402, DRB1*0405-DQB1*0401, DRB1*0901-DQA1*0301-DQB1*0303, DRB1*0802-DQB1*0302, and DRB1*0901-DQB1*0303, also vary among different countries [89–91].

In contrast with classic T1D, the association between HLA and LADA is not as well understood. Studies have demonstrated that HLA genetics are related to LADA in Caucasian populations [16, 21, 23]. A study conducted in a large well-characterized LADA cohort found that patterns of HLA-DRB1 and HLA-DQB1 loci in LADA are similar to that of T1D [26]. The authors found that DRB1*0301-DQB1*0201 and DRB1*0401-DQB1*0302 haplotypes are the main susceptibility haplotypes in LADA, while DRB1*1501-DQB1*0602 is a protective haplotype in LADA. The highest risk HLA genotypes for T1D, DR3/DR4 and DQ2/DQ8, were more prevalent in LADA compared with normal controls; however, the extent of the association was not the same when compared with T1D [16].

When comparing LADA with juvenile-onset T1D, the highest risk genotype DQ2/DQ8 was less frequent in LADA, which is more consistent with late onset diabetes [23, 92]. However, when comparing LADA with adult-onset T1D, there were no consistent differences in HLA class II, which suggest that they may share similar HLA genetic backgrounds [25, 93, 94]. Other reports suggest differences in HLA association between LADA and classic T1D, and some studies have also shown that LADA patients have an increased frequency of DRB1*0602, suggesting that DRB1*0602 may play a protective role in delaying the onset of autoimmune diabetes [29, 95]. HLA-DRB1*03 and HLA-DRB1*04 alleles in T1D patients are higher than in LADA [96]. In contrast to the strong association of HLA with autoimmune diabetes in Caucasians, the most susceptible HLA-DQ genes in Chinese LADA were moderate-risk haplotypes, including DQA1*03-DQB1*0303 and DQA1*05-DQB1*0201 in our studies [14, 30]. This suggests that the immunogenotype association of Chinese LADA is more moderate than that in Caucasians.

In conclusion, HLA associations with T1D and LADA are extremely complex, with many alleles and haplotypes affecting diabetes risk [5]. The mechanisms of HLA-DR and HLA-DQ molecules' association with T1D and LADA may be related to the primary role of HLA in antigen presentation to $CD4^+$ T cells. If HLA cannot present an antigen, the antigen cannot, through classical pathways, become an autoantigen in our body. Different antigens are recognized by the immune system and presented by different HLA molecules. The structural differences between the susceptible and protective HLA molecules may vary with autoantigens and the T cell receptors (TCRs) of autoreactive T cells and thus contribute to disease risk.

Non-HLA Association

Although association studies have shown that the HLA region is the most important genetic factor conferring risk or protectivity toward the development of T1D and related disorders, other T1D susceptibility genes, such as *INS*, *PTPN22*, *CTLA4*, *IFIH1*, *CLEC161*, and *PTPN2*, have also been described (see Table 1).

INS

The first strong non-HLA association with T1D was found at polymorphisms within the *INS* gene on chromosome 11p15.5: a variable number of tandem repeats (VNTRs) located 596 bp upstream of the translational start site of the *INS* gene [54, 97]. The VNTRs in the *INS* gene are found in three forms, class I alleles (20–63 repeats), class II alleles (64–139 repeats), and class III alleles (140–210 repeats) [98]. The class I alleles are associated with susceptibility to T1D, while the class III alleles are associated with protection against T1D. The mechanism by which the VNTRs in the *INS* gene affect the risk of T1D is unknown. However, it has been shown that the VNTRs can regulate insulin messenger RNA (mRNA) transcription in the pancreas and thymus. Class I alleles are associated with high mRNA expression levels in the pancreas and low levels in the thymus, while class III alleles are associated with lower levels of insulin mRNA in the pancreas but higher levels in the thymus [99, 100].

Insulin and its precursor, preproinsulin, are potential target autoantigens for beta cell destruction (Fig. 2). Low levels of proinsulin in the thymus may affect the positive selection of T cells in the thymus, which will cause migration of $CD4^+$, proinsulin-specific T lymphocytes to the periphery and increase the risk for developing T1D. On the other hand, high levels of proinsulin in the thymus may promote negative selection of insulin-specific autoreactive T lymphocytes, leading to immune tolerance [101] and a decrease risk for the development of T1D.

CTLA4

The association between T1D and *CTLA4*, located on chromosome 2q33, was confirmed by several studies [42, 102]. *CTLA4* protein is a costimulatory receptor on $CD4^+$ T cell surface, which can bind B7 ligands that activate CD28, an important molecule in T cell costimulation (Fig. 2). *CTLA4* plays a role in producing a negative signal to inhibit T cell activation and has a crucial role in the function of $CD4^+$ T regulatory cells [103]. The intracellular part of *CTLA4* interacts with the intracellular part of CD3 receptor to initiate phosphorylation of several downstream molecules, leading to activation of T cells after their binding to HLA molecules on antigen presenting cells (APCs) [2].

Changes in expression of *CTLA4* can increase T cell self-reactivity and may play a role in autoimmune diabetes [102, 104]. Studies have shown an association between *CTLA4* A49G polymorphisms and T1D [105]. The A49G polymorphism in exon 1 of *CTLA4* causes substitution of alanine with threonine in the signal sequence, leading to incorrect expression of the mutant protein and the subsequent reduction of *CTLA4* cell surface expression. *CTLA4* has been implicated in multiple autoimmune diseases [102, 106], including, but not limited to, rheumatoid arthritis (RA), systemic lupus erythematosus, and Addison's disease [107].

