

Molecular Basis for HLA-DQ Associations With IDDM

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Autoimmune diabetes is the clinical end point for a sequential cascade of immunologic events that occur in a genetically susceptible individual. Structural and functional analysis of the HLA class II susceptibility genes in IDDM suggests likely molecular mechanisms for several of the key steps in this cascade of autoimmune events. We outline a pathway in which the HLA-DQ genes associated with IDDM bias the immunologic repertoire toward autoimmune specificities, creating an autoimmune-prone individual, followed by amplification and triggering events that promote subsequent immune activation. There are several direct links between genetics and autoimmune disease in this pathway: the developmental maturation of T-cells in a genetically susceptible individual occurs through molecular interactions between the T-cell receptor and the HLA-peptide complex. Selection of T-cells with receptors likely to contribute to autoreactivity may preferentially occur in the context of specific HLA-DQ alleles that are diabetes prone, because of inefficiencies in the peptide-MHC structural interactions of these molecules. Subsequent activation of these T-cells in the context of recognizing islet-associated antigens can trigger a poorly regulated immune response that results in progressive islet destruction. These subsequent diabetes-specific events are also directed by specific HLA genes, most prominently by the binding of specific antigenic peptides by the disease-associated HLA molecules. In this sequential cascade, opportunities for environmental influences and modulation by non-HLA genes are identified that likely act in concert with the predominant genetic susceptibility contributed by the HLA molecules themselves. Clarification of the steps in this pathway extends our understanding of the prevailing role of HLA genes in IDDM pathogenesis and suggests opportunities to intervene at discrete initiating, disease-promoting, or regulatory steps in IDDM development. *Diabetes* 47:1177-1184, 1998

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APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; Th1, pathogenic T-cells involved in cellular immune activation; Th2, helper T-cells.

The *HLA-DQB1* gene is the principal genetic susceptibility locus for IDDM in humans. This gene encodes one polypeptide chain (DQ β) of HLA-DQ, a class II HLA molecule that binds and presents antigens to lymphocytes of the immune system. Several other genes are capable of modifying the genetic risk associated with *HLA-DQB1*—some increase and others decrease risk—although the specific disease-related functions of these modulatory genes are not well understood. HLA-DQ genetic associations with IDDM have been thoroughly studied for over a decade, and function of the HLA-DQ molecules encoded by these genes has also been intensely scrutinized, providing opportunities to better understand the link between genetics and autoimmune diabetes. This article will outline an explicit mechanistic pathway in which HLA-DQ susceptibility genes are proposed to underlie the maturation and activation of disease-initiating events. The sequence of events in this diabetogenic pathway suggests opportunities to better understand the interaction of modulatory genes with HLA genetic susceptibility, the role of genetic protection in IDDM, and the overriding influence of HLA-susceptibility genes in immune selection and activation.

The role of HLA class II molecules in lymphocyte maturation and recognition. The major known function of HLA class II molecules is to bind peptide antigens, which are derived from intracellular proteolysis of both foreign proteins and self proteins. When foreign peptides are bound by class II molecules, they are presented to T-cells as a signal to initiate an immune response, a key adaptive element in generating immunity against infectious and other environmental stimuli. When self peptides are bound by class II molecules, they are presented to T-cells as a signal to educate the immune system to generate a functional repertoire that can distinguish self from non-self, a key adaptive element in maintaining homeostasis and preventing autoimmunity. There are a host of important distinctions between presentation of self and foreign peptides, such as the nature of the cell that expresses the class II-peptide complex (the antigen-presenting cell [APC]), the location in the body where this presentation occurs, and the presence or absence of accessory inflammatory signals or costimulators that also communicate between the T-cell and the APC. This complex interaction is fraught with potential danger, since miscommunication can lead to confusion, inappropriate immune activation, and autoimmune disease.

To understand how such miscommunication can occur, it is useful to consider the basic pathway by which T-cells normally are educated to recognize self peptides. Immature T-

cells that develop in the thymus undergo a series of hard-wired molecular events that culminate in the expression of a cell-surface molecule called the T-cell receptor (TCR). This receptor samples the surface of surrounding cells, and contacts the HLA-peptide complexes that are present on thymic APC. The fate of each T-cell is dictated in large measure by this interaction; T-cells with receptors that fail to bind the HLA-peptide complex die from inattention, whereas T-cells with receptors that engage the HLA-peptide complex receive signals that trigger a functional process called positive selection in which T-cells mature and become immunocompetent (1–4). The peptides bound by HLA molecules, which are engaged by the TCR during selection, are derived from self proteins within the thymic APCs, and there is thus an inherent process of generating autoreactive T-cells during this selection process. The way in which this autoreactivity is kept in check during T-cell thymic development is called negative selection. It is also based on the interaction of the TCR for the HLA-peptide complex and makes use of an ability on the part of the T-cell to sense a threshold of affinity. High-affinity interactions between the TCR and the HLA-peptide complex in the thymus trigger the deletion of those T-cells, leaving only T-cells that have a moderate affinity to mature into immunocompetent T-cells.

