A Unified Hypothesis for the Complex Genetics of HLA Associations With IDDM

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Our understanding of the role of HLA genes associated with insulin-dependent diabetes mellitus (IDDM) is in disarray, despite recent improvements in the definition of specific alleles and haplotypes. Some genes are highly associated with IDDM, other genes are associated with resistance to IDDM, and some highly associated susceptibility genes are markedly influenced by trans-associated synergistic effects (DR3/4 heterozygotes) or protective effects (DR2/4 heterozygotes). This plethora of genetic associations has spawned the notion that there are many contributing susceptibility genes, which, in turn, has led to the search for shared structural features among different genes on IDDM-associated haplotypes. From a more mechanistic point of view, however, the wide range of variable IDDM associations, with both cis- and trans-encoded protective and/or synergistic effects, suggests a different approach. This article proposes a hypothesis in which the different HLA associations with IDDM can be simply explained by a single unifying concept: a hierarchy of affinities determines the interaction between a diabetogenic peptide and different class II molecules, and an individual is susceptible to IDDM if the class II molecule in that individual with the highest affinity for such a peptide is a DQβ susceptibility gene. The explicit formulation of this proposal and its genetic implications provide an explanation for HLA-encoded dominant “protection” and for some of the more subtle genetic observations related to cis and trans influences in IDDM susceptibility. Diabetes 39:1153–57, 1990

Human leukocyte antigen (HLA) associations with insulin-dependent diabetes mellitus (IDDM) are complex. The HLA-DR4, -DR3, -DR1, and -DR8 specificities are all positively associated with IDDM, in descending order (1–4). The HLA-DR2 and -DR5 specificities are negatively associated with IDDM (1.5–8). The DR2 “negative” effect may predominate over the DR4 “susceptible” effect, because DR2/4 heterozygotes are disproportionately underrepresented in IDDM patients (1). In other cases, the DR4 effect is predominant, although here, too, there are important caveats: different DR4+ haplotypes that carry a diabetes-associated DQβ gene have different relative risks for IDDM, suggesting some sort of cis-interacting genetic modifier (9,10). In addition, the well-known DR3/4 heterozygote synergistic risk in diabetes suggests the presence of an important trans-modifying genetic element (11,12).

During the last few years, detailed molecular analysis of haplotypes associated with each of these specificities has implicated individual HLA class II genes as likely contributors to susceptibility. This has greatly improved the precision with which genetic issues can be addressed, but it has so far failed to provide a satisfactory explanation for the genetic complexities. Thus, it is now accepted that the DQ3.2 gene accounts for the DR4 association with IDDM and is likely to be a candidate susceptibility gene in the disease (13,14); however, 30% of IDDM patients do not carry the DQ3.2 gene, and the exact susceptibility gene in these patients, usually associated with DR3, -1, or -8, is not known. The “protective” effect of DR2 in IDDM seems to be predominantly associated with DR2+ haplotypes that carry the DQ1.2 DQB1 and Dw2 DRB1 genes, but the mechanism for this apparent protection remains completely obscure (15). Perhaps most puzzling is the synergistic effect observed in DR3/4 heterozygotes, in which it has often been proposed that, because the susceptibility associated with DR4 is caused by the DQβ gene, perhaps the DR3 haplotype is contributing a DQα polypeptide to form a unique DQαβ heterodimer in these heterozygotes (11,12). Structural studies have shown that these dimers indeed exist (11,16); however, identical DQαβ

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Received for publication 4 June 1990 and accepted 13 June 1990.
heterodimers form in other genetic combinations, notably DR5/4 heterozygotes, not associated with increased risk of IDDM. Nucleotide comparisons among different class II genes in each of these cases have provided some basis for a rough correlation of susceptibility with specific codon usage such as at position 57 (17,18); however, this type of analysis does not address the complex interactions suggested by the combined genetic data set and does not satisfactorily explain many exceptions to these general-sequence correlations (19,20).

