A Molecular Basis for MHC Class II–Associated Autoimmunity

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Class II major histocompatibility (MHC) molecules have an immunoregulatory role. These cell-surface glycoproteins present fragments of protein antigens (or peptides) to thymus-derived lymphocytes (T cells). Nucleotide sequence polymorphism in the genes that encode the class II MHC products determines the specificity of the immune response and is correlated with the development of autoimmune diseases. This study identifies certain class II polymorphic amino acid residues that are strongly associated with susceptibility to insulin-dependent diabetes mellitus, rheumatoid arthritis, and pemphigus vulgaris. These findings implicate particular class II MHC isotypes in susceptibility to each disease and suggest new prophylactic and therapeutic strategies.

THE CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX (MCH) molecules are cell-surface receptors that bind peptide fragments of foreign and self proteins and present these bound peptides to T lymphocytes (*1*). Class II molecules are capable of binding a large number of peptides of different sequence and are thus general, relatively nonspecific, peptide receptors (*1*). These molecules cannot bind all peptides, however, and the structure of a given class II allelic variant has a major effect on binding particular peptides, which in turn can determine the immune response to that peptide (*1*).

Helper T cell receptors recognize peptide fragments of foreign proteins only when they are bound to a self class II MHC molecule. A given T cell is both peptide-specific and MHC-restricted in that it recognizes that peptide only when bound to a particular class II molecule (*2*).

Immune responsiveness is therefore determined by the array of these highly polymorphic class II gene products encoded on human chromosome 6. Class II genotype also contributes to susceptibility to over 40 different autoimmune diseases, including rheumatoid arthritis (RA), insulin-dependent diabetes mellitus (IDDM), and multiple sclerosis (*3*). In these diseases, the body's immune system attacks particular self protein antigens. Major issues in understanding predisposition to autoimmunity include, first, whether primary disease susceptibility is determined by class II genes or by different closely linked genes, and second, how the pathologic gene product contributes to disease development.

Our purpose is to review recent studies (*4–7*) concerning the correlation of particular polymorphic residues of class II human MHC molecules, referred to as human leukocyte antigens (HLA), with disease susceptibility and with what is currently known about MHC molecular structure and function (*1, 2*). These sequence correlations provide a rational basis for the role of class II molecules in certain autoimmune diseases, including IDDM, RA, and pemphigus vulgaris (PV), and suggest new prophylactic and therapeutic strategies.

Class II Molecules: Structure and Function

The MHC class II molecules are heterodimeric membrane glycoproteins that are expressed on the surface of macrophages and B lymphocytes. The molecules consist of one chain with a molecular size of ~33,000 kilodaltons, the α chain, noncovalently associated with a second chain, the β chain, of molecular size ~28,000 kD (*8*). As shown diagrammatically in Fig. 1, these polypeptides each have two extracellular domains. The membrane proximal domains (α_2 and β_2) are thought to fold to form immunoglobulin-like structures. The structures of the NH_2-terminal extracellular domains (α_1 and β_1) are not known, but it is likely, as judged from the crystallographic structure of the related HLA class I molecule, HLA-A2, that they fold together to form a peptide-binding site (*9*). This peptide-binding site is a groove in which the sides are α helices and the floor is composed of several antiparallel β-pleated sheets (*9*). The molecule is anchored in the plasma membrane and has two small intracellular domains.

In humans the class II genes are contained within the MHC in the HLA-D region (Fig. 1A), which spans about 1100 kilobases of the short arm of chromosome 6 (*10*). The HLA-D region contains three subregions—DP, DQ, and DR (Fig. 1B). Each subregion has at least one expressed α and β chain gene. Class II molecules are among the most polymorphic proteins known, and each gene has many alleles (*4, 11–13*). The polymorphism has been defined by the use of class II–specific antibodies (alloantisera and monoclonal antibodies), by the ability of T cells to recognize and proliferate in response to foreign or allogeneic class II molecules in vitro [referred to as the mixed lymphocyte reaction (MLR), which defines Dw subtypes (*14*)], and more recently at the DNA level by analysis of restriction fragment length polymorphisms (RFLP) (*15*) and direct gene

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sequencing (*4, 11–13*). Many HLA antigens occur together more often on the same chromosome than is expected by chance. This nonrandom association is termed linkage disequilibrium. Several DR antigens are in strong linkage disequilibrium with one of the four serologically defined DQ alleles (a particular combination of DR and DQ alleles on chromosome 6 is referred to as a haplotype); the major Caucasian haplotypes are shown in Table 1. The DR and DQ αβ heterodimers constitute the major serologically defined antigens—the class II allodeterminants, DR1 through DRw14 and DQw1, DQw2, DQw3, and DQ Blank. Several DP antigens have also been defined by cellular typing (*14*). Except for the DR_α chain, all the chains are polymorphic. The $DR_{\beta I}$ gene is the most polymorphic, with over 20 alleles (*13*). Most class II polymorphism is located at the first domain (α_1 and β_1) (*4, 11–13, 16*). Although almost half of the 95 amino acid residues of the first domain are polymorphic, most of these are clustered into two to four allelic hypervariable regions (HVR) (*13, 17, 18*).