The outcome of this selection process is that circulating T-cells have an innate bias toward recognition of peptide-HLA complexes, shaped by this early encounter with self peptides and HLA molecules in the thymus. Thus, potential autoreactivity is an inherent property of the immune system. Keeping this autoreactivity in control, and avoiding autoimmunity, is based on an intricate system of checks and balances in which the ultimate fate of these lymphocytes—whether they will be activated or not—depends on the subsequent events that the T-cell encounters during its life span.

This early maturation of T-cells creates the potential for subsequent T-cell autoreactivity. In this sense, the specific HLA molecules that are involved in binding peptides during T-cell maturation contribute toward a propensity to autoimmunity. This step represents the first of several possible ways in which particular HLA molecules are directly involved in susceptibility to autoimmune diseases. Indeed, as discussed below, the HLA-DQ molecules associated with IDDM have structural properties that suggest poor communication during recognition by the T-cell, consistent with the hypothesis that the HLA-DQ associations with IDDM are explained, in part, by inappropriate early T-cell selection and maturation.

Once autoreactive T-cells that have the potential for autoimmunity have left the thymus, they constitute the peripheral immune system, meaning that they circulate and provide immune surveillance and response. Subsequent activation of such autoreactive T-cells to trigger autoimmunity appears to involve a multistep process. As illustrated in Fig. 1, after the initial T-cell thymic selection, there is an amplification step in the autoimmune pathway in which the numbers of T-cells with specificity for particular HLA-peptide complexes are expanded. One of the consequences of this amplification step is to increase the numbers of autoreactive cells above a threshold required to trigger subsequent pathological events.

Amplification may be triggered by specific antigenic recognition, involving restimulation with the autoantigen, as occurs in situations with chronic low-level antigenic exposure. More interesting, and perhaps more prevalent, is amplification trig-

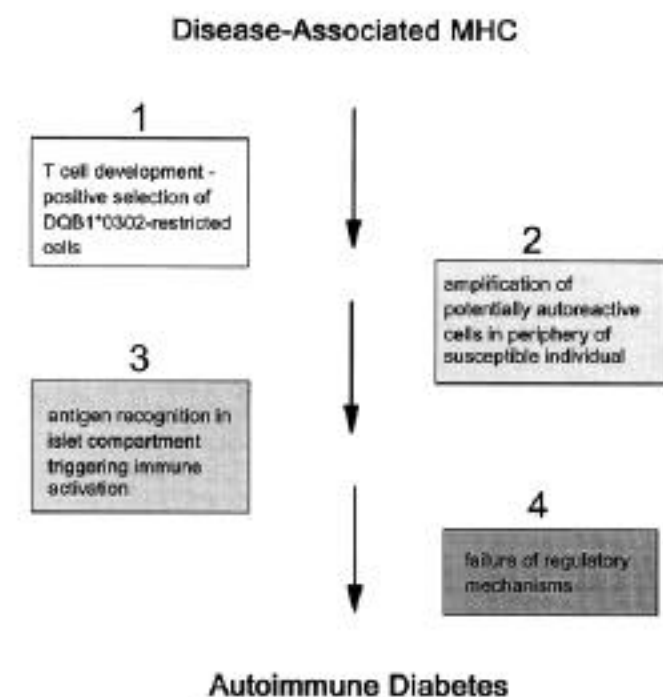


FIG. 1. Stepwise immunologic pathway in autoimmune diabetes. In step 1, presence of disease-associated HLA molecules leads to selection of potentially autoreactive T-cell specificities during T-cell development, characteristic of a genetically at-risk individual. In step 2, the individual becomes autoimmune-prone by virtue of peripheral amplification of the potentially autoreactive T-cells, likely influenced by environmental and infectious immunologic challenges. In step 3, target organ-specific events and local immune activation occur, creating the setting of actual autoimmunity, including specific antigen recognition by the autoreactive T-cells. Step 4 determines whether this autoimmunity will be self-limited or whether it will progress to clinical disease, since potent regulatory mechanisms are capable of modulating autoimmunity for long periods of time. The failure of these regulatory mechanisms, in step 4, is likely to involve the contribution of non-MHC genes associated with diabetes and also provides a promising clinical target for late-stage therapeutic intervention.

gered by stimuli unrelated to the original selecting antigen. Examples are viral or superantigen stimulation of T-cell subsets (5,6), which contain, among many other specificities, some of the autoreactive clones. In other words, when a diverse set of peripheral T-cells are stimulated to expand by virtue of reactivity with viral antigens, some of those T-cells carry the self-peptide recognition receptor that they acquired during their earlier thymic development. In this way, as a consequence of repeated environmental stimuli, numbers of potentially autoreactive cells are amplified. In the autoimmune pathway, this results in the expansion of T-cells with particular HLA-peptide recognition potential, and a threshold required for subsequent activation is achieved. This amplification step, like the earlier thymic selection step, has the potential to explain part of the HLA association with autoimmune diseases. If the environmental stimuli, such as viral infections, that accomplish the expansion of particular autoreactive T-cells are represented by a small set of viral proteins that resemble self proteins, or if they are limited in their preference for complexing with particular HLA genes (HLA-DQ, as opposed to HLA-DR, for example), the particular HLA type of the individual may control the level of autoimmune amplification response that ensues after infection.