PTPN22

PTPN22, located on chromosome 1p13, encodes lymphoid tyrosine phosphatase (LYP), and mutations of this gene have been associated with T1D [108]. LYP is mainly expressed in T cells and plays a role in inhibiting TCR signaling by dephosphorylation of three kinases in the TCR signaling pathway. LYP also interacts with C-terminal Src tyrosine kinase (Csk) to downregulate T cell activation (Fig. 2) [109]. The nonsynonymous single nucleotide polymorphism (SNP) C1858T results in a substitution mutation of arginine for tryptophan (R620W). Functional studies have found that the gain-of-function R620W mutation can increase phosphatase activity [110]. This mutation leads to increased inhibition of TCR signaling, which will reduce $CD4^+$ T cell activation and potentially leads to increased autoimmunity [111]. In addition to T1D, *PTPN22* gene has also been reported to be associated with other autoimmune diseases [112], including RA, Grave's disease, and SLE [113, 114].

Other Susceptible Genes

Interleukin 2 receptor alpha (IL2RA), which encodes the alpha chain of the IL-2 receptor complex locus (CD25) and locates on chromosome 10p15, was identified as a major non-MHC risk gene associated with T1D. CD25 is expressed on regulatory naive T cells, memory T cells, and activated monocytes [115]. CD25 is responsible for binding of IL-2 and plays a role in the proliferation of regulatory T cells. CD25 regulates the activity of effector T cells through regulatory cells, and mutations in CD25 may potentially lead to the development of autoimmunity. Expression of CD25 on the surface of the regulatory T cells is important in regulating T cell proliferation in response to an immunogenic stimulus [2, 71, 116].

Small ubiquitin-like modifier 4 (SUMO4) has also been reported to be a risk factor for T1D [50]. However, inconsistent results have also been reported [117]. The substitution of methionine to valine (M55V) in SUMO4 has been proposed as a causative variant associated with T1D. This substitution causes a significant reduction of sumoylation capacity and higher NF-κB activity as well as elevated secretion of IL12B [50]. Subsequent studies have found that SUMO4 sumoylates IκBα and negatively regulates NF-κB transcriptional activity [118]. The transcription factor NF-κB has a central regulatory role in the immune response [119] and is involved in the development of autoimmune diabetes.

Several studies have found polymorphisms in signal transducers and activators of transcription (STAT) to be associated with T1D [120]. STAT4 is expressed in activated peripheral blood monocytes cells (PBMCs), dendritic cells (DCs), and

