

REVIEW

Molecular aspects of type 1 diabetes

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Type 1 diabetes is a T cell mediated autoimmune disease, characterised by the selective destruction of pancreatic β cells, and susceptibility is determined by a combination of genetic and environmental factors. The environmental agents implicated include viruses and dietary factors, although none has yet been shown to be directly responsible for triggering β cell autoimmunity. The genetic factors that influence disease risk have been subjected to more intensive study and two gene regions of major importance have been identified: the human leucocyte antigen locus and the insulin gene. This review will focus on the mechanisms by which these genes might influence the risk of developing type 1 diabetes.

Type 1 diabetes is a multifactorial autoimmune disease, which is characterised by T cell mediated destruction of the insulin secreting β cells of the islets of Langerhans in the pancreas. The destructive process leads to severe insulin depletion, which results in hyperglycaemia, because of hepatic overproduction of glucose by glycogenolysis and gluconeogenesis and decreased cellular uptake of glucose from the circulation. In the absence of insulin, there is also an increase in fat breakdown and fatty acid oxidation, resulting in the excessive production of ketones. If left untreated, these metabolic disturbances lead progressively to central nervous system depression, coma, and death. Therefore, the disease requires life long treatment with exogenous insulin for survival. The rate of β cell destruction varies from patient to patient, but tends to be more aggressive in infants and young children.¹ Hence, type 1 diabetes usually presents during childhood or adolescence, although it may develop much later in life. The variation in age at onset could be indicative of disease heterogeneity, with different mechanisms leading to β cell destruction in childhood onset versus adult onset diabetes. This might reflect the involvement of different genetic and/or environmental susceptibility determinants.

“Animal studies have also shown that T cells play an important role in the disease pathogenesis”

The early stages of the disease process leading to type 1 diabetes are characterised by insulinitis, the infiltration of the pancreatic islets by mononuclear immune cells, including dendritic cells, macrophages, and T cells.^{2,3} Although this could

reflect a normal inflammatory response to tissue damage, perhaps induced by exogenous factors such as viral infections, the lymphocytic infiltrate is thought to contribute directly to β cell destruction. In support of this hypothesis, autoreactive T cells specific for β cell proteins (including insulin, glutamic acid decarboxylase (GAD), and the protein tyrosine phosphatase, IA-2) have been isolated from the peripheral blood of newly diagnosed individuals with diabetes.^{4–8} Some of these T cells have been shown to be capable of destroying β cells in vitro.⁹ Animal studies have also shown that T cells play an important role in the disease pathogenesis. The non-obese diabetic (NOD) mouse spontaneously develops insulin deficient diabetes that shares many immunological and pathological features with type 1 diabetes.¹⁰ The development of disease in this animal has been shown to be thymus dependent and to require both CD4 positive and CD8 positive T cells. Furthermore, some of the autoreactive T cells isolated from diabetic NOD mice are capable of transferring the disease to non-diabetic animals and accelerating the onset of diabetes in NOD neonates.^{11–13}

The autoimmune aetiology of type 1 diabetes is also reflected by the presence of circulating autoantibodies, specific for β cell proteins including insulin, GAD, and IA-2. These autoantibodies are detectable in 85–90% of subjects with diabetes at the time of diagnosis.^{14,15} It is unclear whether they participate directly in β cell destruction or arise secondary to the release of autoantigens from islets damaged by other components of the immune system. They are, however, good markers of the underlying disease pathogenesis. The appearance of the autoantibodies precedes the clinical onset of disease, often by several years. Indeed, they can develop as early as the 1st year of life, with most individuals possessing autoantibodies directed towards multiple β cell targets by the time clinical symptoms become apparent.¹⁶ Therefore, the presence of multiple autoantibodies can be used as a sensitive marker to predict the risk of developing type 1 diabetes,^{17,18} although there are some autoantibody positive individuals who do not progress to the disease. Such individuals are particularly useful for the study of factors that protect against the development of diabetes.

Abbreviations: GAD, glutamic acid decarboxylase; HLA, human leucocyte antigen; IDDM, insulin dependent diabetes mellitus; IGF, insulin-like growth factor; MHC, major histocompatibility complex; NOD, non-obese diabetic; TCR, T cell receptor; VNTR, variable number of tandem repeats

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SUSCEPTIBILITY DETERMINANTS OF TYPE 1 DIABETES

Susceptibility to type 1 diabetes is influenced by both genetic and environmental factors. The importance of inherited risk determinants is demonstrated by the clustering of the disease within families. The life time risk of diabetes among first degree relatives of diabetic individuals is 5–6%, compared with approximately 0.4% in the general white population.¹⁹ Furthermore, the concordance rate for the disease is much higher among monozygotic twins (30–40%) than dizygotic twins (6%).^{20–21} Although this observation is indicative of a large genetic contribution to disease risk, the relatively low concordance rate among identical twins suggests that the susceptibility genes have low penetrance; that is, not all individuals who are genetically “at risk” of type 1 diabetes will develop the disease. Discordance between identical twins may reflect the generation of disparate immunological repertoires, through random rearrangement of the genes encoding T cell receptors and B cell receptors, stochastic events, or somatic mutations. Alternatively, it may indicate an important non-genetic (environmental) input to disease susceptibility. The importance of environmental determinants of disease risk is further supported by the seasonal variation in the incidence of diabetes, with most new cases occurring in autumn and winter,²² and the geographical variation in disease incidence. For example, the incidence of type 1 diabetes among French and Jewish children living in Canada has been reported to be higher than among their counterparts living in France or Israel.²³ Overall, environmental factors are thought to account for up to two thirds of disease susceptibility.