The first two steps of this proposed cascade, autoreactive T-cell selection and subsequent amplification, have the functional consequence of converting an autoimmune-susceptible individual to an autoimmune-prone individual. Recently, animal models of autoimmunity have been engineered that create animals with this autoimmune-prone phenotype (7). In these experiments, the TCRs from autoreactive T-cells have been cloned and then transferred into mouse embryos. The mice subsequently develop with a large number of T-cells that carry the autoreactive TCR. Essentially, this genetic manipulation bypasses the first two steps of selection and amplification for autoreactive T-cells outlined in Fig. 1. These genetically altered mice are prone to autoimmunity, which can become full-blown autoimmune disease. However, as pointed out in these experiments, there are still important checkpoints in the subsequent activation and regulation of these autoreactive cells that must be overcome for the full expression of autoimmunity (8).

Activation of autoreactive T-cells, step 3 in this cascade, represents one of these checkpoints. This step involves an intricate interplay of factors, such as antigen density, HLA density, and accessory stimuli from other cell-surface receptors and from cytokines that help determine the outcome of a T-cell-inductive antigen-specific response (9). As discussed below, this is a step in which specific characteristics of susceptibility genes contribute to activation in a very precise way: each HLA molecule determines the binding of particular antigenic peptides. The affinity of this binding and the stability of the HLA-peptide complex will determine the overall avidity of the HLA-peptide-TCR interaction. The extent of TCR ligation will in turn determine the fate of the T-cell (10,11). Importantly, the fate of T-cells activated in this way is determined, in part, by the environment in which the HLA-peptide-TCR interaction occurs. General inflammatory stimuli and tissue damage, which increase antigen expression on the target tissue and increase the release of inflammatory mediators locally, result in chemoattraction and migration of T-cells and may be crucial in the clinical distinction between minor autoimmune manifestations and significant autoimmune disease.

The outcome of autoimmune T-cell activation is not inevitably autoimmune disease. Regulatory T-cells with autoimmune specificity may be a common feature of autoimmune-prone individuals who have not progressed to autoimmune disease. Of all the autoimmune disorders, autoimmune diabetes has the clearest illustrations of this important regulatory step: autoantibody-positive relatives of diabetic individuals, many of whom have compromised insulin secretory function, do not inevitably progress to diabetes (12). Indeed, the many so-called nonprogressor individuals attest to the potential to arrest the immune cascade after activation but before tissue destruction. In the NOD mouse models, many investigators have pointed to the peri-insulinitis characteristic of nondiabetic sibling animals as evidence for a similar regulatory checkpoint (13), illustrated as step 4 in the pathway shown in Fig. 1.

These last two steps in the autoimmune cascade, which involve T-cell activation and failure of regulation, also involve many complex signaling events that are controlled by non-HLA genes. Experimental deviation of the activation-regulation axis can be accomplished by a wide spectrum of cytokine agonists and/or inhibitors, raising the likely possibility that some of the important non-HLA genetic contribu-

tions to diabetes may impact primarily at these stages. Although it might be quite a while before the genetics of human nonprogressors are identified, there are numerous opportunities for genetic and/or therapeutic modulation, by drugs or environmental stimuli, of this final regulatory step.

Progression through the autoimmune cascade. The above description of the four steps in an autoimmune pathway have been outlined in rather general terms to emphasize the utility of this paradigm for understanding many autoimmune diseases not limited to diabetes. Indeed, different autoimmune diseases likely reflect quite different degrees of emphasis on each of these four steps. For example, autoimmune diseases with specific allelic HLA associations, such as diabetes and rheumatoid arthritis, may very well be controlled primarily by steps 1 and 3, the selection and activation steps, while autoimmune diseases with lesser HLA associations may be dominated by environmental stimuli or mimicry mechanisms that contribute to step 2, amplification. Other autoimmune diseases with predominant non-HLA genetic contributions may be characterized by rapid progression through step 4, dysregulation of the autoimmune cascade. One of the useful features of this model is that it allows for a context to test explicit steps in the progression from susceptibility to disease. In the sections that follow, the way in which specific HLA-DQ interactions promote progression through this pathway in autoimmune diabetes is suggested, emphasizing first the selection and second the activation steps that are most likely to be influenced by the specific structural features of the HLA-DQ susceptibility genes themselves.

HLA-DQ as an inefficient selection element in IDDM susceptibility. Alleles at the HLA-DQB1 locus are responsible for the majority of HLA-encoded genetic susceptibility in IDDM, and one of these, the DQB1*0302 allele in particular, is the single most highly associated allele in many different ethnic populations (14–16). The degree of susceptibility conferred by this gene, however, is subject to many modifying influences, including some encoded in the HLA region. Various other HLA-DQA1, HLA-DQB1, and even HLA-DRB1 alleles have been described that modify the risk conferred by DQB1*0302 (16,17).