The human, mouse, and rat class II alleles are homologous, and the distribution of polymorphic residues is similar (Fig. 2). A significant part of the polymorphism appears to have been generated by a mechanism analogous to gene conversion or intralocus double crossovers (or both). Such genetic recombination "shuffling" events have resulted in the creation of new alleles that differ from the progenitor alleles by either single amino acids or entire HVRs—for example, residue 57 of certain DR_β and DQ_β alleles (*4, 11*), DR_β chain residues 9 to 13 (*13*), and the bm12 mutation of the mouse A^b_β allele (*17, 18*).

T helper cells require class II molecules as restriction elements during peptide recognition (*1, 2*). Studies in the mouse have shown that T helper cell–dependent immune responses are (i) determined by class II molecules (*19*), (ii) correlated with the polymorphic amino acid differences between class II molecules (*17, 18, 20*), and (iii) also correlated, in most cases, with the ability of the immunogenic peptide to bind in vitro to the particular class II molecule (*1*). The function of human class II molecules is presumably similar.

The crystallographic structure of the HLA-A2 molecule provides evidence for a direct role for polymorphic residues in peptide binding and presentation to T cells. Significantly, most polymorphic residues that affect reactivity of the molecule with antibodies and recognition by T lymphocytes are in or near the putative antigen-binding cleft of this MHC molecule (*9*). When class II sequences are modeled on the class I crystal structure, almost all of the allelic HVR residues are on the β strands in the floor of the antigen-binding cleft or on the sides of the α helical walls of the cleft.

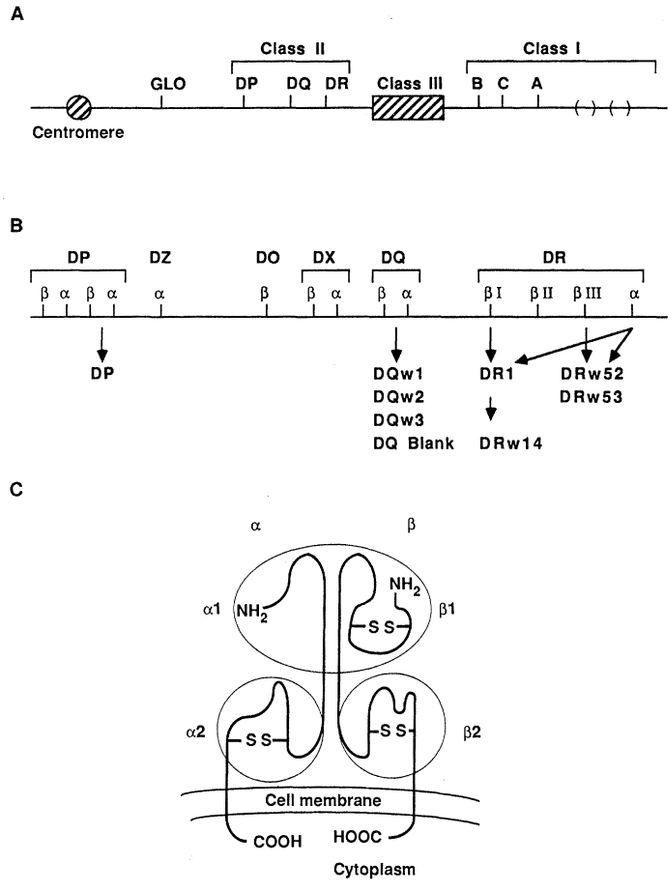

Fig. 1. (**A**) A portion of the short arm of human chromosome 6 and location of the class I, II, and III genes (*52*). (**B**) Map of the class II genes of the human HLA-D region (*8, 10*). The $DR_{\beta I}$ and DR_α gene products associate to form a heterodimeric glycoprotein expressed on the cell surface. This molecule reacts with the alloantisera that define the major serological allotypes HLA-DR1 through DRw14. The relatively nonpolymorphic $DR_{\beta III}$ and DR_α gene products encode the DRw52 and DRw53 serological determinants, which are each associated with several DR allotypes. On the DR3 and DR4 haplotypes (*8, 16*), the $DR_{\beta II}$ gene is not expressed. The DQα and DQβ genes encode the DQ serological specificities (DQw1, DQw2, DQw3, and DQ Blank). The DX_α and DX_β and the second DP_α and DP_β genes are not expressed, and the DO_β and DZ_α genes are expressed only at low levels. Dw cellular subtypes may reflect polymorphism from more than one class II subregion. The Dw specificity of the DR4-Dw subtypes appears to be determined by only a few polymorphic residues in the $DR_{\beta I}$ NH_2-terminal domain (*11, 14*). (**C**) Schematic diagram for the structure of a class II molecule. These transmembrane glycoprotein molecules are organized into domains that correspond to the exon-intron structures of the genes. Their membrane proximal domains (α_2 and β_2) have structural homology with immunoglobulin constant region domains (*8, 9*). We do not know the structure of the class II α_1 and β_1 domains, but, by analogy with the class I crystal structure (*9*), it is possible that the class II α_1 and β_1 domains fold together to form an antigen-binding cleft.