Environmental factors

Several environmental agents have been suggested to contribute to the risk of developing type 1 diabetes. These include viral infections, dietary factors in early infancy, vaccination, climatic influences, toxins (for example, nitrosamines), and stress.^{24–27} It is generally believed that the environmental agents trigger disease development in genetically susceptible individuals. However, recent observations suggest a more complex model in which exposure to multiple environmental factors throughout life influences the penetrance and expression of genetically determined immune dysregulation. This model is supported by the observation that multiple infections during the 1st few years of life are associated with a decreased risk of developing type 1 diabetes, whereas an increased risk is associated with perinatal infections.^{27–29} This suggests that environmental factors may modify the developing immune system in an age dependent manner and may therefore promote or attenuate disease at different stages of development, depending upon the timing and number of exposures.

“Infection with rubella virus or coxsackie B4 virus has been frequently associated with an increased risk of developing the disease”

It is possible that type 1 diabetes has a heterogeneous aetiology, with different environmental factors promoting β cell destruction via different mechanisms. Furthermore, these disparate environmental factors may interact with different genetic determinants to influence the overall risk of developing disease. This potential heterogeneity hampers the identification of disease susceptibility determinants.

Therefore, despite much research no environmental agent responsible for triggering type 1 diabetes has been conclusively identified, although infection with rubella virus or coxsackie B4 virus has been frequently associated with an increased risk of developing the disease. Indeed, circulating T cells specific for these viruses are more prevalent among patients with type 1 diabetes than healthy subjects.^{30–31}

However, the mechanism by which the viruses lead to β cell destruction is yet to be determined.

Genetic factors

The genetic determinants of susceptibility to type 1 diabetes are better understood than the environmental risk factors. The first diabetes susceptibility genes to be identified were the human leucocyte antigen (HLA) genes, located on chromosome 6p21.^{32–34} Subsequent studies demonstrated an association between the disease and the insulin gene region on chromosome 11p.^{35–36} In the mid to late 1990s, high throughput screening of the entire human genome in families with two or more affected siblings was used to identify additional chromosomal regions that may contain susceptibility genes for type 1 diabetes.^{37–40} Over 20 loci showed evidence for linkage with the disease in different data sets. All the studies consistently reported linkage to the HLA gene region (designated IDDM1). Several genome screens, in combination with family based association studies, also supported a role for the insulin gene region (designated IDDM2) in disease susceptibility.^{37–39–41} Linkage to eight additional loci was replicated in independent data sets: IDDM4 (chromosome 11q13), IDDM5 (chromosome 6q25), IDDM7 (chromosome 2q31), IDDM8 (chromosome 6q27), IDDM10 (chromosome 10p11–q11), IDDM12 (chromosome 2q33), IDDM13 (chromosome 2q35), and IDDM15 (chromosome 6q21).^{37–40–42–43} In addition, the locus designated IDDM6 (chromosome 18q21) showed consistent evidence for association with the disease in family studies.⁴⁴ Although the chromosomal locations of these loci are known, the precise identity of the susceptibility genes in these regions remains to be determined.

The genome screens confirmed that the IDDM1 locus (the HLA gene region) is the major genetic determinant of disease risk, accounting for 42% of the familial inheritance of type 1 diabetes.³⁷ The IDDM2 locus (the insulin gene region) contributes a further 10% of genetic susceptibility.³⁷ The remainder of this review article will focus on the mechanisms by which these genes might influence the risk of developing type 1 diabetes.

IDDM1 (HLA) GENES AND TYPE 1 DIABETES

The IDDM1 susceptibility locus encompasses the HLA genes, located within the major histocompatibility complex (MHC). The MHC spans a 3.5 megabase region of chromosome 6p21 and consists of over 200 genes arranged into three subregions, class I, class II, and class III (fig 1).

(1) The class I genes encode α peptide chains, which associate with β_2 microglobulin to form the class I molecules (fig 2A). These are expressed on the surface of all nucleated cells and play a crucial role in the restriction of cytotoxic T cell activity. The HLA class I molecules bind to peptide fragments derived from endogenous antigens and present them for recognition by the T cell receptors (TCRs) of CD8 positive T cells.

(2) The class II (HLA-D) loci are subdivided into at least one A and one B gene. These encode the α and β peptide chains, respectively, which combine to form the heterodimeric class II molecules (fig 2B). The expression of these molecules is normally restricted to professional antigen presenting cells, B cells, and activated T cells. The HLA-DR, HLA-DQ, and HLA-DP molecules are involved in the activation of helper T cells. The $\alpha 1$ and $\beta 1$ domains of these molecules form a cleft into which peptide fragments derived from exogenous antigens can bind. These peptides are then presented for recognition by the TCRs of CD4 positive T cells (fig 3). The recognition process activates the responding T cells and initiates an immune response.

