Abstract

There has been much progress in the identification of genes and molecular pathways involved in the pathogenesis of type 1 diabetes. Human histocompatibility (HLA) complex genes are the most powerful susceptibility determinants. The stronger effect is from alleles coding for selected HLA class I and class II antigen-presenting molecules, which are restricting elements for autoreactive CD8 and CD4 T cells, respectively. Insulin is an autoantigen in type 1 diabetes and insulin gene polymorphisms have been linked to diabetes susceptibility and shown to regulate insulin expression in the thymus. Thus, predisposing HLA and insulin gene variants may drive autoimmunity towards pancreatic islets by synergistically influencing the selection and restriction of T cells reacting with insulin. Several non-HLA loci are associated with type 1 diabetes susceptibility. Among these are CTLA-4, PTPN22 and IL2RA, all modulating basic pathways of T cell activation, function and regulation. Predisposing variants at these loci create a significant propensity to immune reactivity and less effective control over T cell selection, activation and perhaps differentiation into memory and regulatory phenotypes. Polymorphisms at these loci are not necessarily disease-specific, rather providing a generic predisposition to autoimmunity and often conferring increased risk for multiple autoimmune disorders. Finally, the IFIH1 locus may control the magnitude of viral-induced responses, linking the innate immune system to disease pathogenesis. Further studies to fine-map additional susceptibility genes and better define functional effects on immune regulation are ongoing and will help to identify novel therapeutic targets.

Key words:
Type 1 diabetes, human histocompatibility (HLA) complex, INS, CTLA-4, IL2RA, PTPN22, IFIH1, autoimmunity, self-tolerance

Introduction

Type 1 diabetes is a complex disease resulting from the autoimmune destruction of pancreatic β-cells. Both humoral and cellular autoimmune responses target multiple islet autoantigens, including (pro)insulin, glutamic acid decarboxylase 65 (GAD65), insulinoma-associated antigen 2 (IA-2) and the cation efflux transporter zinc transporter 8 (ZnT8) [1–3]. While most patients report no family history, type 1 diabetes is approximately 15 times more common in siblings than in the general population, demonstrating the importance of genetic factors and the existence of significant familial aggregation [4]. Basic but practical estimates of disease risk are based on the relationship to the proband, which reflects the degree of allele sharing and genetic identity [5]. Siblings have an average risk of 6%, although this varies greatly depending on sharing of predisposing genes and shared allele variants. The risk to the offspring of affected individuals is about 3–6% [6, 7]. Disease concordance rates are about 10% in dizygotic twins (50% gene sharing) and 30–50% in monozygotic twins (100% sharing) [8]. Concordance rates are higher with prolonged follow-up. In time, persistent autoantibody positivity or overt diabetes develops in almost 80% of initially discordant monozygotic twins [9].

The genetic basis of type 1 diabetes does not fit simple inheritance patterns. It is best explained by a model that predicts a single, major susceptibility locus together with several other genes conferring smaller risk [5]. This model has been largely validated in the past decade, during which much progress has been made in identifying several risk loci.
The human histocompatibility (HLA) complex: the main susceptibility locus

The human histocompatibility (HLA) complex contributes 40–50% of the overall susceptibility [10]. It is, by far, the susceptibility locus with the largest influence on risk. The HLA complex is located on the short arm of chromosome 6 and is divided into three main regions: class I, class II and class III, harboring genes involved in antigen presentation and the regulation of the immune response. The HLA complex harbors several loci that independently contribute to diabetes risk. These are often inherited together as extended haplotypes, in effect functioning as a multi-gene susceptibility locus [11].

The association with the HLA complex was first noted in the mid-1970s, specifically with the HLA class I antigens B8 and B15 [12]. Several class I alleles are independently associated with susceptibility, including HLA-A*0101, HLA-A*3002 [13, 14] and HLA-B39 [15]. HLA-A24 also confers increased risk and influences the age of onset and the rate of β-cell destruction [15, 16]. It was later shown that selected HLA-DR and HLA-DQ class II alleles have the strongest association with disease (Table I). It has long been known that most Caucasian patients carry the HLA-DR3 and/or DR4 serological types. About 30–50% of patients are DR3/DR4 heterozygotes and have the highest risk (1/15 vs. 1/300 in the absence of this genotype) [17]. However, several populations have been showing a declining number of patients carrying the DR3/DR4 high-risk genotype during recent decades [18–22]. This trend was accompanied by increasing numbers of patients bearing moderate- to low-risk HLA types and genotypes. Together with the increasing disease incidence [23], it appears that type 1 diabetes is becoming more common in subjects with HLA genotypes previously associated with moderate risk. A potential explanation may be an increased pressure from environmental factors on a wider spectrum of HLA types.