Class II Molecules and Disease

Particular class II molecules are found more frequently in patients with certain diseases than in normal healthy controls. Some of these associations (quantified in terms of the relative risk) of HLA class II antigens with autoimmune disease are shown in Table 2 (*3*). Therefore, susceptibility is at least in part a genetic trait mapping to the MHC. In most class II–associated diseases, autoimmune phenomena can be demonstrated. Class II molecules are logical candidates for gene products associated with susceptibility to autoimmune disease because of their regulatory role in the immune response. In families with certain diseases, including IDDM (*21*) and RA (*22*), affected members tend to share class II haplotypes more frequently than would be expected for independently segregating traits, an indication that a susceptibility gene is linked to the HLA-D region. In addition, the association between the class II antigen (or antigens) and disease is often maintained in different ethnic groups. This is strong support for direct participation of class II genes in disease susceptibility, since it is unlikely that a non–class II susceptibility allele would have remained linked to a particular class II allele during recent evolution. However, most individuals with disease-associated class II alleles do not develop autoimmune disease. The low concordance rates for IDDM in HLA-identical siblings (10% to 20%) and genetically identical monozygotic twins (30% to 70%) (*21*) indicate that other genes contribute to disease susceptibility. Furthermore, the disease discordance between monozygotic twins indicates that environmental effects also play a role in susceptibility.

DNA sequencing of class II genes from autoimmune disease patients, in combination with serological and RFLP studies, provides compelling correlative data indicating that, for IDDM and RA