(3) The class III genes encode a range of molecules with a variety of functions, including complement components (C2, C4, and Bf), tumour necrosis factor, and heat shock protein, Hsp70.

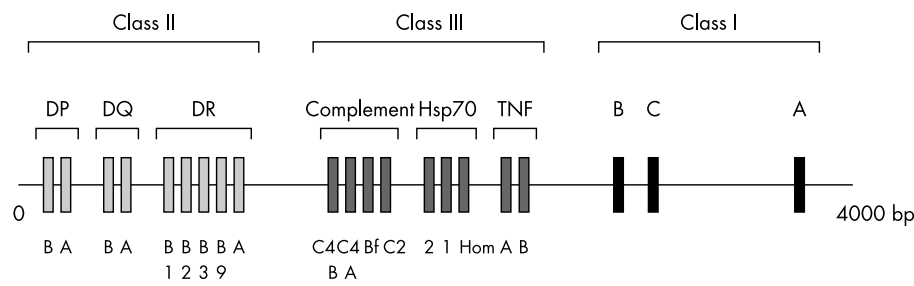


Figure 1 A simplified map of the major histocompatibility complex on chromosome 6p21, showing the genes arranged in three classes. Hsp, heat shock protein; TNF, tumour necrosis factor.

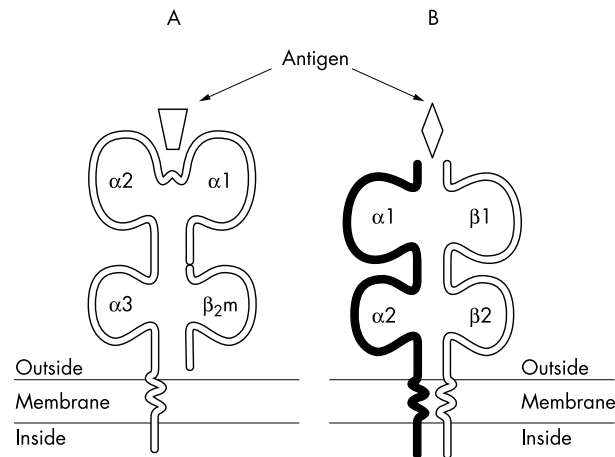


Figure 2 A diagrammatic representation of (A) a human leucocyte antigen (HLA) class I molecule and (B) an HLA class II molecule, showing the antigen binding clefts.

Many of the HLA genes are highly polymorphic. The class I and class II molecules encoded by these allelic variants show great variability in their three dimensional structure, particularly in the antigen binding regions of the molecules. This has important functional consequences, because the structure of the antigen binding site determines the way in which the molecule interacts with a particular antigenic peptide and T cell receptor.

“The identification of the primary disease susceptibility determinants within the major histocompatibility complex region is confounded by strong linkage disequilibrium between the genes”

Because the HLA class I and class II molecules play a pivotal role in the activation of T cell responses, the genes encoding these molecules have been implicated in susceptibility to several T cell mediated autoimmune diseases, including type 1 diabetes. However, the identification of the primary disease susceptibility determinants within the MHC region is confounded by strong linkage disequilibrium between the genes—that is, particular alleles at distinct loci are inherited together more frequently than expected by chance. The combination of alleles inherited together on the same chromosome is known as a haplotype. It is difficult to distinguish the primary associations with disease susceptibility from associations occurring secondary to linkage disequilibrium. Despite this complication, a recent fine mapping study of the UK population suggested that the HLA class II genes, DRB1 and DQB1, are the major determinants of IDDM1 encoded susceptibility to type 1 diabetes.⁴⁵ However, the disease risk conferred by these genes may be modified by other MHC loci, including the class I HLA-B gene and the class II HLA-DPB1 gene.^{46 47}

Susceptibility to type 1 diabetes is associated with two combinations of DQA1 and DQB1 alleles, namely: DQA1*0501.DQB1*0201 and DQA1*0301.DQB1*0302, which encode the HLA-DQ2 and HLA-DQ8 molecules, respectively. Two DRB1 alleles, DRB1*03 and DRB1*04 (which encode the DR3 and DR4 molecules, respectively), are also associated with