In Caucasians, the HLA-DQ8 and HLA-DQ2 heterodimers (encoded by the DQA1*0301, DQB1*0302 and DQA1*0501, DQB1*0201 alleles, respectively) are considered the main HLA class II susceptibility determinants [24], although they are in linkage disequilibrium with HLA-DR4 and HLA-DR3, respectively. HLA-DR4 haplotypes carrying DQB1*0302 are strongly predisposing while HLA-DR4 haplotypes carrying DQB1*0301 are considered neutral. Unlike the HLA-DR molecule, in which the α-chain is invariant, both the HLA-DQ α- and β-chains are polymorphic. This higher diversity is further increased by trans-complementation of DQ chains encoded on both chromosomes. Thus HLA-DQ8/DQ2 heterozygotes can express by trans-complementation the strongly predisposing

<table>
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<tr>
<th>DR type (serology)</th>
<th>DRB1 alleles</th>
<th>DQA1 alleles</th>
<th>DQB1 alleles</th>
<th>Odds ratio</th>
<th>Controls (%)</th>
<th>Proband (%)</th>
<th>Effect on risk</th>
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<td>11.37</td>
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HLA-DR/HLA-DQ haplotypes are ranked as conferring susceptibility (S1–S5) or protection (P1–P5). The frequency of these haplotypes for affected family-based controls and probands is shown as a percentage. Adapted from a recent report of the Type 1 Diabetes Genetics Consortium [26].

### Table I: Risk hierarchy of the most common HLA class II DR/DQ haplotypes.
DQA1*0501/DQB1*0302 heterodimer [25, 26]. There is also a hierarchy of relative predisposition effects conferred by different HLA-DR/HLA-DQ haplotypes, as HLA-DQ susceptibility is modulated by alleles at the HLA-DRB1 locus. For example, DQA1*0301, DQB1*0302 haplotypes confer different risk based on the HLA-DRB1*04 subtype (Table I) [26, 27].

Certain HLA-DR/HLA-DQ haplotypes are associated with disease resistance. The HLA-DR2 (DRB1*1501), DQA1*0102, DQB1*0602 haplotype is negatively associated with disease in different populations, even in the presence of high-risk HLA alleles [28]. Other HLA-DR2 haplotypes lacking DQB1*0602 are neutral or moderately predisposing. Two other strongly protective haplotypes are DRB1*1401, DQA1*0101, DQB1*0503 and DRB1*0701, DQA1*0201, DQB1*0303 [29], but these are less common in the population.

Since both class I and class II antigens are involved in antigen presentation, it is likely that allelic variation at the HLA-A, -B, -DR and -DQ loci will affect their functional properties and in turn presentation of islet peptide antigens to CD8 and CD4 T cells. The modulation of antigen presentation can have profound effects on both T cell tolerance and the activation of the immune response. Predisposing HLA-DQB1 alleles (DQB1*0302, DQB1*0201) have alanine instead of aspartic acid at position 57 [24]. This and other amino acid residues contribute to determine the structure, function and shape of the key molecular pockets that interact with the antigen [30], in turn influencing antigen-binding affinity, stability and presentation. Protective HLA molecules may have higher affinity for some islet autoantigen peptides than predisposing ones, and perhaps form more stable complexes [31]. Figure 1 illustrates potential scenarios in which predisposing or protective HLA molecules may influence antigen presentation to T cells. Protective HLA molecules may enhance presentation of autoantigens in the thymus and lead to efficient deletion of autoreactive T cells, while predisposing molecules may