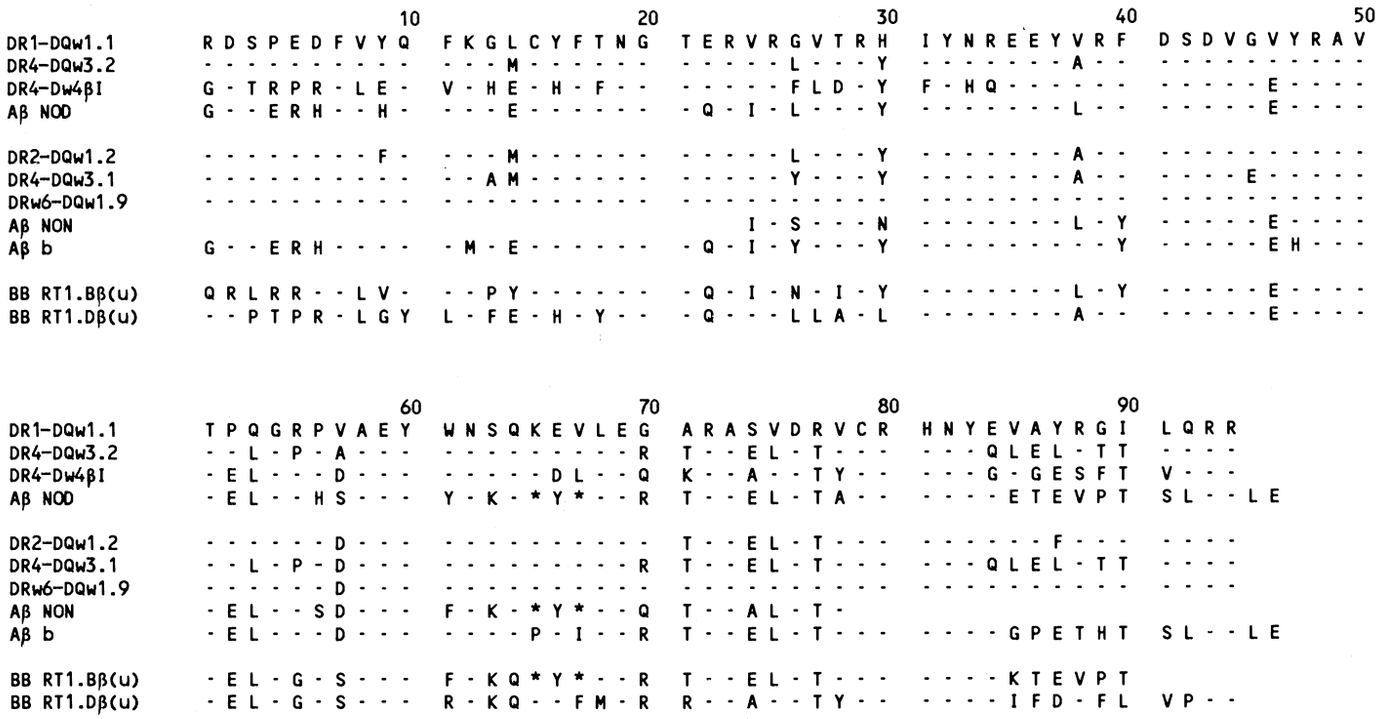

Fig. 2. Comparison of the first domain amino acid sequences of certain human DQ_β (*4, 5, 11, 53*), $DR_{\beta I}$ (*11, 13, 16*), mouse A^b_β (*17*), mouse NOD A_β (*31*), mouse NON A_β (*54*), rat $RT1.B^u_\beta$ (homologous to mouse A_β and human DQ_β genes), rat $RTI.D^u_\beta$ (homologous to mouse E_β and human DR_β genes) alleles. The Lewis rat strain class II β chain sequences also have Ser-57 (*55*). The rat sequences are from the diabetes sensitive (BB-prone) and diabetes resistant (BB-resistant) strains. The BB-prone rat develops a spontaneous form of diabetes (*23*). The class II sequences analyzed from both strains that have the rat u haplotype were identical (*54*). The human and mouse sequences are placed in two groups—those commonly found in diabetics and those rarely found in diabetics (lower group). A dash indicates identity with the DR1-DQw1.1 sequences and a blank indicates that no information is available. The one-letter code for amino acids is: F, Phe; L, Leu; S, Ser; Y, Tyr; C, Cys; P, Pro; H, His; Q, Gln; R, Arg; I, Ile; M, Met; T, Thr; N, Asn; K, Lys; V, Val; A, Ala; D, Asp; E, Glu; G, Gly; and W, Trp.

at least, class II disease-specific polymorphic residues exist and directly contribute to susceptibility. We undertook direct cloning and sequencing analysis as the only approach that would reveal sequence polymorphisms not detected by other typing methods. By using the rapid and powerful approach of in vitro DNA amplification invented and developed by Erlich and colleagues (*12*) (DNA polymerase Klenow fragment is used with synthetic oligonucleotide primers to produce large quantities of the specific target DNA sequence in vitro), we sequenced the HLA DQ_β, DQ_α, $DR_{\beta I}$, and $DR_{\beta III}$ gene segments that encode the NH_2-terminal polymorphic domains isolated from patients with autoimmune disease (Table 2) (*4, 5*).

Insulin-Dependent Diabetes Mellitus

IDDM affects about 0.5% of Caucasian populations. The disease usually appears in individuals before age 20 and is the result of the selective destruction of the insulin-producing cells in the pancreas (beta cells). Insulin regulates the cellular uptake and metabolism of glucose, and its deficiency leads to hyperglycemia, diabetic acidosis, and diabetic coma. The presence of autoantibodies (directed against pancreatic beta cells and insulin) and infiltration of the islets by both B cells and T cells of the immune system indicate that beta cell destruction is probably due to islet cell–specific autoimmunity (*23*). Evidence from spontaneous forms of the disease in mouse and rat models of human IDDM indicates that the disease is T cell–dependent (*23, 24*).

Population and family disease data for IDDM indicate that one or more disease susceptibility genes are linked to the HLA-D subregion. The DP genes can be excluded because DP alleles have not shown association with IDDM (*25*). DP antigens may, however, play a role in other diseases. Analysis of DR and DQ RFLPs indicate that the DQ subregion is more closely linked to the disease susceptibility gene (or genes) than are the DR loci (*15*).

All the nucleotide sequences present in IDDM patients, and patients with other diseases (Table 2), were also found in normal controls (*4*). In other words, disease susceptibility is not caused by mutant class II alleles found exclusively in patients; this implies that alleles found in the healthy population can participate in the autoimmune process.

It was important to ascertain which DR or DQ allele may encode the class II molecule directly mediating susceptibility or resistance to IDDM. The association of HLA with IDDM is unusual in that at least two DR antigens have consistently been shown to be negatively associated with the disease. This indicates that an HLA-linked gene can confer resistance, as well as susceptibility, to the disease. Hence, we examined the known sequences (*4*) for polymorphic residues or allelic HVRs that are shared among all the haplotypes that are positively associated with Caucasian IDDM (DR4-DQw3.2, DR3, DR1, DR2-AZH, and DRw6-Dw19) but that are different from those haplotypes that confer resistance to IDDM (DR2-Dw2, DR2-Dw12, DR5, and DR4-DQw3.1) (Fig. 2 and Table 3). Only residue 57 of the DQ_β chain correlated strongly with IDDM susceptibility and resistance (*4*).