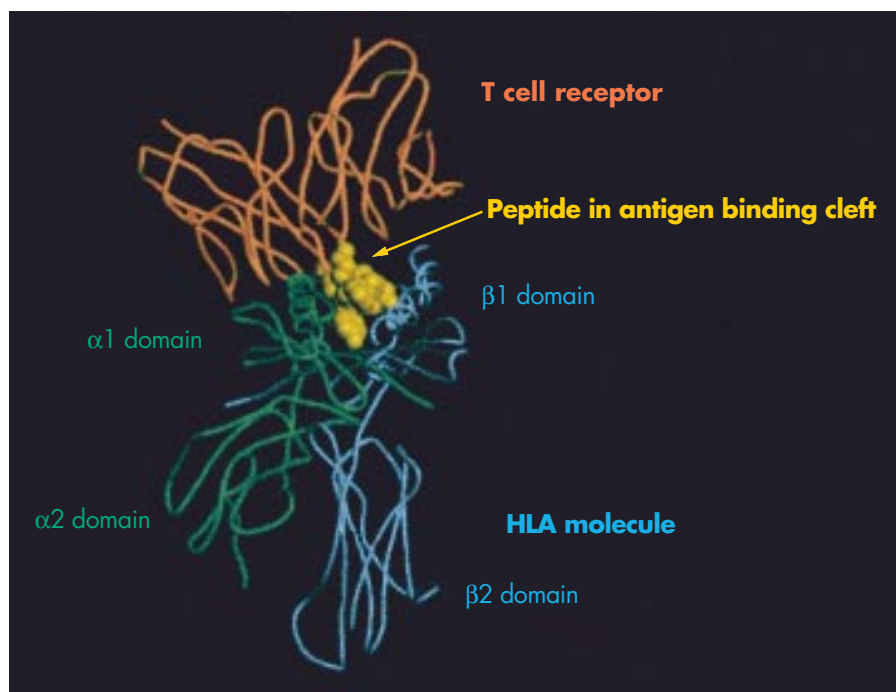


Figure 3 Antigen presentation to the T cell receptor of a CD4 positive helper T cell by a human leucocyte antigen class II molecule.

an increased risk of disease. DRB1*03 is in linkage disequilibrium with the DQA1*0501.DQB1*0201 allelic combination (forming the DR3.DQ2 haplotype), whereas DRB1*04 is in linkage disequilibrium with DQA1*0301.DQB1*0302 (forming the DR4.DQ8 haplotype). Up to 90% of patients with diabetes carry one or both of these haplotypes and the highest genetic risk of the disease is conferred by the DR3.DQ2/DR4.DQ8 heterozygous genotype. Although the DQ locus has been suggested to be more strongly associated with the disease than the DRB1 gene,⁴⁸ it is clear from recent studies that both loci are important for determining overall disease risk. This is illustrated by the DR4.DQ8 haplotype; although DQ8 is the principal disease determinant on this haplotype, its influence on disease risk may be modified by the DRB1 subtype present.⁴⁹ The DRB1*0401, DRB1*0402, and DRB1*0405 subtypes have been reported to increase the risk of diabetes independent of DQ8, whereas DRB1*0403 and DRB1*0406 confer protection from the disease.^{48 50 51} The protective effect of DRB1*0403 can override the susceptibility conferred by DQ8, even in subjects carrying the high risk DR3.DQ2/DR4.DQ8 genotype.^{52 53}

Strong natural protection against type 1 diabetes is also conferred by the DQA1*0102.DQB1*0602 haplotype, which encodes the HLA-DQ6.2 molecule. This molecule occurs in approximately 20% of the healthy white population, but is rarely found among patients with diabetes.⁵⁴ The protection provided by DQ6.2 appears to be dominant over the susceptibility conferred by other HLA markers, because individuals heterozygous for DQA1*0102.DQB1*0602 and a high risk HLA haplotype, such as DR3.DQ2 or DR4.DQ8, do not usually develop diabetes.⁵⁵ The DQ6.2 molecule can prevent progression to overt diabetes even after the onset of islet autoimmunity, suggesting that it may have an immunomodulatory role.⁵⁶ However, its influence is not absolute because patients with diabetes who are positive for DQ6.2 have been reported.^{57 58}

Although the DR3.DQ2 and DR4.DQ8 haplotypes are the major genetic determinants of disease risk, approximately 10% of white patients with diabetes carry neither of these markers. This figure is higher in non-white populations (up to 30%). Among these individuals, susceptibility to diabetes is conferred by other HLA haplotypes, including DRB1*0801.DQA1*0401.DQB1*0402 (DR8.DQ4), DRB1*0101.DQA1*0101.DQB1*0501 (DR1.DQ5), and DRB1*0901.DQA1*0301.DQB1*0303 (DR9.DQ9).⁵⁹⁻⁶¹ It is unclear whether type 1 diabetes in individuals lacking the DR3.DQ2 and DR4.DQ8 haplotypes results from the same mechanism as that in patients with one or both of the high risk markers, although the clinical profile of the disease is the same in both groups. It is possible that the different HLA associations reflect an interaction with different environmental triggers of the disease.

In conclusion, the HLA associations with type 1 diabetes are complex, with many haplotypes influencing disease risk. These form a hierarchy ranging from strongly protective (DQ6.2) to highly predisposing (DR3.DQ2/DR4.DQ8 heterozygosity).