Detailed structural studies of the HLA-DQ molecule provide some basis for these observations. The HLA protein molecule that is encoded by the DQB1*0302 allele is called DQ3.2. Like all HLA class II molecules, DQ3.2 is an $\alpha\beta$ heterodimer. DQ3.2 exists as a dimer encoded by DQA1*0301 and DQB1*0302 when present on the disease-associated haplotype commonly linked to HLA-DR4. HLA typing for the presence of HLA-DR4 or, more precisely, for HLA-DQB1*0302, have, therefore, historically been used to identify individuals who potentially encode this disease-associated molecule. The molecular structure of HLA class II molecules such as DQ3.2 includes a groove on the surface of the molecule, which is the site at which antigenic peptides bind. By analyzing and mutating the sites on the DQ3.2 molecule that border this groove, it has been possible to determine the molecular features that distinguish DQ3.2 from other HLA-DQ molecules. The DQ3.2 molecule has a characteristic structural motif for peptide binding in which there are four main contact points that discriminate between the specific amino acid side chains on the antigenic peptide (18). These four amino acids on the peptide are called "anchor residues" of the antigen, and they are important for determining the affinity of HLA-peptide

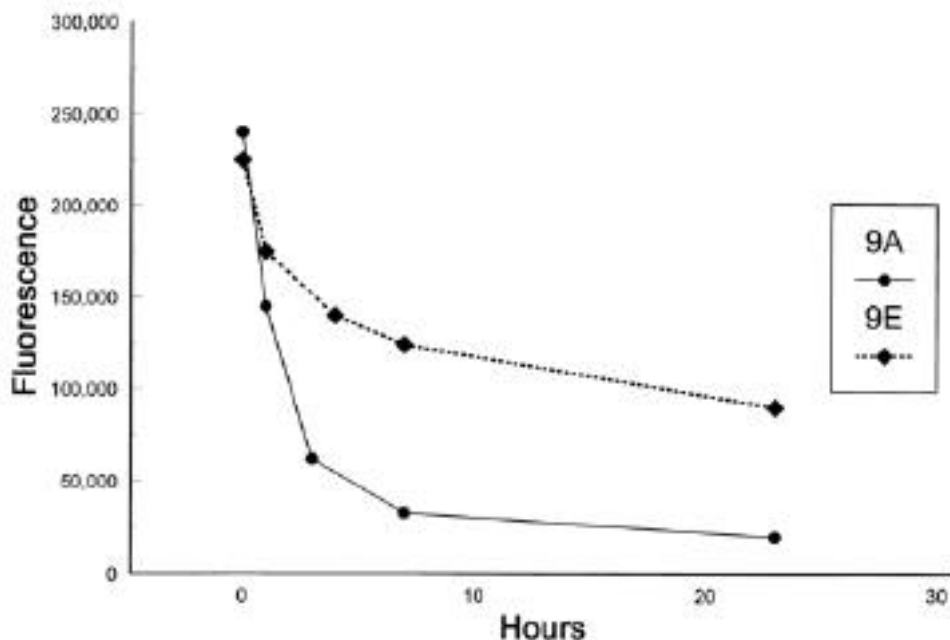


FIG. 2. Stability of the DQ3.2-peptide complex is influenced by specific binding site interactions. Binding of the HSV-2 VP16 433–445 analog to the DQA1*0301/DQB1*0302 class II dimer is illustrated for two cases in which the peptide anchor position 9 is either an alanine (9A) or a glutamic acid (9E). After initial binding, the 9E peptide–DQ complex has a gradual slow decay, whereas the 9A peptide–DQ complex dissociates very rapidly, consistent with its failure to stimulate VP16-specific T-cell clones (20).

interaction. These key anchor residues are spatially separated by other amino acids on the peptide and occur at positions 1, 4, 6, and 9; the corresponding sites on the HLA molecule that interact with these anchor residues are known as “pockets,” and are referred to as pockets 1, 4, 6, and 9. Comparisons of peptides binding DQ3.2 with peptides that bind closely related but non–diabetes-associated alleles have identified pockets 4 and 9 as the sites of key disease-associated polymorphisms (18,19). Peptides that bind DQ3.2 with high affinity have large aliphatic side chains in pocket 4 and negatively charged side chains in pocket 9, forming tight interactions and a stable HLA-peptide complex.

Some insight into the significance of this motif for T-cell selection on DQ3.2 has come from studies of peptide binding. Figure 2 illustrates a measurement of peptide binding to DQ3.2 molecules, comparing two peptides that differ only in the charge of the side chain at anchor residue 9. Peptides that contain a negative charge at residue 9 (i.e., Fig. 2, peptide 9E) form stable complexes with DQ3.2 that remain bound with a fairly long half-life. On the other hand, the same peptide with an alanine substitution at residue 9 dissociates quickly from the class II complex (i.e., Fig. 2, peptide 9A). Thus, the interaction between the DQ3.2 molecule and a single residue on the peptide makes a crucial contribution to the stability of the HLA-peptide complex.