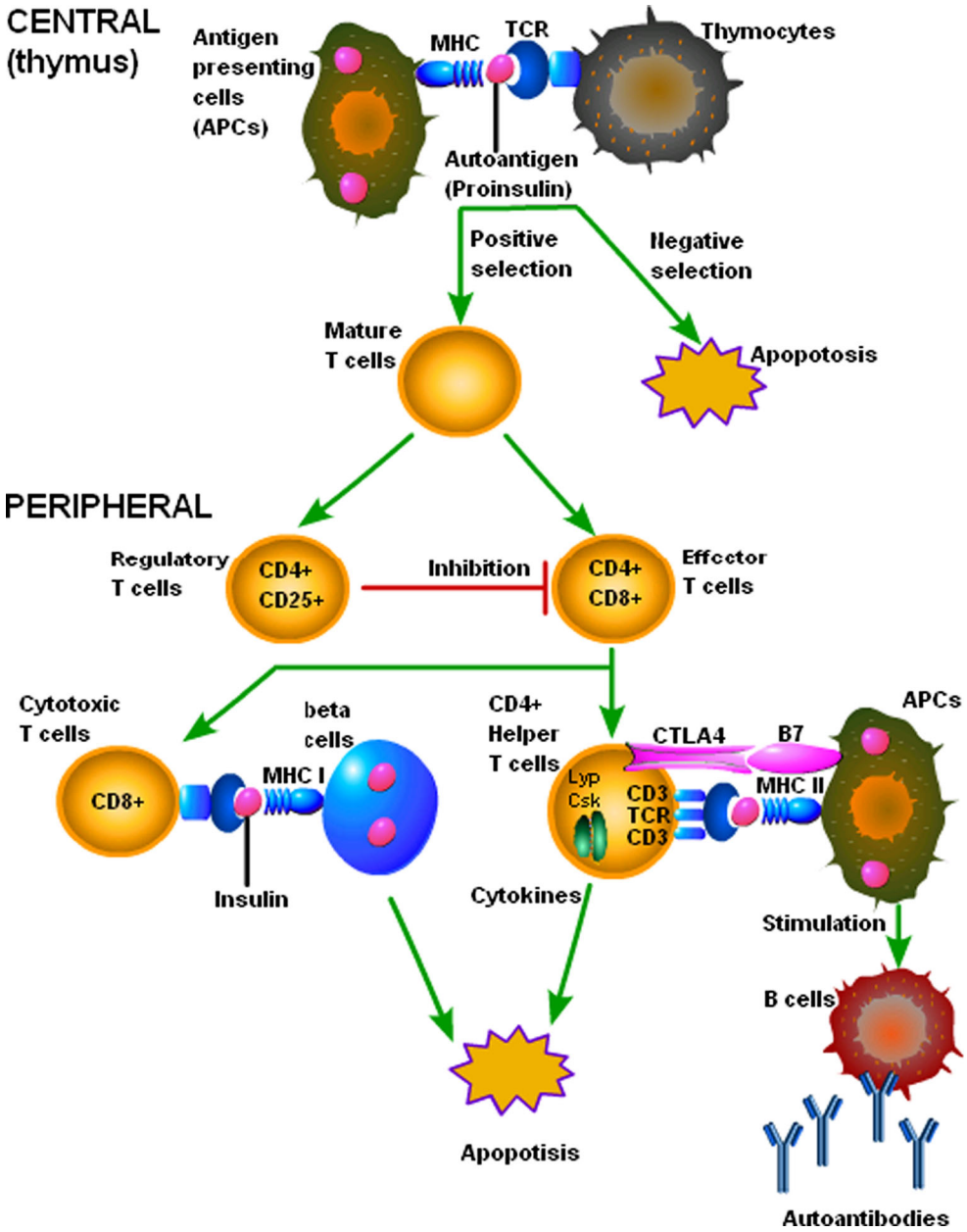

Fig. 2 Development of the autoimmune response. During thymocyte maturation, positive and negative selection takes place in the thymus. This process requires the interaction between the MHC molecules on the APCs, autoantigens (proinsulin as an example here), and the TCR on the surface of thymocyte. Large percentages (98 %) of thymocytes are removed by apoptosis (negative selection). The rest (2 %) thymocytes are positively selected and then migrated into peripheral as mature T cells. The mature T cells will then develop into $CD4^+$ and $CD8^+$ T cells and regulatory T cells. The balance between regulatory and effector T cells plays an important role in the development of both T1D and LADA. Note that susceptibility genes, including *HLA*, *CTLA4*, and *PTPN22* are involved in this process. Adapted from Ounissi-Benkalha 2008 [2]

macrophages at sites of inflammation [121]. STAT4 directly interacts with the IL-12 receptor and plays an important role in the IL-12 signaling pathway [122]. IL-12 is an immunoregulatory cytokine which takes part in the generation of Th1 cells and cytotoxic lymphocytes and leads to the production of proinflammatory cytokines [123].

Although the *INS* gene is the only non-HLA gene consistently demonstrated to be associated with LADA [27], other associations have been reported [28, 124]. The influence of non-HLA genes on LADA may, in fact, be more significant than in T1D. A study from Germany showed that *PTPN22*, *STAT4*, *CTLA4*, *IL2RA*, *INS*, *ERBB3*, *SH2B3*, and *CLEC16A* are all associated with LADA [125]. Other studies suggest that HLA-related genes play more of a role in the onset of T1D in children, whereas non-HLA genes play more of a role in the onset of LADA and T1D in young adults [29, 126]. Different HLA associations could not explain the differences between LADA and classic T1D, and further studies are needed to clarify the role of genetics in LADA.

Environment

Environmental factors may not only impact disease but can also interact with genetic factors to affect the development and progression of human disease. The importance of environmental factors in the pathogenesis of T1D is suggested by (1) the low concordance rate (30–40 %) in monozygotic twins

[127, 128], (2) only 10 % of genetically individuals with susceptible HLA genes eventually progress to diabetes [129], (3) a 15-fold difference in the disease incidence among Caucasians living in Europe, and (4) population studies which show that the incidence of diabetes increased after migration to a high-incidence region [130]. Studies have shown that the proportion of patients with high-risk HLA has decreased, while the proportion of patients with low-risk and protective HLA has increased [131, 132]. These data suggest an increased environmental risk. In Europe, the lowest annual rate occurs in Macedonia, amounting to 3.2/100,000 under 15 years, while the highest rate is 60/100,000 in Finland. The 15-fold difference cannot be explained by genetic factors alone [133].

Environmental factors that have been attributed to autoimmune diseases include a wide range of chemicals, pathogens, drugs, toxins, diet, stress, viral infection, organ phosphates, heavy metals, and solvents [134–138]. Other non-environmental factors that can contribute to the pathogenesis of T1D and other autoimmune diseases, and which may be affected by the environment, include weight, puberty, increased linear growth, body mass index, and other parameters of body habitus. Studies have examined the role of chemicals in the environment in the development of T1D, such as N-nitroso compounds, air pollutants, and persistent organic pollutants [139, 140]. Environmental chemicals may affect the development and function of the immune system, leading to autoimmunity and contributing to the development of T1D [141].

Diet is associated with T1D, with particular risk factors being cow's milk and wheat gluten, while the protective effects of breastfeeding and various nutrients have also been demonstrated [142, 143]. The gut immune system plays an important role in the development of autoimmune diabetes, and the intestinal walls of patients with T1D have been found to be more leaky than those of non-diabetic patients [144].

Psychological stress is associated with T1D-related autoimmunity at early ages. Studies suggest that psychological stress may accelerate the appearance of T1D, contribute to the induction or progression of T1D-associated autoimmunity, and induce beta cell stress or adversely impact the immune system, as a mechanism for the development of the autoimmune state [145].

Height, weight, and BMI have been associated with an increased incidence of T1D [146]. A number of viral infections have been associated with T1D and/or autoantibodies in humans, including enterovirus, rubella, mumps, rotavirus, and cytomegalovirus (CMV). A meta-analysis found a clinically significant association between enterovirus infection and T1D autoimmunity [147]. Other studies also found increased enterovirus RNA in the blood of children with T1D compared to control children, suggesting that enterovirus plays a role in the pathogenesis of T1D, by initiation of the process leading to beta cell damage [148, 149].

How exactly these factors influence the development of T1D is unclear. However, evidence has shown that the mechanism by which these environmental factors induce T1D may include epigenetic modification (DNA methylation, histone modification, and microRNA), reaction with the self-component to generate novel antigen molecules, and molecular mimicry [150], which is based on the cross-reactivity between environmental antigens and autoantigens. This has been supported by the detection of serum autoantibodies that also recognize pathogenic epitopes. For example, the anti-GAD autoantibody obtained from T1D patients also reacts with CMV.