Functional evidence for the role of HLA-DR and HLA-DQ molecules in disease pathogenesis

A direct role for HLA-DR and HLA-DQ molecules in the pathogenesis of type 1 diabetes was recently demonstrated in studies of HLA transgenic mice. The expression of human DR3 (DRB1*03) and/or DQ8 (DQA1*0301.DQB1*0302) in the B10 strain of mice was shown to induce a loss of immune tolerance to GAD, a potential β cell autoantigen.^{62 63} Mice expressing both HLA molecules also developed spontaneous insulinitis, although they did not progress to overt diabetes.⁶² No immune reactivity to GAD was seen in transgenic mice expressing DR3 or DQ8 in combination with DQ6.2 (DQA1*0102.DQB1*0602),

however.⁶³ This finding is consistent with the dominant protective role reported previously for DQ6.2. In a separate study of C57BL/6 HLA transgenic mice, the expression of DQ8 or DR4 (DRB1*0401) alone was shown to induce spontaneous autoimmune diabetes, but only in mice expressing the T cell costimulatory molecule, B7.1, on their pancreatic β cells.⁶⁴

Although these studies support a direct role for the DR and DQ molecules in disease pathogenesis, they have not helped us to understand how the molecules influence disease risk. Furthermore, the findings of the different studies are not directly comparable because of the different methodological approaches taken. Therefore, further investigations are required to gain an insight into the mechanism by which the HLA molecules influence the development of diabetes.

The effect of molecular structure on the function of HLA-DR and HLA-DQ

The influence of the HLA-DR and HLA-DQ molecules on the risk of type 1 diabetes is probably related to their central role in antigen presentation and the activation of a helper T cell mediated immune response. This function is largely determined by the precise three dimensional structure of the antigen binding clefts of the molecules, formed by the $\alpha 1$ and $\beta 1$ domains of the peptide chains. This dictates the way in which each molecule interacts with antigenic peptides and the TCRs of responding T cells.

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Studies using *x* ray crystallography and computer modelling have suggested that HLA molecules associated with susceptibility to type 1 diabetes share similar chemical and geometric properties in their antigen binding clefts. These characteristics are strikingly different from those of protective HLA molecules, which again are similar to each other.^{65 66} The structural differences between the predisposing and protective molecules are reflected by functional differences in (1) peptide selectivity and binding affinity, (2) the interaction with TCRs, and (3) molecular stability on the surface of the antigen presenting cell.

Peptide binding

The architecture of the antigen binding clefts of the DR and DQ molecules is very similar (fig 4). Both molecules bind to peptides of 12–25 amino acids in length, using conserved residues distributed throughout the binding sites. These form hydrogen bonds with the amino and carbonyl groups along the backbone of the peptide.⁶⁷ Amino acid side chains of the peptide also slot into a series of deep cavities within the binding cleft, termed “pockets”. These pockets are highly polymorphic and their structure provides the basis for the “peptide binding motif” of the molecule; that is, the preference for particular amino acid residues at crucial anchor points along the peptide. Structurally distinct HLA molecules favour different peptide binding motifs,⁶⁸⁻⁷⁰ dictated by the shape and size of the pockets, and may therefore interact differently with a given antigenic peptide. The key determinants of the binding motif are pockets 1, 4, and 9 (P1, P4, and P9, respectively).^{65 66}

P1

In the HLA molecules that predispose to type 1 diabetes, the P1 pocket is generally much deeper compared with that seen in the protective molecules. For example, P1 in the predisposing DR4 molecules (encoded by DRB1*0401 or DRB1*0405) contains a glycine residue at position 86 of the β chain ($\beta 86$), which confers a preference for binding to large aromatic side



Figure 4 The antigen binding cleft of a human leucocyte antigen class II molecule showing the binding pockets (P1, P4, and P9) and the amino acid residues implicated in peptide binding and T cell activation.

chains in the peptide. In contrast, the valine residue encoded at position $\beta 86$ in the protective DR4 molecule (encoded by DRB1*0403) creates a preference for small or medium sized hydrophobic residues in the peptide.⁶⁵ The P1 pockets of the predisposing DQ2 and DQ8 molecules are similarly much deeper than that of the protective DQ6.2 molecule.⁶⁵

P4

The P4 pocket is an important determinant of peptide binding selectivity in the DR molecule. The alanine residue at position $\beta 74$ in the diabetes permissive DR4 (DRB1*0401) molecule produces a P4 pocket with a high affinity for acidic residues. In contrast, peptides containing acidic residues at this anchor position are unable to bind to the protective DR4 molecule, encoded by DRB1*0403, because of the presence of an incompatible glutamate residue at position $\beta 74$.⁷¹ The P4 pocket in the DQ molecule is unlikely to play a crucial role in determining susceptibility to diabetes because its predicted structure is similar in DQ2, DQ8, and DQ6.2.^{65, 66}