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**Fig. 1:** Mechanisms of HLA susceptibility and protection. The figure depicts the interface between an antigen (Ag)-presenting cell (APC) and a CD4 T cell to illustrate the putative effects of predisposing (DQ8, DQ2) and protective HLA-DQ molecules (DQ6) in the thymus and peripheral compartments. HLA molecules influence presentation of peptide antigens to T cells and may influence both thymus selection processes as well as peripheral autoimmune responses. TCR, T cell receptor.
allow a higher number of autoreactive T cells to escape negative selection. In the periphery, when autoreactive T cells are triggered, protective HLA molecules may favor immune deviation and regulation as opposed to the activation of more aggressive immune responses (usually sustained by Th1 and Th17 cytokines) [32]. For example, autoantibody-positive first-degree relatives with the protective HLA-DQ6 molecule encoded by DQA1*0102, DQB1*0602 rarely progress to overt diabetes. Their autoantibody response is mostly limited to the development of GAD autoantibodies, rarely including other autoantibodies [33–35]. These findings suggest a predominantly humoral response, perhaps expression of a Th2 bias, or better regulation of cellular responses.

Other susceptibility loci

High throughput genotyping has allowed genome-wide scans in large datasets to identify additional susceptibility loci. While these studies identified a large number of loci with small individual effects on risk, associations were not always replicated in independent datasets or in all populations, perhaps reflecting genetic heterogeneity but also the limited power of smaller datasets. To address these challenges, the Type 1 Diabetes Genetics Consortium (www.t1dgc.org) has assembled large datasets from several populations worldwide [36]. The most extensive genome-wide analysis to date included 7514 cases, 9045 controls and over 2000 affected sibling-pairs. Overall, 41 genomic regions were associated with type 1 diabetes [37 (reviewed in the International Diabetes Monitor 2010; 22(2): 90–3)]. While several loci need further mapping and functional analysis, the best characterized susceptibility loci besides the HLA are insulin (INS), CTLA-4, PTPN22, IL2RA and IFIH1.

INS susceptibility maps to a polymorphic repeat sequence (VNTR, variable number of tandem repeats) [38]. VNTR allele classes are defined as smaller class I alleles (30–60 repeats), larger class III alleles (120–170 repeats) and intermediate class II alleles (<2% in Caucasians). Homozygosity for class I VNTR alleles is found in approximately 75–85% of patients compared with 50–60% in the general population. It predisposes to type 1 diabetes and increases the probability that identical twins will be concordant for disease development [39]. The relative risk of the class I/I genotype vs. I/III or III/III genotypes is estimated in the 3–5 range, with the INS locus accounting for about 10% of the observed familial clustering. Only about 15% of patients carry a class III VNTR allele in heterozygosity, while class III homozygous patients are very rare (<1%). Thus class III VNTR alleles confer significant protection from type 1 diabetes [40–42]. The VNTR or other polymorphisms in the INS region do not alter coding sequences, suggesting that these will influence transcriptional regulation. INS is reportedly transcribed in the thymus [43, 44], by both medullary thymic epithelial cells and dendritic cells [45, 46], noting that thymic expression of self-molecules with tissue-restricted expression is critical for the development of immunological self-tolerance [47]. Insulin mRNA levels in the thymus are modulated by VNTR alleles (Fig. 2). Protective class III VNTR alleles are associated with two- to threefold higher INS transcription than class IV VNTR alleles [43, 44]. Such increased transcription explains the protection associated with class III VNTR alleles, as it likely leads to more efficient negative selection of insulin-specific T lymphocytes or improved selection of insulin-specific regulatory T cells [48]. These processes should be less effective in the presence of predisposing VNTR alleles. Reduced insulin thymic levels may synergize with reduced peptide-binding affinity and stability of predisposing HLA molecules in impairing presentation of insulin peptides and, in turn, thymic selection of insulin-specific T cells. There is also evidence that epigenetic mechanisms may influence INS transcription in the thymus by silencing INS transcription from one chromosome. Silencing of the class III VNTR allele is reported in about 20% of thymus samples with a class I/III heterozygote genotype and results in much reduced INS transcription [43, 44]; this may prevent the protective effect associated with class III VNTR alleles [49] and explain the parent-of-origin effects noted in the transmission of VNTR alleles to patients with type 1 diabetes [38].