Epigenetics

Environmental factors can induce epigenetic changes, which regulate gene expression and affect immune cell function. For this reason, epigenetics provides a source of molecular mechanisms that can explain the environmental effects on the development of autoimmune diabetes [150, 151]. Epigenetics focuses on the mechanisms that influence gene expression and cell function without a change in the DNA sequence. There are three main epigenetic modifications, including DNA methylation, histone modification, and microRNA. All of them are associated with transcriptional regulation and determination of the cellular transcriptome, thereby contributing to cell function [152].

DNA Methylation

DNA methylation is a biochemical process involving the addition of a methyl group to the fifth carbon of cytosine DNA nucleotides in CpG dinucleotide islands by using *S*-adenosylmethionine (SAM). DNA methylation is carried out by specific enzymes called DNA methyltransferases (DNMTs) including DNMT1, DNMT3a, DNMT3b, DNMT3L, and DNMT2. 5-Methylcytosine can be converted to 5-hydroxymethylcytosine and cytosine by the ten-eleven translocation (TET) family of proteins [153]. Generally, DNMTs have two classes, including maintenance DNA methyltransferases and de novo methyltransferases.

DNMT1 is responsible for the maintenance of methylation patterns during DNA replication, while DNMT3a and DNMT3b are responsible for de novo methylation [154]. Methyl-CpG-binding domain (MBD) proteins also regulate methylation together with DNMTs [128]. There are two types of demethylation—passive demethylation and active demethylation. The maintenance methyltransferase DNMT1 has a preference for hemi-methylated DNA. If DNMT1 is inhibited or absent during DNA replication, the newly synthesized DNA will not be methylated, and this will cause passive demethylation. Active demethylation can occur through the

enzymatic replacement of 5-methylcytosine (5meC) with cytosine with the help of TET and thymine-DNA-glycosylase (TDG) [155]. The balance between methylation and demethylation is important to the growth and development, as aberrant methylation may cause serious diseases [156, 157], such as cancer, autoimmune diseases, and neurodegenerative diseases.

A genome-wide DNA methylation analysis of diabetic nephropathy in T1D was performed in 2010. Nineteen CpG sites were found to correlate with the development of diabetic nephropathy in T1D [158]. Another genome-wide DNA methylation profile was performed by using $CD14^+$ monocytes from 15 T1D-discordant monozygotic twin pairs. It was discovered that 132 different CpG sites significantly correlated with the diabetic state, including 58 hypermethylated T1D–methylation variable positions (MVPs), such as TNF and TRAF6, and 74 hypomethylated T1D–MVPs, such as GAD65 and HLA-DQB1 [159]. DNA methylation has also been found to decrease TLR9 stimulation of *FOXP3* expression, through attenuation of IRF-7 binding activity in T1D [160].

Specific DNA methylation changes in T1D have also been found [161]. It has been proposed that the methylation level in the human *INS* gene acts as a biomarker in predicting beta cell death [162, 163]. Our group found that the genomic DNA methylation in $CD4^+$ T cells from LADA patients was significantly increased compared to controls. DNMT3b mRNA levels were higher in $CD4^+$ T cells from LADA patients, whereas *FOXP3* expression was decreased, and the *FOXP3* promoter region was hypermethylated in $CD4^+$ T cells [162]. All these studies suggest the importance of aberrant DNA methylation in the development of autoimmune diabetes [164]. This mechanism may explain the effect of DNA methylation in the expression of autoimmune diabetes-related genes, such as *INS* and *FOXP3*.

Histone Deacetylation

Histone proteins are subjected to a wide variety of posttranslational modification, including lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, lysine ubiquitination, and sumoylation. These modifications work together to alter the function of the nucleosome. Among histone modifications, acetylation/deacetylation is the most common gene expression regulatory mechanism. This process is catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes respectively [165].

Another modification is lysine methylation within the histone tail. The number of methyl groups in lysine residues has an impact on gene expression. For example, three methyl groups on the lysine 4 residue of histone H3 has been associated with transcriptional activation. However, the triple methylation of residues 9 or 27 has been associated with transcriptional inhibition [166]. Other modifications, such as phosphorylation, ubiquitination, and sumoylation can also modulate gene expression. A chromatin immunoprecipitation (ChIP) linked to microarray (ChIP-on-chip) approach [167] was used to analyze histone methylation patterns in blood lymphocytes and monocytes from patients with T1D. The expression of a series of genes was found to be significantly increased in H3K9me2 in lymphocytes but not in monocytes from patients with T1D. Many of these genes were associated with autoimmune and inflammation-related pathways, such as TGF-beta, NF-κB, p38 MAPK, toll-like receptor, and interleukin 6.

Expression of the T1D susceptibility gene *CTLA4* was also increased in their study [168]. Another study analyzed the T1D mellitus (T1DM)-specific gene expression and the relationship with T1DM autoimmunity. It was found that $CD4^+$ T cells of patients with T1DM were downregulated, specifically affecting key immune functions and the cell cycle. At the same time, gene expression of HDAC was decreased [169]. The H3K9Ac status of HLA-DRB1 and HLA-DQB1 has also been shown to be associated with T1D [170]. This study suggests that the promoters and/or enhancers of key susceptible genes may be important determinants in their functional association to T1D susceptibility, suggesting that the interaction between genetics and epigenetics may play a role in the development of T1D.