P9

In general, HLA class II molecules that confer protection against type 1 diabetes carry an aspartate residue at position 57 in the β peptide chain (Asp $\beta 57$), whereas those that predispose to the disease carry an uncharged amino acid residue at this position (non-Asp $\beta 57$), although there are some exceptions to this rule.^{72–74} Residue $\beta 57$ is located within P9, where it plays an important role in determining the structure of this pocket. In molecules carrying Asp $\beta 57$, a salt bridge is formed between this negatively charged residue and a conserved positively charged residue (arginine) at position $\alpha 76$ (in DR molecules) or $\alpha 79$ (in DQ molecules) (fig 5).^{66, 75, 76} This alters the shape of P9 relative to that seen in non-Asp $\beta 57$ molecules and hence alters the preference of the molecule for particular anchor residues in the bound peptide. Several stud-

ies have suggested that the non-Asp $\beta 57$ molecules preferentially bind to peptides with an acidic (negatively charged) residue at the P9 anchor point, because this residue can form a stabilising salt bridge with the unopposed Arg $\alpha 76$ or Arg $\alpha 79$ residue.^{66, 69, 77–79} However, this does not hold true for the DQ2 molecule, which prefers large hydrophobic residues in P9.⁸⁰ This could be attributed to the neighbouring residues, which produce a larger P9 pocket than that found in other non-Asp $\beta 57$ molecules—for example, DQ8.⁶⁶ This highlights the importance of the morphology of the entire pocket, rather than the influence of a single residue. Nevertheless, the amino acid residue at position $\beta 57$ does have a profound impact on the peptide binding affinity and selectivity of DR and DQ molecules.^{69, 78, 81, 82}

In summary, the structural differences between the predisposing and protective HLA molecules may result in differences in their ability to bind to diabetogenic antigens. This may determine whether diabetes develops or not, although the mechanisms involved are unclear.

T cell interaction

Crystal structures of class II HLA-DR-peptide- $\alpha\beta$ TCR and class I HLA-A-peptide- $\alpha\beta$ TCR complexes have been elucidated.^{83, 84} These clearly show that different TCRs can bind in a similar orientation to different HLA molecules. The TCR presents a relatively flat surface, which is tilted at an angle to bind to the HLA-peptide complex by avoiding peaks or α helical borders of the antigen binding cleft (fig 3).⁸⁴ The activation of a particular T cell, via its receptor, is influenced by the structure of both the HLA molecule and the peptide being presented.

The T cell receptor is thought to have several docking points on the HLA class II molecule that are conserved in all DR and DQ heterodimers. In addition, it interacts with some of the polymorphic residues that distinguish one HLA molecule from another.⁸⁴ Thus, the unique combination of amino acid



Figure 5 The antigen binding cleft of a human leucocyte antigen class II DQ molecule, showing the salt bridge between residues $\beta 57$ and $\alpha 79$.

residues that characterises the antigen binding cleft of a particular HLA molecule will determine which T cell populations can respond to peptides presented by that molecule. Functional studies of DR and DQ molecules have suggested that residues $\beta 70$ and $\beta 71$ may be crucial contact points for T cell recognition of the HLA–peptide complex, and that residues $\beta 57$ and $\beta 30$ may also influence T cell activation.^{85–87}

“The unique combination of amino acid residues that characterises the antigen binding cleft of a particular HLA molecule will determine which T cell populations can respond to peptides presented by that molecule”

Residues of the peptide presented in the antigen binding cleft are also contacted by the TCR. The amino acid side chains of the peptide residues that bind to the P2, P5, and P8 pockets in the HLA molecule (termed p2, p5, and p8, respectively) have been suggested to be crucial for T cell activation.⁸⁸ Substitution studies have identified the central residue, p5, as the primary TCR contact point for many peptides. For example, Nepom and colleagues⁸⁹ showed that a conservative substitution at the p5 residue of a GAD peptide had little effect on binding to the DR4 (DRB1*0401) molecule, but rendered the DR–peptide complex incapable of stimulating GAD specific T cells from patients with diabetes. In contrast, De Oliveira *et al* showed that TCR interaction with the p5 residue is not always essential for antigen recognition and can be compensated by interactions with the residues at p2 and p8.⁸⁸ Clearly, further research is necessary to understand TCR–peptide–HLA interactions fully.

Molecular stability

In general, the HLA-DQ molecules associated with protection from type 1 diabetes are more stable on the cell membrane

than those associated with susceptibility.⁹⁰ This could lead to an extended half life of “protective” DQ–peptide complexes, which might influence disease risk by altering the strength of the interaction with pathogenically relevant T cells and ultimately affecting their activation status. The stability of the DQ molecule may be determined, in part, by the amino acid residue at position 57 in the β peptide chain. We, and others, have shown that the Asp $\beta 57$ residue is crucial for the stability of the protective DQ6.2 molecule.^{91–92} In contrast, however, soluble DQ heterodimers encoded by DQA1*0201.DQB1*0302 and DQA1*0201.DQB1*0303 were shown to have similar stability, despite differing only at residue $\beta 57$ (Ala versus Asp, respectively).⁸² This suggests that other residues must also play an important role in maintaining the integrity of the class II molecule.

Possible mechanisms by which HLA molecules influence the development of type 1 diabetes

Clearly, the structural differences seen between the predisposing and protective HLA molecules will affect their ability to interact with diabetogenic antigens and the TCRs of autoreactive, β cell specific T cells. Several mechanisms have been proposed to explain how this might influence the risk of developing autoimmune type 1 diabetes.⁹³

(1) Antigen binding in the periphery: predisposing HLA molecules may bind well to diabetogenic antigens in the periphery and hence activate an autoimmune T cell response, whereas protective HLA molecules may not. Alternatively, the protective molecules may bind to the autoantigens with a higher affinity, thus competing with the predisposing molecules. In this last scenario, the threshold of binding required for T cell activation restricted by the predisposing molecules may not be reached.