Approaching the questions posed by such genetic variability from a mechanistic and immunologic, rather than a strictly structural, context provides an opportunity to develop some insight into the genetics of the disease and into the disease process. HLA class II molecules function as peptide-binding proteins (21–23). The affinity of the binding reaction and whether a peptide will bind a class II molecule are dictated by the specific sequence of the peptide and by the specific polymorphisms present in the class II structure. Thus, a single peptide can bind to many different class II molecules but with different relative affinities (24–26). The nature of the polymorphisms within the class II molecule, which perturb the functional consequences of peptide binding, are just beginning to be identified. Site-directed mutagenesis of class II polymorphic sites predicted to be important for peptide contact have been used to influence the role of the DQ3.2B class II molecule in stimulating T-lymphocyte clones; individual mutations at selected sites of allelic variability are critical determinants of immune activation (27). Finally, the understanding that the intracellular antigen-processing pathway defines a compartment where endocytosed peptides can bind class II molecules en route to the cell surface for presentation to T lymphocytes (28) sets the stage for posing a key mechanistic question in IDDM and (potentially) other autoimmune disorders: How does the complex array of class II molecules present in individual antigen-presenting cells interact with peptides? This article outlines how focusing on this issue provides a simple solution to the problem of interpreting the complex genetics associating HLA with IDDM.

PEPTIDE-AFFINITY MODEL FOR HLA ASSOCIATIONS WITH IDDM

There are five tenets of the peptide-affinity model for HLA associations with IDDM.

1. Class II molecules are peptide-binding proteins.
2. Diabetogenic peptides exist and bind to different class II molecules with a hierarchy of affinities.
3. IDDM susceptibility is caused by peptide presentation by a class II gene product that binds a diabetogenic peptide.
4. In an individual with a permissive class II gene, susceptibility occurs when the product of this gene is the most efficient peptide binder among the different class II molecules in that individual.
5. An individual with a permissive class II gene is functionally unsusceptible to IDDM when products of other class II genes in that individual are more efficient and bind the same peptide with higher affinity.

Basic model. The central concept of the peptide-affinity model is that a few class II susceptibility genes exist and that the class II molecules encoded by these genes must compete with other class II molecules for binding specific peptides. If a susceptibility gene product successfully binds the diabetogenic peptide by outcompeting other class II genes in the same individual, then susceptibility results. For example, the most prevalent susceptibility gene known is the DQ3.2 DQB gene. This model predicts that the DQB molecule encoded by DQ3.2 binds and presents a diabetogenic peptide with a predictable affinity. It also predicts that there will be other class II molecules that bind the same peptide with a higher or lower affinity. A result of the model is that class II molecules with a higher affinity for the same peptide afford genetic protection by outcompeting DQ3.2 for binding to this peptide. As an explicit example, genes associated with the DR2,DQ1.2,Dw2 haplotype account for DR2-associated dominant protection. For simplicity, I assume that the DQB gene product is responsible; the model predicts that the relative peptide affinity for the inferred diabetogenic peptide is

\[
\text{DR2} > \text{DQ3.2} \uparrow \text{susceptility}
\]

In an individual heterozygous for each of these two genes, the two class II molecules will be expressed in the same endosomal compartments and will compete for binding to the diabetogenic peptide. Because the DR2 molecule will preferentially outcompete the DQ3.2 molecule, the DQ3.2 susceptibility gene will not be able to present this peptide and an unsusceptible phenotype will result. This simple competition model is consistent with genetic evidence of protection in such heterozygotes (Fig. 1).

In a similar way, this model can be explicitly extended to account for other related observations. The relative peptide affinity becomes

\[
\text{DR2} > \text{DR5} > \text{DQ3.2} > \text{DR4,DR3,DR7,DR6} > \text{DQ3.1} \uparrow \text{susceptility}
\]

This extended formulation predicts a mechanism for the observed protective effect associated with DR5 haplotypes, similar to the DR2 effect. This protective effect is probably caused by the DR5 DRB1 molecule rather than the DQ molecule, because the DQA and DQB genes associated with DR5 haplotypes are also found on other haplotypes that are not protective. Several other HLA molecules not associated with IDDM are also indicated in Formula 2. They presumably have lower affinity for binding to the diabetogenic peptide or do not bind at all; thus, they do not compete with a susceptibility gene, e.g., DQ3.2.