MicroRNAs

MicroRNAs are short non-coding RNA sequences (22 nucleotides) found in plants and animals. MicroRNAs are transcriptional and posttranscriptional regulators of gene expression [171]. MicroRNA binds to the complementary sequences in the 3′ UTR of multiple target mRNAs, causing the degradation of the target gene. They are crucial regulators of immune function, including development, differentiation, proliferation, and apoptosis [172–174]. Dysregulated microRNA expression patterns have been found in patients with T1D [175, 176]. It has been shown that dysregulated microRNA will cause aberrant immune function and may be involved in the development of T1D.

Increased expression levels of miR-326 in the peripheral blood lymphocytes from patients with T1D have been reported. The predicted targets are involved in immune regulation, indicating that miR-326 may be associated with ongoing islet autoimmunity [177]. Increased expression of miRNA-510 and decreased expressions of both miRNA-342 and miRNA-191 were identified in regulatory T cells of T1D patients [178]. Another study found that miR-21a and miR-93 were downregulated in PBMCs from patients with T1D [179].

More recently, a study compared the expression level of serum microRNAs from new onset T1D children and healthy controls. Twelve increased human microRNAs in T1D patients (miR-152, miR-30a-5p, miR-181a, miR-24, miR-148a, miR-210, miR-27a, miR-29a, miR-26a, miR-27b, miR-25, and miR-200a) were identified. Several of these microRNAs

were linked to apoptosis and beta cell function [180]. MicroRNA-21 was reported to prevent T1D by blocking pancreatic beta cell death. It has been proposed that the NF-κB-microRNA-21-PDCD4 axis plays a vital role in T1D and represents a unique therapeutic target for disease treatment (Table 3) [182]. The contribution of microRNA to immune system function and the development of autoimmunity are becoming more and more evident [183]. It is believed that the study of microRNA in T1D will elucidate new mechanisms involved in the development of T1D.

Environmental factors can affect epigenetic mechanisms of gene expression and the development of T1D. These mechanisms can regulate gene expression and thus affect the development and function of immune and islet beta cells. The increased research on epigenetics in recent years will provide a new perspective and a possibility that epigenetic modification can act as a potential diabetes therapeutic target [184].

Mechanisms of Autoimmunity in T1D

Molecular Mimicry

Viral infections are thought to be a major environmental factor influencing the development of T1D. Common viruses identified to be associated with T1D include enteroviruses such as coxsackievirus B [185], rotavirus [186], mumps virus [187], and CMV [188]. It is unknown whether viruses act as an accelerator during the ongoing immune process initiated by other factors [189] or whether they are able to initiate the entire autoimmune process. It is difficult to establish which immunological processes link viral infections to disease initiation and progression. A commonly discussed mechanism by which viruses may take part in the development of T1D is molecular mimicry. Molecular mimicry is based on structural similarity (amino acid sequence or conformational structure) between pathogen and autoantigen [190]. The virus, which may share a similar epitope with certain structures on the beta cells, can mimic autoantigens and thus activate T cells, inducing a cross-reactive autoimmune response [189].

Molecular mimicry has been thought to be a primary mechanism contributing to many autoimmune diseases, including T1D, systemic lupus erythematosus, multiple sclerosis, and Sjogren's syndrome. The potential cross-reactivity between the non-structural P2-C protein of coxsackievirus and the autoantigen GAD65 was found in T1D [191]. Another study showed that the homologous peptides for cross-reactivity are immunogenic. However, the homology between GAD65 and P2-C was not associated with significant functional consequences [192].

Similarity and cross-reactivity between the VP1-protein of enteroviruses and the beta cell autoantigen tyrosine phosphatase IA-2 have also been reported, and it was postulated that enterovirus infection may also alter immune responses [193]. The similarity between GAD65 and human CMV (hCMV) major DNA-binding protein has also been demonstrated. The hCMV-derived epitope can be naturally processed by dendritic cells and recognized by GAD65 reactive T cells [194].

All these data suggest that molecular mimicry may play a role in the development of T1D. However, functional studies, which involve experimentally inducing T cells with a viral peptide to mimic the islet autoantigen, are still needed to demonstrate the role of molecular mimicry role in the development of T1D. Whether molecular mimicry can only enhance autoimmunity, or can initiate autoimmunity, or both, is unknown. We should also keep in mind that molecular mimicry between virus and autoantigens may not be the sole mechanism for the development of autoimmunity; other mechanisms may also play a major role in initiating the immune response and in the pathogenesis of T1D.

Autoantigens and Autoantibodies

One of the characteristics of T1D is the recognition of beta cell proteins as autoantigens by autoreactive $CD4^+$ and $CD8^+$ T cells and autoantibodies. Several autoantigens have been attributed to T1D. These autoantigens include propreinsulin, GAD65, IA-2 [195], ZnT8, non-specific islet cell autoantigens (ICAs), imogen 38, pancreatic duodenal homeobox factor 1 (PDX1), chromogranin A (CHGA), islet specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), heat shock protein 60 (hsp60) [196], and islet cell antigen 69 (ICA69). It has been shown that many proteins mentioned above are potential targets of the immune system.

Several methods have been used to characterize autoantigens in T1D [197]. The first method involves the detection of islet cell autoantibodies, such as GAD65, ICA69, IA-2, and carboxypeptidase H (CPH). The second method is based on the detection of islet autoreactive T cells. Imogen 38 and IGRP were identified as beta cell autoantigens by detection of pancreatic islet autoreactive $CD4^+$ T cells. The third method employed a technique used in cell biology, which is based on selective expression of β cell proteins defined by complementary DNA subtraction libraries or microarrays. Previously reported autoantigens, imogen 38, IGRP, IA-2, IA-2β, and ZnT8, were confirmed or identified by this method. The fourth method is through a process known as inverse translation or adoptive transfer. GAD65 and insulin were confirmed by adoptive transfer of autoreactive T cells. Proteomic strategy was used as a fifth method for identifying islet autoantigens [198].