(2) Molecular stability and thymic deletion of autoreactive T cells: protective HLA molecules may form stable complexes with self antigens in the thymus, leading to efficient deletion of potentially autoreactive T cells. In contrast, the less stable complexes formed by the predisposing HLA molecules may result in inefficient T cell removal and the release of autoreactive T cells into the periphery. Negative selection of diabetogenic T cells by protective class II molecules has been demonstrated in one mouse model,⁹⁴ but not in another.⁹⁵

(3) Influence on T cell phenotype: predisposing and protective HLA molecules may interact differently with the TCRs of autoreactive T cells, affecting the phenotype of the T cells (proinflammatory versus regulatory) or their activation status (proliferative versus anergised). This immunomodulatory hypothesis is supported by the observation that DQ6.2 can protect against the development of diabetes, even after the onset of β cell autoimmunity.⁹⁶

It is unclear exactly which mechanisms are involved in determining the risk of type 1 diabetes. Currently, functional studies aiming to deal with this question are limited because the antigenic peptide(s) and T cell populations involved in initiating and perpetuating the autoimmune attack on the β cells are yet to be identified. Advances in understanding the environmental influences on disease development may be necessary before progress can be made in identifying the crucial components of β cell destruction. To identify pathogenically relevant T cell populations, it may be necessary to study individuals at the onset of the autoimmune attack, rather than at the time of clinical diagnosis, when such T cells may no longer be present. Suitable prediabetic subjects for these studies could be recruited from among first degree relatives of diabetic probands, although intensive monitoring would be necessary to determine the onset of β cell autoimmunity.

IDDM2: THE INSULIN GENE VNTR AND TYPE 1 DIABETES

Several studies of white populations have shown an association between type 1 diabetes and polymorphisms within the insulin gene region on chromosome 11p15.5.^{35 36 96} The primary association is thought to be with a variable number of tandem repeats (VNTR) region, located 596 bp upstream of the translational start site of the insulin gene.⁹⁷ Three classes of VNTR alleles have been identified, segregated according to the number of repeats of a 14–15 bp sequence: class I alleles (20–63 repeats), class II alleles (64–139 repeats), and class III alleles (140–210 repeats).

The class I alleles are generally associated with susceptibility to type 1 diabetes, with the highest risk conferred by class I homozygosity, whereas the class III alleles are associated with dominant protection,⁹⁷ although there are exceptions to this pattern for both class I and class III alleles. Some class I alleles are not predisposing.^{97 98} It has recently been suggested that the class I alleles can be subdivided into three groups, termed IC+, ID+, and ID–, based on a combination of variant repeat distributions and flanking haplotypes. All class I alleles are equally predisposing to diabetes except the ID– alleles, which are protective when transmitted from class I ID–/class III heterozygous fathers.⁹⁹ The class III alleles can also be subdivided into two highly diverged lineages, designated IIIA and IIIB.⁹⁹ The class IIIA alleles are protective against type 1 diabetes, whereas the class IIIB alleles are very protective. A recent study also identified a subgroup of rare class III alleles that appear to predispose to diabetes.¹⁰⁰

Functional relevance of the insulin VNTR and diabetes susceptibility

The mechanism by which the insulin VNTR polymorphisms influence the risk of type 1 diabetes is unclear. However, this locus has been shown to regulate the expression of two downstream genes that may be relevant to disease pathogenesis,

namely: the insulin gene and the insulin-like growth factor 2 (IGF2) gene.

Insulin and its precursors are potential target autoantigens for β cell destruction. Transcripts of the insulin gene have been detected in the human thymus, in addition to pancreatic islets, and the amounts of insulin mRNA are reported to correlate with allelic variation at the VNTR locus. Protective class III alleles are associated with increased transcription of the insulin gene in the thymus compared with predisposing class I alleles. In contrast, higher amounts of insulin mRNA in the pancreas are associated with the predisposing class I alleles.^{101–103} Raised concentrations of preproinsulin in the thymus may promote the efficient deletion of autoreactive T cells specific for this protein, leading to immune tolerance to a key autoantigen in the pathogenesis of diabetes. This mechanism may explain the dominant protective effect of the class III VNTR alleles. In subjects homozygous for the predisposing class I alleles, the combination of lower intrathymic insulin expression and higher expression in the pancreas would be expected to increase the risk of insulin driven pancreatic autoimmunity. This immune tolerance hypothesis is supported by the finding of two rare class III alleles, which are associated with complete silencing of thymic insulin transcripts and are reported to predispose to type 1 diabetes.¹⁰⁰ Furthermore, a recent study of a mouse model in which there was graded thymic insulin deficiency showed an inverse correlation between thymic insulin concentrations and peripheral T cell reactivity to insulin.¹⁰⁴ To date, however, there is no evidence of a correlation between insulin VNTR class and induction of tolerance to insulin in humans.¹⁰⁵

“Raised concentrations of preproinsulin in the thymus may promote the efficient deletion of autoreactive T cells specific for this protein, leading to immune tolerance to a key autoantigen in the pathogenesis of diabetes”