CTLA-4 polymorphisms were linked to type 1 diabetes susceptibility in several studies, but not in all populations [50–53] nor in studies that included pooled datasets from multiple countries [36, 54]. This probably reflects genetic heterogeneity and a quite small contribution to type 1 diabetes risk (relative risk is estimated at 1.1–1.4) [53, 55]. However, CTLA-4 confers stronger risk to autoimmune thyroid disease and also predisposes to several autoimmune diseases [53, 56]. CTLA4 is expressed by activated T cells. CTLA4 binds the same ligands B7-1/B7-2 (CD80/CD86) on antigen-presenting cells as CD28 but with higher affinity. While CD28
provides the co-stimulatory signals required for T cell activation, CTLA4 downregulates T cell responses. The known effect of CTLA-4 polymorphisms is on the synthesis of an alternatively spliced transcript which translates into a soluble form of CTLA4 (sCTLA4). The protective haplotype is associated with 50% higher levels of sCTLA-4 mRNA in unstimulated CD4 T cells. It is possible that sCTLA4 competes with CD28 for binding the co-stimulatory B7-1/2 molecules expressed by antigen-presenting cells (Fig. 3). In the presence of reduced levels of sCTLA4, immune responses may be less regulated by inhibitory signals, which may predispose to autoimmunity.

The PTPN22 gene codes for the tyrosine phosphatase non-receptor type 22, or Lyp, a negative regulator of T cell receptor (TCR) signals. Lyp binds to the signaling molecule Csk and the Lyp-Csk complex downregulates TCR signaling. Susceptibility maps to a missense mutation resulting in an arginine to tryptophan change at position 620 (R620W) [57, 58]. In vitro, T cells from patients carrying the R620W mutation secrete less interleukin-2 (IL-2) and show reduced phosphorylation of TCR signals compared with patients carrying the wild-type allele [59]. Thus the disease-predisposing R620W mutation is a gain-of-function variant that may predispose to autoimmunity by suppressing TCR signaling more potently (Fig. 3). It has been suggested that reduced TCR signaling may impair thymic selection processes and favor the survival of autoreactive T cells. The mutation may also affect T cell function in the periphery, as well as the function of regulatory T cells and B lymphocytes [60, 61]. Given an odds ratio of approximately 1.7, PTPN22 contributes the most susceptibility after the HLA and INS loci. The predisposing allele confers increased risk in those with low-risk HLA genotypes [62–64]. However, the combined risk of diabetes for individuals carrying high-risk HLA and PTPN22 genotypes is higher than that for those carrying low-risk HLA genotypes and high-risk PTPN22 genotypes. The predisposing allele also influences insulin autoimmunity, is associated with persistent autoimmunity [65] and favors pro-

**Fig. 2: Mechanisms of insulin gene susceptibility and protection.** The figure depicts the interface between an antigen-presenting cell (APC) and a CD4 T cell to illustrate the influence of INS polymorphisms. INS variants regulate levels of insulin mRNA expression in the thymus, which is transcribed by both medullary epithelial cells and dendritic cells. INS should ultimately modulate the abundance of insulin peptides available for presentation to developing lymphocytes in the thymus. Thymic selection processes are likely to be more efficient in the presence of higher levels of antigen, determined by protective INS variants, facilitating the establishment of self-tolerance. In contrast, predisposing variants will reduce the availability of insulin and impair thymic selection processes.
Autoantibody-positive children carrying the predisposing allele have a four-fold higher risk of developing an additional autoantibody [62]. Patients with the predisposing mutation have lower residual C-peptide at diagnosis compared with those with the wild-type allele [66]. A study from Finland suggests that PTPN22 susceptibility interacts with early exposure to cow’s milk, as it was predominantly associated with autoantibodies and disease development in children exposed to cow’s milk formula before 6 months of age [67]. Similar to CTLA-4, the PTPN22 predisposing variant also increases susceptibility to other autoimmune disorders, such as rheumatoid arthritis, lupus erythematosus, Graves’ and Addison’s disease [68].