The site on the DQ3.2 molecule responsible for this interaction with peptide anchor residue 9 was determined by analysis of additional HLA-DQ molecules. We have previously shown that this alanine-substituted peptide can be presented and recognized by T-cells if presented in the context of DQ3.3, rather than DQ3.2, molecules (20). The only structural difference between these two alleles is the presence of an aspartic acid encoded by codon 57 in DQ3.3. We interpret these results to mean that a stable peptide-DQ interaction is facilitated by a charge interaction at pocket 9: in the case of the alanine-substituted peptide with DQ3.3, the aspartic acid from DQ3.3 provides a negative charge, which probably forms an ionic-bond salt bridge with an available positively charged arginine at position 79 on the nearby DQ α chain. In the case of DQ3.2,

which lacks this aspartic acid at codon 57, the alanine-substituted peptide is insufficient for binding stability in the absence of a similar charge interaction. However, an analogous contribution to overall stability is provided for interactions with the DQ3.2 molecule when the peptide contains a glutamic acid at residue 9, as in the 9E peptide. In this case, the peptide residue 9 is positioned near codon 57 of the DQ molecule and apparently is able to form a surrogate charge interaction with Arg79, resulting in a stable complex with a long half-life.

What is the significance of this interaction for understanding the role of DQ molecules in IDDM? In the context of thymic selection for autoreactive T-cells restricted by DQ3.2, these data suggest an interesting conclusion. Self peptides with a negative charge at residue 9 (i.e., the good binders) are likely to bind with high affinity and a long half-life (Fig. 2, peptide 9E). Self peptides with similar motifs except for the charge at residue 9, such as the alanine substitution in the peptide 9A, are likely to bind with moderate to low affinity. Because positive selection of developing T-cells is based on low-to-moderate avidity interactions, this leads to the paradoxical hypothesis that the key autoreactive peptides responsible for selection of potentially autoimmune T-cells are those that, like peptide 9A in the example, explicitly lack the high-affinity binding motif.

One of the important implications of this paradigm is that positive selection of T-cells on a self-peptide repertoire will generate T-cells with moderate avidity for peptides, such as the 9A analog used in this illustration. As discussed below, exposure of the same T-cells to the 9E analog at a later time, perhaps in the second or third step of the autoimmune pathway, when the T-cells are in the periphery, is likely to result in a high-avidity interaction and T-cell activation. In this case, the TCR-peptide-HLA interaction would be enhanced by the overall stability of the peptide-HLA complex and, therefore, the duration of TCR-peptide-HLA signaling. Thus, the overall threshold for T-cell activation would be shifted from a high threshold (normal) to a low threshold (autoimmune-prone) based on the peptide-induced change in stability of the DQ3.2-peptide complex.