The IGF2 gene product (IGF-II) may also contribute to IDDM2 associated susceptibility to diabetes. Thymic IGF-II has been suggested to influence the risk of pancreatic autoimmunity, because it plays an important role in T cell development and negative selection. It may also act as a selecting peptide for insulin reactive T cells, as a result of its homology to proinsulin.¹⁰⁶ This mechanism is unlikely to account for the disease associations observed with the insulin VNTR alleles, however, because the class I and class III alleles are associated with similar levels of IGF2 expression in the pancreas and thymus.^{106 107} However, the predisposing class I alleles are associated with increased expression of IGF2 in the placenta.¹⁰⁸ This has been suggested to influence intrauterine growth and birth size, which are both risk factors for type 1 diabetes.¹⁰⁹

SUMMARY

The HLA genes are the strongest genetic determinants for type 1 diabetes identified to date, and the insulin VNTR also makes a contribution to disease risk. Although these risk factors are fairly well characterised at the genetic level, it is still unclear exactly how they influence susceptibility to the disease. Further functional studies are required to rectify this lack of knowledge. However, studies aiming to elucidate the mechanism by which HLA molecules influence disease risk are currently hampered by a lack of knowledge regarding the key autoantigenic peptide(s) and T cell populations responsible for the initiation and amplification of β cell destruction. Despite considerable research work, these vital components are yet to be identified. The influence of environmental susceptibility factors also remains unclear. Further research work aimed at identifying susceptibility determinants (both genetic and

Take home messages

- Type 1 diabetes is a T cell mediated autoimmune disease, characterised by the selective destruction of pancreatic β cells
- Susceptibility to the disease is determined by a combination of genetic and environmental factors
- Several environmental agents have been implicated in disease risk, including viruses and dietary factors, although none has yet been shown to be directly responsible for triggering β cell autoimmunity
- The genetic factors that influence disease risk have been subjected to more intensive study and over 20 chromosomal regions have been reported to contain susceptibility genes for type 1 diabetes
- Of these, only two loci have been well characterised: the human leucocyte antigen (HLA) locus and the insulin gene
- The HLA class II genes, DRB1 and DQB1, provide the strongest genetic risk component, although their influence may be modified by other genes within the major histocompatibility complex
- The molecules encoded by the DR and DQ genes are thought to play a central role in the thymic selection and subsequent activation of the autoreactive T cells responsible for β cell destruction, and their influence on disease risk is probably related to the presence of specific structural features, which determine the ability of the HLA molecules to interact with diabetogenic T cell populations
- The variable number of tandem repeats (VNTR) region located upstream of the insulin gene also makes an important contribution to diabetes susceptibility. The molecular mechanism involved is unknown although it may be related to the selection/deletion of insulin specific T cells in the thymus
- Further research is necessary to elucidate the precise mechanisms by which these genetic factors influence the risk of developing type 1 diabetes and to determine how they might interact with environmental susceptibility factors
- Such knowledge will further our understanding of the molecular pathology of the disease and may contribute to the design of novel therapeutic strategies

environmental) may benefit from better definition of disease phenotype, because disease heterogeneity is a significant confounding factor in such studies. Although advances may be slow, it is nevertheless important to improve our understanding of the molecular pathology of type 1 diabetes and the role played by the major susceptibility factors. This knowledge will facilitate the development of novel treatment strategies, aimed at preventing the onset and/or progression of the autoimmune process.

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ECHO

Enzymes aid invasion of arthritic joints



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Recent evidence suggests that enzymes which destroy cartilage aid invasive growth of fibroblast-like synoviocytes (FLS) in rheumatoid arthritis (RA). An in vitro study has compared potential invasive properties of FLS from patients with RA, osteoarthritis (OA), and avascular necrosis (AVN) and found that FLS from RA had significantly more matrix metalloproteinases (MMPs).

Growth through an artificial matrix was greater for FLS from RA (median cell number 4788 v 1875 for OA, v 1530 for AVN), and so was growth rate (0.27 /day v 0.22 /day v 0.25 /day, respectively). However, growth rate showed no correlation with cell number.

FLS expressing MMP-1, MMP-3, or MMP-10 were significantly more invasive (median number of invasive cells 3835, 4248, and 4990, respectively) whether from RA or OA. But the odds of having MMP-1 and MMP-9 and RA were significant, 6.5 and 10.7, when compared with OA. Other attributes—expression of cathepsin-K and tissue inhibitors of MMP-1 and MMP-2—did not influence invasiveness.

FLS were cultured from tissue obtained from joint replacements or synovectomy in patients with RA (30), OA (17), and AVN (nine). Invasiveness was assayed in a Matrigel transwell culture system, by counting cells that migrated through the matrix after three days' incubation. Growth rate was determined from cell counts of cultures harvested at intervals after seeding. Expression of cathepsin-K, tissue inhibitors, and MMPs was indicated by reverse transcriptase-PCR.

Activated FLS invade the synovium, articular cartilage, and bone in RA. Whether this is through increased growth or invasiveness has not been studied directly before now.

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