This formulation also provides an interpretation to account for the synergistic susceptibility associated with DR3/4 heterozygotes. As mentioned before, the DQA polypeptide associated with DR3 forms an efficient trans-associated class II dimer with the DQB3.2 gene product (11,16). This heterodimer present in DR3/4 heterozygotes would be an attractive candidate for contributing to susceptibility except for the confounding genetic paradox that the same DQA gene present on DR5 haplotypes does not contribute to synergistic
**IDDM RESISTANCE**

**IDDM SUSCEPTIBILITY**

**FIG. 1. Interactions of diabetogenic peptides and class II molecules in endosomal compartments:** DR2, high affinity; DQ3.2, moderate affinity; DR"other," low affinity. In actual heterozygous individual, as many as 8-12 different class II molecules may be present, competing for binding to peptides. In this model, binding diabetogenic peptide to DQ3.2 molecule is key permissive event leading to autoreactive T-lymphocyte activation in insulin-dependent diabetes mellitus; in presence of high-affinity competitor (DR2), this event does not occur.

susceptibility in DR5/4 heterozygotes. The model easily explains this observation, however, because the array of class II molecules competing for peptide in a DR3/4 individual would include the DQ3.2 dimers with (DQ3α)cis- and (DQ2α)trans-associated α-chains as the highest-affinity binders for that peptide. In contrast, in a DR5/4 heterozygote, the DR5 DRB1 molecule would outcompete the DQ molecules for the peptide, blocking susceptibility. Thus, in the formulation as developed, two forms of the DQ3.2-associated class II molecule would be permissive for disease, the DQ2α/3.2β and DQ3α/3.2β molecules; presumably, the DQ2α/3.2β heterodimer is the most effective trigger of diabetogenic T-lymphocyte activation. The relative peptide affinity is

\[
\text{DR2} > \text{DR5} > \frac{\text{DQ2αDQ3.2β}}{\text{DQ3αDQ3.2β}} > \text{DR4, DR3, DR7, DR6} > \text{DQ3.1} \quad (3)
\]

**Embellishments to the basic model.** The peptide-affinity model also provides a straightforward interpretation for some of the more subtle aspects of HLA genetics in IDDM. One example is the differing associations of distinct DR4* haplotypes that each carry a DQ3.2 susceptibility gene. DQ3.2* haplotypes with a cis-linked DRB gene (Dw4, Dw10, or Dw14) are all associated with IDDM but with decreasing relative risk, in the order listed. A simple interpretation is that these DRB alleles have some affinity for the same diabetogenic peptide that binds DQ3.2 in IDDM; however, the relative affinities for peptide binding are reversed (Dw14 > Dw10 > Dw4). In this way, the Dw14 partially competes with the DQ3.2 molecule for peptide binding and therefore is relatively "protective" compared with the other DRB alleles.

It is also clear that this model relies on the function of the class II dimer as a whole rather than any single α- or β-chain. This reliance has already been discussed for the DQ2α and DQ3α polypeptides complexed with DQ3.2β, but it presumably also applies for the contributions of other α-chains. The DRB gene functions as the key permissive allele in IDDM, but its ability to bind peptide with appropriately high affinity is intimately influenced by the nature of the associated α-chain.

Other IDDM-associated susceptibility genes exist on non-DQ3.2* haplotypes. Although none have been specifically characterized, it can be predicted that some of the class II molecules on DR3-, DR1-, DRw16-, and DR8-associated haplotypes prevalent in IDDM will play a role similar to that of DQβ3.2. Although not studied as thoroughly as the preceding examples, class II susceptibility genes on each of these haplotypes will presumably fit into a formulation with the same relative peptide affinity as DQ3.2. These other susceptibility genes may be either DR or DQ products, because the hypothesis is independent of the genetic locus, based only on a hierarchy of peptide-binding affinities.

The nature of the key diabetogenic peptides acting in this model is unknown. Most likely, they are processed fragments of antigenic proteins important for immune activation. Alternatively, the key peptides in this model could be derived from self-proteins and act in more subtle ways. For instance, self-peptides from a processed DR2 polypeptide, if bound as a peptide by an intact DQ3.2 molecule, could conceivably compete with exogenous peptide and thereby protect from disease in heterozygotes. Another alternative could involve...
self-peptides that fail to bind DQ3.2 molecules at a critical time in tolerogenesis because of competition from other disease-associated alleles, e.g., Dw4 (DR4) or DR3, thus precipitating failure of normal tolerance to an autoantigen. The proposed model does not specifically address any of these more subtle mechanisms, but it does point to the importance of a specific hierarchy of affinities for peptide among class II molecules expressed in each individual as the central issue determining susceptibility.