It has been proposed that β cell loss is caused by lymphocytic infiltration of the islet by dendritic cells, macrophages, and T lymphocytes [2]. Autoreactive T lymphocyte cells specific for beta cell autoantigens, such as insulin, GAD, IA-2, and ZnT8 have been identified [199, 200]. Studies have

Table 3 Epigenetic alterations involved in type 1 diabetes

Source	Study design	Main findings	References
DNA methylation			
$CD14^+$ monocytes	Genome-wide DNA methylation profiles from 15 T1D-discordant MZ twins	Fifty-eight hypermethylated genes, including TNF, CD6, and TRAF6. Seventy-four hypomethylated genes, including GAD65, HLA-DQB1, and NFKB1A	[159]
Whole blood	Genome-wide DNA methylation profiles from 96 patients with T1D and nephropathy, 96 controls with T1D but no renal disease.	Nineteen CpG sites associated with diabetic nephropathy. Among them, hypermethylation of CpG 18 bp upstream of UNC13B gene was identified.	[158]
Whole blood cell	Bisulfite-PCR method was used to measure DNA methylation of 7 CpGs in the INS promoter in 485 T1D patients and 317 controls.	Compared with controls, hypomethylation of CpGs at miR-19, miR-135, and miR-234, hypermethylation of CpG miR-180.	[161]
$CD4^+$ T cells	Genomic DNA methylation and specific gene methylation in 15 LADA patients and 11 controls.	Global DNA methylation was increased. The FOXP3 promoter region was hypermethylated in LADA patients.	[162]
Histone modifications			
Blood lymphocytes and monocytes	ChIP-on-chip was used to compare genome-wide H3K9me2 patterns from T1D patients and healthy controls.	A series of genes, including CTLA4, TGF-β, p38, and IL-6, showed increased promoter H3K9me2 in lymphocytes but not in monocytes.	[168]
$CD4^+$ T cells	DNA microarray data from new onset T1D patients, long-term T2D patients, and controls.	Histone deacetylase gene expression is reduced.	[169]
Blood monocytes and lymphocytes	CHIP-on-chip microarray was used to screen histone PTMs within the T1D susceptible loci from T1D patients and controls.	H3K9Ac status upstream of HLA-DRB1 and HLA-DQB1 was associated with T1D.	[170]
MicroRNAs			
Peripheral blood lymphocytes	The expression level of miR-326 from T1D patients was measured.	Increased expression of miR-326 in T1D patients associated with ongoing islet autoimmunity.	[177]
Regulatory T cells (Tregs) and T cells	miRNAs profiling from five T1D patients and six healthy controls.	Higher expression of miRNA-510 and lower expression of miRNA-342 and miRNA-191. Tregs had higher expression of miRNA-146a and lower expression of eight miRNAs compared with T cells.	[178]
PBMCs	qPCR analysis of miR-21a and miR-93 in 20 T1D patients and 20 healthy controls.	Both miR-21a and miR-93 were decreased in T1D patients.	[179]
Serum	MicroRNA sequencing analyses were performed from T1D cohorts and control group.	Twelve increased microRNAs was identified. Among them, miR-25 was negatively associated with residual β cell function.	[180]
Serum	Stem-loop RT-PreAmp Real-time PCR and Taqman Low Density Array was used to check expression profile of 384 miRNAs in 20 newly diagnosed T1D patients and 20 controls.	Sixty-four miRNAs were found differentially expressed, with some miRNAs involved in immune processes (miR-155, miR-181a miR-146a, miR-31, and miR-199a) or involved in the regulation of the beta cell function (miR-34a and miR-9).	[181]

shown that $CD4^+$ helper and $CD8^+$ cytotoxic T lymphocytes play an important role in the pathogenesis of T1D [201, 202] (Fig. 2). The main factors initiating autoreactive responses are not clear; however, it is well accepted that specific autoantigens are processed by APCs. APCs include DCs, macrophages, and B cells in the pancreatic islets. The autoantigens are then presented to naive T cells by diabetes-associated HLA molecules to contribute to priming and expansion of pathogenic T cells and generation of autoreactive $CD4^+$ T cells. These activated $CD4^+$ T cells will then produce cytokines and subsequently activate beta-cell-specific cytotoxic $CD8^+$ T cells. The activated T cells will be recruited to islets and stimulate macrophages and other T cells, contributing to the destruction of islet beta cells [196, 203].

Fundamental molecular mechanisms still remain unclear [204]. For example, what is the role of the autoantigen-specific $CD4^+$ T cell response [196]?. Which autoantigen is primary in initiating T1D? A series of studies suggest that proinsulin or insulin is the primary autoantigen. The specific expression of insulin in islet beta cells makes it a good candidate. Other autoantigens are expressed elsewhere. We should also keep in mind that the true primary autoantigen in T1D has not yet been definitively identified. The importance of identifying T1D-associated autoantigens will help us understand the molecular mechanisms by which beta cells are destroyed by the immune system during the development of T1D. Identification of autoantigens is also important for the development of autoantigen-specific tolerance induction immunotherapy and for establishing diagnostic and predictive markers of T1D.

The autoimmune nature of T1D is supported by the appearance of autoreactive T cells and autoantibodies. Most patients with T1D develop humoral and cellular immune responses to islet autoantigens such as GAD65 and insulin [205, 206]. The presence of antibodies to islet autoantigens can occur many years before clinical diagnosis. Autoantibodies against these islet autoantigens are present in the serum of 90 % of patients with T1D [207–209]. It is not clear if these autoantibodies play a pathogenic role in the development of T1D or if they are merely an epiphenomenon.