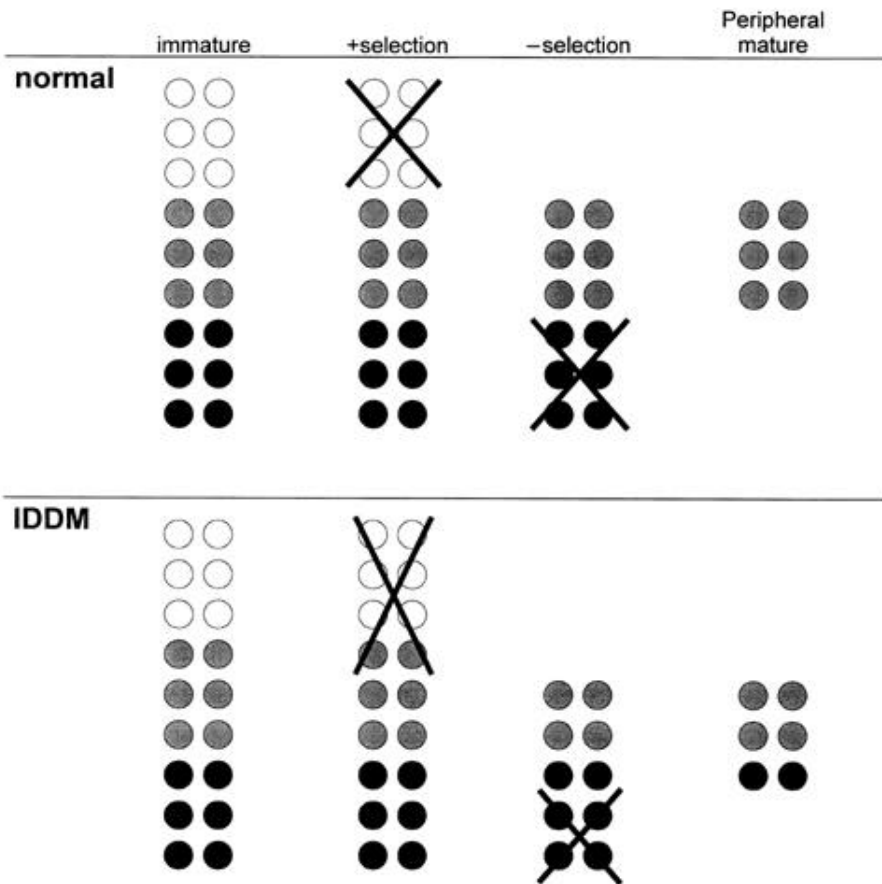


FIG. 3. Generation of autoimmune T-cells in IDDM. The top panel summarizes the current paradigm for normal T-cell development in the thymus, in which a gradient of TCR-HLA-peptide interactions, based on overall avidity, determines T-cell selection outcome. Immature T-cells that fail to interact with MHC-peptide complexes are not selected; TCRs that surpass an avidity threshold are positively selected, while the high avidity subset of these cells is subsequently deleted. These three groups of T-cells are represented by ○, ●, or ●, respectively. The lower panel is a hypothetical interpretation of the impact on this model if selection were to occur in the context of an intrinsically unstable MHC class II molecule. The peptide compartment with high-avidity interactions leading to negative selection is envisioned to be smaller, with many of these peptide-MHC combinations resulting in positive selection due to an overall change in the avidity threshold. The result is that mature T-cells capable of interaction with self peptides in the periphery are altered in terms of specificity and perhaps also increased in number.

Structural observations support this hypothesis. The DQ3.2 molecule is a very unstable class II dimer under most experimental conditions. Compared with other class II molecules, it has a fall-apart phenotype on SDS-containing polyacrylamide gels, particularly at neutral pH (21). This propensity to intrinsic instability probably accounts for the difficulty in affinity purification and crystallization of this particular molecule as well. A similar phenotype has been attributed to the IA⁹⁷ molecule, the disease-associated class II molecule in the NOD mouse, and it has been suggested that this intrinsic instability may contribute to autoimmunity by inefficient negative selection on self peptides (22,23). In other words, negative selection functions as a key barrier to the maturation of autoreactive T-cells, since T-cells with high-affinity interactions with HLA-peptide complexes in the thymus are normally deleted, as described above. Unstable HLA-peptide complexes, such as the DQ3.2 complex with peptide 9A in Fig. 2, are of short duration and unlikely to sustain the interactions required for negative selection.

Figure 3 is a model that illustrates these concepts. For any given HLA molecule, self-peptide-HLA interactions can be functionally defined as no HLA-peptide interaction, low-to-moderate interaction with a short half-life, or strong interactions with a long half-life, represented in the figure by clear, shaded, or black circles, respectively. The number of immature T-cells in the thymus that interact with the HLA-peptide complex in each of these three categories is not known, but it is generally felt that most developing T-cells in an individual contain TCRs that fail to interact with HLA-peptide complexes and therefore die of neglect. T-cells with moderate-avidity TCRs are positively selected, and progress

through functional maturation. Because of the intrinsic instability of DQ3.2-peptide interactions, as discussed above, it is likely that a subset of self peptides are functionally suitable for positive selection in DQ3.2-positive individuals, even if these same peptides promote high-affinity interactions in individuals with other HLA types. In other words, some self peptides that normally negatively select T-cells are likely, in the context of HLA-DQ3.2, to lead to positive selection. In this model, T-cells that are negatively selected in the thymus are those that recognize DQ3.2-peptide complexes in a stable high-affinity configuration, such as the 9E peptide in Fig. 2. In a DQ3.2-positive individual, therefore, the result is the release from the thymus of mature T-cells with low-to-moderate affinity for self-derived peptides, which then establish a potentially autoimmune repertoire in the periphery (24–26).

What are the consequences for autoimmunity when the DQ class II molecule is intrinsically unstable? In the scenario outlined above, T-cells are positively selected on self peptides analogous to the 9A peptide in our example. These cells are dangerously autoreactive if they encounter a peptide like 9E, which compensates for the instability of the DQ3.2 molecule by a high-affinity binding interaction. These T-cells are also potentially autoreactive if they subsequently encounter a peptide like 9A under conditions that can compensate for the short half-life of this complex, such as increased density of the HLA-peptide complex on an APC. Viewed in this manner, the principal role of the DQ susceptibility gene in initiating the autoimmune pathway is to skew the specificity, and perhaps the number, of potentially autoreactive cells exiting the thymus and subsequently populating the peripheral repertoire.

There are several additional important implications of this model. First, it seems unlikely that there is a single self peptide responsible for selecting the autoimmune repertoire in such a scenario. Recent studies using mice that express predominantly a single major histocompatibility complex (MHC)-peptide combination in the thymus have shown a diverse T-cell repertoire (27), consistent with the notion that potentially autoreactive T-cells could result from many different self-peptide selection events. A second intriguing implication is that it seems unlikely that repertoire generation on an unstable class II molecule will be disease specific. In other words, DQ3.2-positive susceptible individuals in this model might be considered potentially autoreactive against a wide repertoire of self peptides, with disease specificity dependent on subsequent steps in the autoimmune pathway. Thus, to dissect the diabetes-specific nature of the autoimmune pathway, we must also look to subsequent disease-specific events in the amplification, activation, and regulation steps (Fig. 1), which predominantly occur in the peripheral immune and target organ compartments.