All the DQ_β chains found with increased frequency in diabetics (DQw3.2, DQw2, DQw1.1, DQw1.19, and DQw1.AZH) have Ala, Val, or Ser at position 57. In contrast, DQ_β chains found with decreased frequency (less than 10%) in Caucasian IDDM patients (DQw1.2, DQw1.12, DQw3.1, DQw1.18, DQw1.9, and DQ

Blank) have Asp at this position. Table 3 shows the distributions of the DQ_β chains in patient and control populations for each of the three informative haplotypes—DR2, DR4, and DRw6. For all three, the IDDM patients are significantly different from controls. Similar data have been obtained by Erlich and colleagues (*26*).

The associations of certain DR and DQ antigens can be very different in different ethnic groups, presumably because of recombination between the DR and DQ genes. For example, in American black populations 40% to 50% of the DR3 haplotypes contain DQ Blank (Asp-57 positive), whereas the others have DQw2 (Asp-57 negative) (*27*). The DQw2 antigen is associated with 100% of American white DR3-positive individuals. Preliminary data (*28*) indicate that all 28 black IDDM patients had the DQw2 antigen. In the Japanese population almost all DQ_β chains observed are Asp-positive (*29*) and, in accord with this, the prevalence of IDDM in Japan is 5% to 10% of that observed in the United States (*30*).

The strength of the correlation of DQ_β with IDDM (the increased frequency of Asp-negative DQ_β alleles on each of the informative haplotypes in patients compared to controls) (Table 3) leads us to conclude that certain DQ_β alleles are susceptibility genes (*4*).

This correlation extends to the nonobese diabetic (NOD) mouse. The NOD mouse develops a spontaneous form of IDDM that has many immunological and pathological similarities with the human disease. An IDDM susceptibility gene has also been mapped to the mouse MHC region (*31*). The murine homolog of the DQ_β gene, A_β, was previously isolated and sequenced. The NOD A_β gene, with Ser at codon 57, differs from the gene in other mouse haplotypes (*32*). A_β chains from nondiabetic mouse strains, including the closely related nonobese normal (NON) strain that has been commonly used in studies of genetic crosses with NOD, have Asp at position 57 (Fig. 2). Furthermore, the NOD A_α chain sequence was identical to an A_α chain sequence previously isolated from a normal nondiabetic mouse strain. Because the I-A heterodimer is the only major class II molecule expressed by the NOD MHC (NOD is I-E negative), it seems likely that the NOD A_β chain is responsible for the autoimmune response.

A model for the class II structure based on the class I crystal structure (*9*) places β chain residue 57 at position 143 of the class I α_2 domain. This residue is part of the α_2 domain helix that forms one side of the putative antigen-binding cleft. In the model, amino acid 57 of the class II β chain occupies a similar position and points into the site. In addition, the Asp-57 side chain is in a position to form a salt bridge with a conserved Arg at position 79 of the class II α chain (equivalent to the class I residue 84 of the α_1 domain) (*9*). This model helps explain how one residue (position 57) could potentially have a pleiotropic effect on the structure, function, or α,β chain pairing and expression of the class II molecule.

This correlation with position 57 of the DQ_β chain does not explain all of the HLA associations with IDDM. For example, the DR7 haplotype has a DQw2 DQ_β chain identical to that associated with DR3 (*33*), and yet DR7 frequency is not increased in IDDM (*3*). The DR7 DQ_α chain sequence is unique and may negate the susceptibility provided by the DQw2 β chain. This exception to the correlation highlights the importance of the other differences between "susceptible" and "protective" or "neutral" DQ_β chains and also α chain differences. It has been demonstrated experimentally in the mouse that both chains of the class II heterodimer are important for T cell recognition and antigen presentation (*20*). The DQw3.2 and DQw2 α chains are strikingly different from the DQw1 group of α chains between residues 47 and 56 (*4, 12, 33*). Multiple amino acid substitutions in both the α and β chains could account for the differences between DQ-mediated susceptibility on the DR4 and DR3 haplotypes compared to lower susceptibility conferred by the DR1 haplotype. DQ_α chain polymorphism also helps provide an explanation for the observed high-risk status of DR3,4 heterozygotes, which is greater than that of either DR4 or DR3 homozygotes, where a DQ_α chain from one haplotype (for example, DR3) forms a hybrid molecule with a β chain from the other haplotype (*3*). Although no statistically significant associations with $DR_{\beta I}$ or $DR_{\beta III}$ polymorphisms were found (*4*), it remains a distinct possibility that other class II genes contribute to disease susceptibility and resistance. In the BB rat, the expressed class II β chains from