**DISCUSSION**

During the last few years, there has been an explosion of information regarding the genetic and structural characteristics of MHC genes associated with IDDM. Studies focused on the most prevalent candidate susceptibility gene, DQβ3.2, have demonstrated the critical role for specific amino acid substitutions determining the functional properties of the molecule. These studies have been interpreted as indicating a critical role for peptide-binding interactions of the DQ3.2 molecule (compared with its closely related but non-diabetes-associated allele DQ3.1), which are responsible for T-lymphocyte activation (27,29,30).

At the same time, the nature of "promiscuous" peptide binding to class II molecules is becoming better understood. There are many examples in which a single defined peptide will bind to multiple different class II molecules with different affinities. Although peptide competition is usually envisioned as a situation in which different peptides compete for binding to the same class II molecule (31–33), it is apparent that the reverse is equally biologically significant—the competition of different class II molecules for a single peptide. Recent studies indicate that this competitive event likely happens at a discrete site in the endosomal recycling pathway of antigen-presenting cells as a fundamental step in antigen processing (28).

The proposed model, in its simplest form, is straightforward. A class II susceptibility gene product such as DQ3.2 binds and presents a diabetogenic peptide. This produces a predisposition to diabetes in individuals in whom DQβ3.2 is the most avid binding element. In individuals in whom DR or other DQ genes outcompete DQ3.2 for binding to the same peptide, however, there is protection from susceptibility.

This explicit model makes several clear predictions that can be tested experimentally. Although the predicted competitive events have been shown to occur in vitro, they need to be studied and the concentrations of the relevant components need to be measured in situ in endosomal compartments. Perhaps more important is the way in which this peptide-affinity model predicts important directions for and new approaches to IDDM. If the key permissive event is a competitive interaction between peptide and class II susceptibility genes, then intervention directed to this event has a therapeutic rationale (34,35). Thus, directing peptides that have a higher affinity for DQ3.2 and thus outcompete other peptides is one approach. The introduction of protective (higher-affinity class II) molecules into the endosomal compartment to outcompete for binding peptides to susceptibility gene products is another approach. Also, by viewing this triggering event as a competitive interaction, it seems likely that quantitative levels of expression of different class II genes will be critical in determining the expression of a susceptible phenotype in a genetically at-risk individual. In other words, one source of variation in diabetes incidence among genetically at-risk individuals may be the control of quantitative levels of the susceptibility allele relative to other class II genes.

An equally intriguing but more speculative possibility is that this peptide-affinity model may provide some insight into individual variation in diabetes susceptibility caused by non-MHC-encoded genes. This possibility is based on the observation in experimental systems that self-peptides efficiently bind to class II molecules and compete for binding with exogenously administered peptides (36). Thus, it is possible that self-peptides, encoded by non-MHC genes, are a potential source for antigenic competition in binding the diabetogenic peptides to class II susceptibility genes. Different individuals with the same HLA genes may have different degrees of susceptibility depending on the relative binding affinities of these peptides.

HLA class II molecules are central to various complex immunological interactions; their role as peptide binders in antigen presentation is only one of several. It is probable that class II molecules are involved in multiple stages of the pathogenic processes in IDDM, perhaps involved in amplification of cellular immune response in the islet, and perhaps involved in establishing a T-lymphocyte repertoire in a diabetes-susceptible mode. Despite this variegated background, however, the primary HLA genetic and structural correlates of IDDM susceptibility can be satisfactorily explained by a rather simple model. In this scheme, the HLA class II susceptibility molecule contributes a key permissive element to autoimmune triggering; this permissive event mechanistically reflects the function of class II molecules as peptide-binding polypeptides. This peptide binding occurs in a competitive milieu and provides a suitable site for intervention in the disease process.

**ACKNOWLEDGMENTS**

This work was supported by grants from the National Institutes of Health and the Benaroya Foundation.

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