Peripheral activation of autoreactive cells. Thymic selection on susceptible HLA molecules leads to a potentially autoimmune T-cell repertoire, but target organ specificity, and hence disease specificity, arises from peripheral amplification and activation events, building on the foundation of the autoreactive repertoire generated during thymic selection.

Viral and environmental stimuli are likely contributors to the amplification of the autoimmune T-cell repertoire, highlighted as step 2 in Fig. 1. There is undoubtedly a significant environmental component to IDDM susceptibility. All HLA-DQ at-risk individuals do not display similar degrees of islet autoimmunity or overt diabetes. In human IDDM, a low frequency of autoreactive T-cells after thymic selection can be changed to a high frequency of autoreactive T-cells by amplification events that stimulate T-cell division. These events are likely to be diverse, in some patients involving viral or bacterial superantigens that amplify entire subsets of T-cells, while in other patients they may be much more subtle, involving more antigen-specific stimuli or chronic antigenic exposure. For example, recurrent tissue damage with islet antigen release may provide this type of cumulative stimulus for autoreactive T-cell amplification. This step also highlights a plausible role for antigen-specific mimicry mechanisms. Antigens that cross-react with islet autoantigens (e.g., Coxsackie virus, bovine serum albumin [28]) may amplify parts of the self-reactive T-cell repertoire, thereby increasing the frequency of autoreactive cells in the peripheral compartment and making it more likely that a threshold for subsequent pathogenic events will be achieved.

In the proposed model, this peripheral amplification of autoreactive T-cells need not occur in the same location as the subsequent autoimmune activation. Thus, systemic exposure to environmental stimuli may amplify the frequency of autoreactive clones that circulate in the periphery and that subsequently activate in a specific tissue, such as the islet (see below). It seems likely that many examples of molecular mimicry may contribute to autoimmunity during this amplification stage by virtue of cross-reactive recognition by the autoimmune T-cell.

There is also a potential genetic contribution to this amplification step, which serves as a link in the pathway between T-cell development and subsequent autoimmune activation. In

IDDM, there is a higher risk for childhood disease in individuals heterozygous for both HLA-DR3 and HLA-DR4 haplotypes. In such heterozygotes, different combinations of α - and β -chain dimers form, increasing the number of class II susceptibility molecules. At the DQ locus, four different α/β heterodimers are generated in these individuals (29), and it is interesting to note that the DQA1*0501/DQB1*0201 dimer that is formed by the DQ genes on the HLA-DR3 haplotype is, like DQ3.2, a relatively unstable class II molecule (R.A. Ettinger, A.W. Liu, G.T.N., W.W.K., unpublished observations), perhaps quantitatively influencing the number of potential autoreactive cells. Because heterozygosity for this combination of susceptible DQ molecules is highly associated with a childhood onset of disease (30), it is quite possible that increased numbers of susceptible DQ molecules in this way lead to increased numbers of autoreactive cells, thereby achieving the threshold required for subsequent autoimmune activation at an accelerated pace and with an earlier disease onset.

The autoimmune-prone individual becomes functionally autoimmune when autoreactive T-cells are triggered to initiate tissue damage. There is a complex set of inductive stimuli that initiate a progressive immune response. Among the most important parameters are quantitative variables such as the concentration of antigen at the site, increased density of HLA molecules, and increased expression of costimulatory molecules, which are cell-surface receptors on the APCs and on the T-cells that augment cell-to-cell contact and signaling. There are also important parameters such as local expression of pro-inflammatory cytokines and chemokines that promote and direct the activation pathways. To achieve a signaling threshold for activation of the T-cell response, both the HLA-peptide density and the duration of the HLA-peptide-TCR signal appear to be key elements in determining the pro-inflammatory activation of the autoreactive T-cell (31).

As discussed above, the interaction between peptides and the DQ3.2 molecule is determined both by the amino acid sequence of the peptide and by the structure of the HLA-DQ molecule. The HLA polymorphisms on the disease-associated DQ molecule are critical at this juncture. A detailed interpretation of the HLA-DQ polymorphisms involved in establishing a hierarchy of peptide binding during this interaction has previously been discussed (32). According to this interpretation, DQ molecules associated with IDDM bind peptides in the periphery, as well as in the thymus, with moderate affinity. Certain disease-associated peptides, derived from diabetes autoantigens, bind to the DQ3.2 molecule but fail to bind to other HLA molecules that are not associated with IDDM. Thus, there is a direct correspondence predicted between the autoantigenic peptides and the disease-associated HLA molecules based on this binding interaction. This is a crucial step in the autoimmune pathway, providing a potential link between systemic predisposition to autoimmunity and the ultimate organ-specific disease-specific immune activation. In the proposed model, the diabetes-specific nature of autoreactive T-cells influenced by HLA-DQ pivots on this key feature of HLA class II structure, namely that the allelic polymorphisms of the disease-associated DQ molecules dictate binding of specific islet-associated peptides that lead to T-cell recognition and activation. One of the major unresolved questions is the precise relationship between these peptides derived from islet antigens, and the self peptides that participated in T-cell selection during

thymic development. As illustrated by the example in Fig. 2, it is possible that they are not identical, but are functionally related through their interactions with HLA-DQ molecules if a self peptide (like peptide 9A) selects moderate-avidity T-cells, which are subsequently stimulated with a high-avidity peptide (like peptide 9E) derived from islet antigens.