A Rheumatoid arthritis

	65				70				75		
DR4-Dw4	Lys	Asp	Leu	Leu	Glu	Gln	Lys	Arg	Ala	Ala	Val	
-Dw14	---	---	---	---	---	---	Arg	---	---	---	---	
-Dw15	---	---	---	---	---	---	Arg	---	---	---	---	
DR1-Dw1	---	---	---	---	---	---	Arg	---	---	---	---	Susceptibility
DRw10	---	---	---	---	---	Arg	Arg	---	---	---	---	
DRw53(βIII)	---	---	---	---	---	Arg	Arg	---	---	Glu	---	
DR4-Dw10	---	---	Ile	---	---	Asp	Glu	---	---	---	---	No positive association

B Pemphigus vulgaris

	65				70				75		
DR4-Dw10	Lys	Asp	Ile	Leu	Glu	Asp	Glu	Arg	Ala	Ala	Val
DR6a	---	---	---	---	---	---	---	---	---	---	---
DR6b	---	---	Leu	---	---	Arg	Arg	---	---	Glu	---

Fig. 3. (**A**) Correlation of $DR_{\beta I}$ and DRw53 third allelic hypervariable regions (amino acids 65 to 75) with susceptibility to rheumatoid arthritis, and (**B**) comparison of $DR_{\beta I}$ third allelic hypervariable regions from the DR4-Dw10 subtype and DR6a and DR6b alleles. Sequences are taken from several sources (*4–7, 11, 13, 16, 56*). A dash indicates identity with the sequence on the first line.

Table 1. Distribution of HLA haplotypes in healthy Caucasian populations (*4, 14*).

DR type	Dw type	Associated DQ type	Haplotype frequencies (%)
1	1	w1.1	20
2	2	w1.2	26
2	12	w1.12	2.5
2	AZH	w1.AZH	1.5
3	3	w2	22
4	4	w3.1	5
4	4	w3.2	9
4	10	w3.2	3
4	14	w3.2	7
4	15	Blank	0.5
5	5	w3.1	15
w6/w13	18	w1.18	10
w6/w13	19	w1.19	3
w6/w14	9	w1.9	3
7	7	w2	24
7		w3.3	1
w8		Blank	6
w8		w1	
		w3.1	2
w9		w3.3	1

IDDM-sensitive and IDDM-resistant strains have identical sequences with Ser at position 57 (Fig. 2). This suggests that MHC-associated IDDM susceptibility and resistance in the rat model may be different from that in the human and mouse.

HLA-associated resistance to IDDM is correlated with the presence of Asp at position 57 of the DQ_β chain. However, about 10% of patients analyzed carried one copy of an Asp-positive DQ_β allele (*4*). The mechanisms responsible for the proposed DQ-mediated resistance are currently unknown. Possible mechanisms have been discussed in detail elsewhere (*34*). One possibility is that Asp-positive DQ molecules mediate T cell suppression for the putative islet cell antigen. DQ molecules participate in MHC-restricted, antigen-specific, T cell–mediated suppression (*35*). In addition, defects in suppression are thought to have a role in IDDM in the human and NOD mouse (*36*). A second possible mechanism, which does not exclude the occurrence of the first, is that Asp-positive DQ molecules cause the elimination of potentially autoreactive T cells during early T cell ontogeny in the thymus (referred to as cross-tolerance). There is evidence for class II–mediated positive and negative selection of T cells in the thymus (*2*). Although it is not currently understood how "thymic education" takes place, it is certain that class II molecules have a major influence on the T cell repertoire (*2*) and hence on the immune responsiveness of the individual and susceptibility or resistance to autoimmune disease.