The first autoantibodies reported were islet cell autoantibodies [210]. ICAs are detected by reacting serum with sections of human pancreas and then staining for these autoantibodies. Anti-insulin antibodies were found in patients with T1D without exogenous insulin. Other studies indicate that anti-insulin antibodies are present several years before clinical diagnosis. Anti-GAD antibodies were first reported in patients with stiff man syndrome and were subsequently reported in patients with T1D [211]. ZnT8 was identified as an autoantigen from microarray data; our results and others show that ZnT8 autoantibodies is a predictive and diagnostic marker associated with T1D [212].

Autoantibodies may play an important role in autoantigen processing and presentation by HLA molecules. Several experiments have shown that the T cell response to autoantigen is enhanced or shifted in the presence of autoantibodies. This suggests that disease-associated GAD65 antibody can modulate the GAD65 presentation to the T cells and it may be a potential mechanism for the breakdown of islet beta cell tolerance [213]. Autoantibodies are widely used in disease prediction and diagnosis. It is accepted that the number of positive antibodies in patients is more important in predicting disease than the particular autoantibody. One experiment analyzed 45 new onset patients, 882 first degree relatives, and 217 controls, and found that 98 % had one or two antibodies and 76 % had two or three autoantibodies when they are diagnosed [214]. Our results suggest that combination testing of IAA with GADA and IA-2A may improve the LADA diagnosis rate [215].

T1D is caused by autoimmune destruction of beta cells in genetically susceptible individuals. Several studies have shown that HLA alleles are associated with autoantibodies [216]. This suggests that HLA molecules may participate in regulating the generation of autoantibodies against a specific autoantigen. The relationship between HLA and autoantibodies still needs to be explored further in order to better understand the inter-relationship between these two important pathogenic mechanisms.

Discussion

Accumulating data suggests that autoimmune T1D is the result of interaction between genetic susceptibility and environmental factors. Both genetic and environmental factors are vital for the development of autoimmune T1D. The findings in this field will accelerate our understanding of T1D. The improved understanding will help elucidate new methods of predicting the risk of developing T1D, as well as novel treatment methods and methods to prevent the onset or progression of the disease process. Currently, prediction of T1D is possible by the detection of autoantibodies in relatives of T1D patients [217]. However, negative autoantibody results do not rule out the possibility of developing T1D. Environmental factors are also important factors in the pathogenesis of T1D. Therefore, the modification of environmental exposures in the global population or in populations with high genetic susceptibility, while a massive undertaking, may be a strategy for the prevention of sporadic and familial T1D.

It has been suggested that the HLA genes are, by far, the strongest genetic determinants to T1D. HLA was identified by its role in transplant rejection. HLA has been suggested to be involved in over 100 diseases, including many autoimmune diseases, such as RA, multiple sclerosis, and T1D; infectious diseases, such as AIDS; and other diseases, such as narcolepsy [74]. The main genes associated with susceptibility of

T1D are the HLA class II genes, HLA-DRB1, HLA-DQA1, and HLA-DQB1.

Alleles at the class I locus A and locus B have also been shown to play a role in T1D susceptibility. Previous research has suggested that T1D is associated with class I A*24 alleles [218]. B*3906 alleles appear to be the alleles most commonly associated with T1D [219]. The class III region does not have classical HLA loci but includes several immunologically relevant genes, such as tumor necrosis factor-α (*TNFA*) gene and complement C4-encoding genes *C4A* and *C4Bc* SNPs in the -238 and -308 positions in the promoter region of the *TNFA* gene have been reported to be associated with T1D with conflicting results. It is recommended that all studies regarding genetic susceptibility genes in T1D be accompanied by consideration of HLA genetic susceptibility in the interpretation of the data [74].

About 50 additional non-HLA loci have been found to contribute to the development of T1D. Certain susceptible genes were found based on the candidate gene strategy, which takes into account the immunological or islet-related function of the candidate genes. Many susceptible genes were identified by genome-wide association studies (GWAS). It is estimated that there are about 5–10 million frequent variants in the human genome, with most of them being SNPs. However, only a few dozen SNPs are expected to be involved in susceptibility to T1D. The identification of these SNPs had been a significant challenge until the development of high-throughput SNP genotyping arrays. Given the huge number of SNPs used in GWAS, a very large number of T1D cases and health controls are needed to get a genome-wide statistical significance [2].

GWAS is based on common variants (high-population frequency and low contribution to disease) in the human genome [220, 221]. Several large GWAS have been performed, with the greatest amount of data coming from the T1D genetics consortium (T1DGC) [34, 222]. The most significant GWAS associations were found to be in the HLA region and the insulin gene, which were previously identified by linkage analysis and candidate gene strategy. A repository of GWAS genes associated with T1D can be found at www.t1dbase.org. Most of these genes are involved in immunological and metabolic function [68]. With the advancement of high-throughput next-generation sequencing technologies, rare variant SNPs (low-population frequency and high contribution to disease) can be rapidly identified. These results will represent a significant achievement in the development of methodology that will contribute to our understanding of the genetic susceptibility to T1D.