The IL2RA gene codes for the α-chain of the IL-2 receptor (IL-2Rα, or CD25). Two single nucleotide polymorphisms (SNPs) in the IL2RA intron 1 and 5′ region were initially associated with type 1 diabetes [69]. Three additional associated SNPs were later mapped 5′ to exon 1 and within intron 1 [70]. None of these SNPs lies within known regulatory sequences but might affect IL-2Rα transcription by modifying the chromatin structure. IL-2 is a critical growth factor for lymphocytes. It promotes T cell proliferation and expansion and maintains the homeostasis of CD4+CD25+ regulatory T cells. Clinical studies have provided initial evidence for a functional correlate, since patients homozygous for predisposing IL2RA alleles have lower levels of soluble IL-2 receptor (Fig. 3) [69, 70], which correlate with the number of circulating activated T cells. Thus, lower, soluble IL-2Rα is consistent with fewer IL-2R-bearing cells. Yet it is unknown which population of lymphocytes (effector, regulatory) may be primarily affected, and exactly how. Importantly, the IL2RA locus is also linked to susceptibility to multiple sclerosis and other autoimmune diseases [70–72], although distinct functional mechanisms and allelic variants are associated with different diseases [70, 73, 74].

The IFIH1 gene has been linked to type 1 diabetes risk [75–78] and, at least in one study, to Graves’ disease [79]. It codes for the interferon induced with helicase C domain protein 1, also known as MDA5 (melanoma differentiation-associated protein 5). This helicase is involved in the cytoplasmic recognition of double-stranded RNA viruses. Upon recognition, IFIH1 signals promote interferon and nuclear factor κB responses and, in turn, the production of inflammatory cytokines (Fig. 4). Functional studies associate predisposing variants with increased expression of IFIH1 mRNA in peripheral blood.

**Fig. 3:** Pathways modulated by CTLA-4, IL2RA and PTPN22. The figure depicts the interface between an antigen (Ag)-presenting cell (APC) and a CD4 T cell to illustrate known and possible effects on key pathways and molecules modulated by these susceptibility loci. Polymorphisms in the CTLA-4 and IL2RA genes are associated with reduced levels of the soluble forms of CTLA4 and IL-2Rα. It is plausible that CTLA-4 polymorphisms may impair inhibition of T cell responses. The nature of the effects of IL2RA polymorphisms, presumably of effector and regulatory T cells, remains to be elucidated. PTPN22 predisposition results in greater inhibition of T cell receptor (TCR) signals and thus may impact thymic selection processes, perhaps in synergy with HLA and INS.
mononuclear cells, which include antigen-presenting cells [78]. In contrast, rare IFIH1 variants associated with disease protection [80] are associated with reduced function when experimentally expressed in cell lines [81]. Such variants influence the recognition of double-stranded RNA, signaling, or other functions of IFIH1 [81]. Thus IFIH1 predisposition may be explained by stronger innate responses to viral infection. In the presence of IFIH1 predisposing alleles, viral infection of pancreatic β-cells would lead to more sustained interferon responses and upregulation of HLA class I expression. This in turn increases the potential to expose self-antigens and trigger autoreactive responses leading to β-cell destruction [82]. The discovery of a mechanism of genetic predisposition linked to viral infections epidemiologically associated with type 1 diabetes, together with the frequent presence of enterovirus capsid protein in patients’ pancreatic islets [84], suggests that this pathway may represent an important target for disease prevention.

**Fig. 4:** Mechanisms of IFIH1 susceptibility and protection. The figure depicts the interface between a pancreatic β-cell and a CD8 T-cell to illustrate the influence of IFIH1 polymorphisms. Predisposing IFIH1 variants may favor enhanced innate responses to environmental factors; increased proinflammatory signals induced by viral infections may facilitate the triggering of autoreactive T-cell responses. This occurrence would be much less likely in the presence of protective IFIH1 variants, which are associated with reduced IFIH1 responses. IFN, Interferon; NFκB, nuclear factor κB; Ag, antigen; TCR, T-cell receptor.

**Concluding remarks**

The genetic basis of type 1 diabetes is multifactorial. The HLA complex is the strongest susceptibility determinant and likely controls presentation of islet antigens to T-cells. The recognition
This recognition emphasizes the need for prioritizing critical steps in the genetic control of the maturation of the immune system, suggesting profound influence on thymic selection during the maturation of the immune system. Thus, a significant component of susceptibility involving the HLA, INS and PTPN22 loci may have a profound influence on thymic selection during the maturation of the immune system.

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