Rheumatoid Arthritis

There are similarities between the associations of HLA with RA and with IDDM. Haplotype sharing in affected siblings demonstrates linkage of an RA susceptibility gene to the HLA region. There is also an increased concordance of RA among monozygotic twins in comparison with HLA identical siblings (*22*). Seropositive RA is primarily associated with DR4 in several ethnic groups (*37*). Other antigens that have been shown to be increased in frequency are DR1 and DRw10 (*6, 7, 38*). By analogy to the IDDM correlation with class II sequences, inspection of the $DR_{\beta I}$ (and $DR_{\beta III}$) alleles that occur more frequently in RA patients than in controls have very similar amino acid sequences in the $DR_{\beta I}$ third allelic HVR at residues 65 to 75 (Fig. 3A). In addition, Morel *et al.* (*6*) showed that $DR_{\beta I}$ sequences isolated from RA patients, selected by reactivity with DR4-Dw14–specific T cell clones, are identical in structure to the equivalent gene sequences from normal individuals. The DR4-Dw10 subtype is not positively associated with RA (*37*). The third allelic HVR sequences of the Dw10 $DR_{\beta I}$ chain contains two charged amino acid differences compared to the positively associated third HVRs of the other DR4 subtype βI chains (Dw4, Dw14, and Dw15), implying that residues 70 and 71 in particular may be important for the association of the DR4–non-Dw10, DR1, and DRw10 haplotypes with RA. The highly polymorphic region consisting of residues 65 to 75 in the $DR_{\beta I}$ chain is clearly a target for T cell recognition (*6, 7, 11, 14*) and may also be the structural basis for a serological determinant shared by DR1 and DR4 (originally referred to as MCI) (*38*). An alloantiserum that defines the MCI epitope reacts with lymphocytes in more than 90% of RA patients compared to 74% who are DR4-positive (*38*). In addition, it appears that the monoclonal antibody 109d6 recognizes the third HVR of the DRw53 chain, which has a sequence (residues 65–73) identical to that of the $DRw10_{\beta I}$ chain (*39*). Since the DR_α chain is nonpolymorphic it is possible that the DRw53 molecule also contributes to RA susceptibility. Perhaps DR4–non-Dw10 haplotypes are most strongly associated with RA because they have two predisposing genes, $DR_{\beta I}$ and DRw53 or $DR_{\beta III}$.

These correlations between class II epitopes and disease susceptibility indicate that, for RA and IDDM, HLA genes contribute directly to disease susceptibility. Disease susceptibility class II alleles may create a DR or DQ site that is effective in presenting a self peptide from the synovium or the beta cell to peptide-specific DR- or DQ-restricted T cell clones. Alternatively, they may be defective in presenting a self peptide to T suppressor cells, they may fail to delete autoreactive T cells, or both of these mechanisms may be operating.

For neither RA nor IDDM do we know the nature of the autoantigen or what triggers the autoimmune response to it. No etiologic agent has been identified, such as a virus that might trigger autoimmunity. An immune response to a virally encoded protein

Table 2. HLA class II associations and autoimmune disease (*3*), with the relative risks, DR type of the patients analyzed and the class II genes isolated from these patients and sequenced (*4, 5, 49*). The DQw1.19b β gene, isolated from a Caucasian lupus patient, differs from DQw1.19a by one amino acid at position 30 (His to Tyr) (*50*). Only gene segments encoding the NH_2-terminal domains were sequenced. Methods have been described (*4, 12*). Numbers in parentheses indicate the number of patients analyzed.

Class II antigen	Relative risk	Patient DR type	Genes sequenced
		Coeliac disease	
DR3	8–12	DR3,7	$DR3_{\beta I}$, $DR7_{\beta I}$
DR7			DQw2 (DR3α,β)
DQw2			
		Insulin-dependent diabetes mellitus	
DR3	4–6	DR3,3	$DR3_{\beta I}$, DRw52a
DR4	4–6	DR3,4(2)	$DR4_{\beta I}$-Dw10
DR3,4	20	DR4,w6(2)	$DR4_{\beta I}$-Dw14
DR2	0.25		DRw53
			DQw3.2(α,β)
			DQw2(α,β)
			DQw3.1(β)
			DQw1.18(β)
			DQw1.19a(β)
		Rheumatoid arthritis	
DR4	4–6	DR2,7	$DR2_{\beta I}$
DR1			$DR7_{\beta I}$
			DRw53
			DQw1.2(α,β)
			DQw2(α,β)
		Myasthenia gravis	
DR3	2.5	DR1,3	$DR1_{\beta I}$, $DR3_{\beta I}$
DR7		DR2,7	DRw52a, $DR2_{\beta I}$-AZH
		DR5,9	$DR7_{\beta I}$, DRw53
			DQw1.1(α,β)
			DQw1.2(β)
			DQw2 (DR3,α,β)
			DQw2 (DR7,β)
			DQw3.1 (DR5,α,β)
			DQw3.3 (DR9,α,β)
			DQw1.AZH(β)
			DX (α,β)
		Multiple sclerosis	
DR2	4	DR2,3(2)	$DR2_{\beta I}$-Dw2
			$DR2_{\beta III}$-Dw2
			$DR3_{\beta I}$, DRw52a
			DQw1.2(α,β)
			DQw2(α,β)
		Pemphigus vulgaris	
DR4	24	DR4,w6	$DR4_{\beta I}$-Dw10
DRw6	1.5		DQw1.9(β)
			DQw3.2(β)
		Systemic lupus erythematosus	
DR2	1–3	DR2,3	DQw1.2(β)
DR3	2–3	DR2,w6	DQw1.19b(β)
		DR2,7	DQw2(DR3,β)
			DQw2(DR7,β)
			DQw1.AZH(β)

Table 3. Informative haplotypes for Caucasian IDDM. Data are taken from several sources (*4, 14, 15, 29, 34, 51*). IDDM patients are significantly different from controls for all three groups (Fischer's exact test, $P < 0.05$). NA, no information is available.