Although GWAS have identified many common variants which are associated with T1D, the reason for familial clustering of T1D is largely unknown. A new methodology to help explain this takes advantage of copy number variation (CNV). CNVs are a form of structural variation, leading to the cell having a different number of copies of one or more sections of the DNA. CNVs may affect the expression of surrounding genes. Several studies have reported an association between CNV and autoimmune diseases in humans, such as systemic lupus, psoriasis, Crohn's disease, RA, and T1D [223]. Recently, a genome-wide CNV analysis was performed in 20 unrelated adults with T1D and 20 control subjects. Nine CNVs were identified to be either enriched or depleted in patients with T1D or who were at high risk for T1D [224]. These CNV regions may contain genetic variants which can contribute to disease onset and be used to predict the risk of developing T1D. We believe that knowledge of CNVs involved in T1D could also improve our understanding of the mechanisms of autoimmune diabetes.

The mechanisms of susceptibility genes in T1D may be related to their role in presenting antigens and in autoreactive T cell responses. However, the key autoantigens and T cell populations which are vital in the initiation and amplification of β cell loss are still unclear. This is a significant unmet need in our quest for answers to the questions regarding pathogenesis of T1D. Other susceptibility genes that should be considered in the future include the *BACH2* gene, which is specifically expressed in β cells.

MicroRNAs play an important role in regulating the expression of target genes. These short inhibitory RNA sequences have the capability to influence many biological processes, including the maintenance of immune homeostasis and immune cell differentiation and maturation. This regulatory role of microRNA is essential for the maintenance of physiological systems. MicroRNAs have been associated with aberrant expression in many autoimmune diseases, including multiple sclerosis, RA, and systemic lupus erythematosus [225–227]. These findings suggest that microRNAs play critical roles in the pathogenesis of autoimmune diseases. Aberrant expressions of microRNAs were identified in autoimmune diabetes (Table 3), and these findings may provide a new perspective on molecular mechanism of autoimmune diseases and highlight the development of microRNA-based disease interventions.

Posttranslational modifications (PTMs) have also been proposed to be important in the development of T1D. We know that proteins can be modified after they are translated and result in new antigens, and the antigens which undergo modifications are recognized by T cells or antibodies as new antigens [228]. PTMs have been shown to be involved in several autoimmune diseases, including RA, multiple sclerosis, and celiac disease [229, 230]. Evidence has also shown that PTMs may also be important in T1D. Cells undergoing stress are more prone to PTM changes. The β cell is susceptible to ER and oxidative stress, and it is a good candidate for PTMs. The β-cell-specific autoantigen, insulin, is present in high concentration in β cells, and is thus a target for PTMs [231]. A recent paper demonstrated that β cell proteins which undergo PTMs may be involved in β cell destruction in T1D

[232]. However, this conclusion has been challenged in another paper, suggesting that there is no evidence of a causal effect of PTMs of β cell proteins in T1D [233]. Further experimental studies are required to clarify whether and how PTMs are involved in the pathogenesis of T1D.

One of the characteristics of T1D is the recognition of islet autoantigens by autoreactive $CD4^+$ and $CD8^+$ T cells and autoantibodies. $CD4^+$ T cells and $CD8^+$ cytotoxic T lymphocytes play an important role in the pathogenesis of T1D. It is generally believed that in T1D, specific autoantigens are processed and presented by APCs to naive T cells, leading to activation of $CD4^+$ T cells [234]. This results in the production of inflammatory cytokines leading to the activation of beta-cell-specific $CD8^+$ T cells. These activated T cells are then recruited to islets and stimulate macrophages and other T cells, resulting in the damage to islet beta cells [235].

The primary autoantigen involved is a controversial issue, and it has not been definitively identified. Identification of this autoantigen will help us understand the molecular mechanisms on how beta cells are destroyed by the immune system. It will also help in the development of new strategies for autoantigen-specific tolerance induction immunotherapy and for the diagnosis and prognosis of T1D. Autoantibodies against islet autoantigens may play an important role in autoantigen processing and presentation by HLA molecules.

The gut microbiota refers to the microbe population living in our intestine. One third of gut microbiota is common to the majority of humans, while two thirds are specific to the individual. Both the gut and pancreas are involved in the intestinal immune system, so it is expected that there may be an association between autoimmune diabetes and the gut [236]. The gut microbiota has been implicated in a variety of autoimmune diseases, including RA, T1D, and systemic lupus erythematosus [237, 238].

Significant differences in gut microbiota were found between children with T1D and healthy controls by PCR-DGGE and real-time quantitative PCR methods. In a recent study, the number of *Clostridium*, *Bacteroides*, and *Veillonella* was increased, while the number of *Lactobacillus* and *Bifidobacterium* was decreased in children with T1D. The quantity of bacteria which is critical to maintenance of gut integrity was decreased in the children with T1D [239].

Gut microbiota can affect the function of innate and adaptive immune systems. Studies have shown that the balance between Th17 and Treg cells is dependent on the composition of gut microbiota [240, 241]. Altered gut microbiota can cause increased gut permeability and decreased butyrate and mucus production, imbalance of T cells, and eventually may lead to β cell destruction [242]. More research is still needed to elucidate the role of gut microbiota in the development of T1D. The research of microbiota will provide us with new methods for the prevention and treatment of T1D by targeting the gut immune system.

It is important for us to understand that the interaction between genetic and environmental factors is important not only in the initiation of β cell autoimmunity, but may also be involved in the disease process of T1D. Another consideration to keep in mind is that there may be interactions among environment factors [129].

Conclusions

It is known that the development of autoimmune T1D is a complex process. The molecular mechanisms of autoimmune responses, beta cell autoimmunity, immune tolerance, and the causes of autoimmune diseases are numerous and complicated [243]. The interaction between genetic factors and environmental factors are crucial in the development of autoimmune T1D. Advances in genetics, epigenetics, autoreactive T cells, and new autoantigen discovery are important research goals that will drive new methods of diagnosis and treatment of autoimmune diseases such as T1D.

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