In the context of the peptide binding hypothesis for autoimmune diabetes, some HLA molecules provide protection from diabetes by binding the same peptides with higher affinity and effectively compete for binding with the DQ3.2 molecule. This genetic protection from IDDM is a remarkable feature of this disease that argues for a direct functional role of HLA-DQ molecules in disease outcome. In population studies, even in the presence of the susceptible DQ3.2 molecule, individuals who express a DQB1*0602 gene are strongly protected from IDDM (33,34). More modest degrees of relative protection are also seen with several DRB1 alleles, such as DRB1*0403 and *0406 (35).

How does HLA-encoded protection correspond to the four-step pathway outlined in Fig. 1? In the peptide binding hypothesis (32), protective alleles bind key autoantigenic peptides with higher affinities than susceptible alleles, and a protective outcome ensues. This could be a simple antigenic competition during recognition and activation in the periphery in which the peptide antigen is unavailable for binding to DQ3.2 if it is already bound to another HLA molecule with higher affinity. In this scenario, a threshold of activation on the susceptible allele is not achieved. Another possible mechanism involving peptide binding and protection from diabetes is the possibility that the protective HLA allele is an efficient restriction element for negative selection of autoreactive T-cells. This mechanism is consistent with a description of T-cell development in NOD mice carrying an autoreactive TCR, where maturation of T-cells is influenced by the presence of protective MHC molecules (36). Because the protective HLA element differs from the susceptible HLA element, however, this scenario requires that the TCR undergoing selection be capable of binding peptides complexed to two different HLA molecules. Thus, in this mechanism, the self peptides complexed to DQ3.2 responsible for selecting autoreactive cells may resemble self peptides complexed to protective HLA molecules, and the autoreactive T-cells would be capable of recognizing two different HLA-peptide complexes.

There is, as yet, no convincing evidence to discriminate between these mechanisms for the dominantly protective HLA alleles in IDDM. They share a common feature—the preferential binding of antigens to the protective HLA molecule—that does have some experimental support. Recent studies of peptide-binding motifs by DQB1*0602-encoded class II molecules have highlighted the ability of this molecule to bind many peptides with high avidity in a stable interaction with a long half-life (R.A. Ettinger, W.W.K., unpublished observations).

Analysis of individuals with protective HLA genes also may provide some clues to the final steps in the autoimmune pathway progressing to IDDM. As shown in Fig. 1, there is an additional step between autoimmune activation and clinical disease, attributable to the failure of immune regulatory mechanisms. Evidence that such regulatory mechanisms exist comes from both human and animal studies: many DQB1*0602-positive relatives of IDDM patients express anti-GAD65 autoantibodies (37). This indicates that immune activation to islet antigens has occurred and argues against a sim-

ple T-cell deletion model for dominant protection. Similarly, DRB1*0406-positive individuals are highly associated with the insulin autoimmune syndrome (38), another antibody-dominated response to an islet-related autoantigen. A common feature of these two examples is that of an immune response to an islet antigen dominated by antibodies, rather than by pathogenic T-cells. This is reminiscent of a well-studied regulatory paradigm in immunology, in which antibody responses are controlled by helper T-cells (Th2) that are a different lineage from pathogenic T-cells involved in cellular immune activation (Th1). Cytokines that regulate these two lineages are intertwined in a reciprocal fashion, with cytokines promoting Th2 responses at the same time they suppress Th1 responses, and vice versa.

In animal models, many of the therapeutic interventions that protect NOD mice from diabetes deviate the immune response toward a Th2 profile, leading to the recognition that IDDM is likely to be a Th1-dominated disease. The inhibition of IDDM in NOD mice with Th2-promoting therapies, a potential parallel with Th2-like genetic protection in humans, is perhaps the most hopeful indicator that immunomodulation aimed at blocking step 4 in the pathway to autoimmune diabetes will have practical clinical benefit. The existence of nonprogressor relatives of diabetic patients demonstrates that clinical diabetes is not inevitable, even after immune recognition events have occurred. Arrested disease progression after the diabetes-prone stage (step 2) or even after the autoimmune stage (step 3) demonstrates that selection and activation do not irrevocably progress to disease. Indeed, the four-step model offers multiple opportunities for clinical intervention. Changes in environmental stimuli and childhood disease exposure may modify the amplification of autoreactive cells (step 2); immunomodulatory therapies, such as cytokine blockade or Th2 agonists, offer potential for regulating disease progression, as does the ultimate diabetes-specific therapy, administration of islet antigens in ways that induce the immune system to regulate and block expansion or activation of the autoreactive cells that are pathogenic. Finally, a further understanding of the mechanisms of dominant genetic protection should lead to additional opportunities for intervention late in the autoimmune cascade.

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