DR antigen	DQβ chain	Residue 57	IDDM patients (%)	Healthy controls (%)
DR2	DQw1.AZH	Ser	77	5
	DQw1.12	Asp	NA	5
	DQw1.2	Asp	23	90
DR4	DQw3.2	Ala	95	70
	DQw3.1	Asp	5	30
DRw6	DQw1.19	Val	58	~10
	DQw1.9	Asp	NA	<10
	DQw1.18	Asp	42	~80

that shares a T cell or B cell epitope with an autoantigen may be a sufficient stimulus in the HLA-susceptible individual to initiate an autoimmune T cell response against the self antigen. It is of interest that a striking similarity between a viral antigen and an HLA molecule has been identified. Cytomegalovirus has previously been suggested as an etiologic agent for IDDM (*23*). Residues 52 to 57 of the $DR_{\beta I}$ and DQw1.2 β chains (PQGRPD) show five out of six amino acid identities with residues 81 to 88 (PLGRPD) of the human cytomegalovirus IE2 protein (*40*). It is possible that this viral pentapeptide is part of an IE2 epitope that is recognized by T cells (in DR3 or DR4 individuals lacking this sequence) and may influence disease susceptibility.

An additional possible mechanism for class II–mediated disease susceptibility involves regulation of class II expression. Evidence from experimental allergic encephalomyelitis, an animal model of multiple sclerosis, indicates that disease susceptibility correlates with hyperinducibility of class II molecules on brain astrocytes (*41*).

Pemphigus Vulgaris

Pemphigus vulgaris, an autoimmune disease of the skin, is slightly more common in Jews than other ethnic and racial groups (*42*). In Ashkenazi Jews, the frequency of the DR4 antigen is 95% in PV patients compared to 40% in healthy controls. In non-Ashkenazi Jews and non-Jewish Europeans, approximately 57% of PV patients are DR4 (versus 22% in controls) and 60% are DRw6 (versus 31% in controls) (*43*). Overall, more than 95% of all PV patients are DR4 or DRw6. Most of the DR4 PV patients have the Dw10 subtype, although this allele is also quite frequent in the normal Jewish population (*44*). DR4-Dw10 is relatively rare in North American controls but is present in most North American DR4 PV patients (*45*). The maintenance of the DR4-Dw10 association with PV in different ethnic groups suggests that the Dw10 $DR_{\beta I}$ third HVR may be required for susceptibility to PV in DR4 individuals. However, one of the two known $DRw6a_{\beta I}$ alleles has a third HVR identical to that of DR4-Dw10, and yet this $DRw6a_{\beta I}$ allele is not increased in DRw6 patients versus controls (Fig. 3B) (*45, 46*). Thus, the presence of a Dw10 third allelic HVR in PV is apparently not in itself required to provide susceptibility and suggests that other polymorphic residues may be important.

Recent RFLP studies suggest that DQ is also important in susceptibility to PV in DRw6-positive patients (*41*). Nucleotide sequence analysis identified a DQ_{β} variant in a DRw6-DQw1 PV patient that differs by only one amino acid (position 57) from two other DQw1 alleles (Fig. 2) (*4, 5*). Oligonucleotide dot blot analysis showed that this variant (DQw1.9) is present in 100% of DRw6-DQw1 Israeli PV patients but fewer than 7% of DRw6-DQw1 Israeli controls (*5*). These studies suggest that the DQw1.9 allele or a closely linked gene (for example, $DR_{\beta I}$) is associated with susceptibility to PV.

Implications

Self-peptide recognition by T cells may lead to induction or suppression of an immune response. An essential step in T cell activation is the binding of peptide to a class II molecule. It appears that the class II molecule has only one major peptide-binding site that has high affinity but, paradoxically, low specificity for peptides (*1*). Class II allelic HVRs presumably exert their influence on an immune response at two levels, T cell repertoire selection (*2*) and peptide binding (*1*). It is not surprising, therefore, that over half of IDDM heritability is attributable to an HLA-linked gene (or genes) (*21*). For IDDM and RA, sequence correlations indicate that DQ and DR molecules, respectively, contribute directly to T cell–dependent autoimmune responses, presumably through the interaction of certain class II polymorphic residues with peptide and T cell receptor residues. It is emphasized that the class II polymorphic amino acid residues associated with RA and IDDM are only part of a particular molecular conformation. The influence of a residue or allelic HVR on disease susceptibility is clearly dependent on other allelically variable residues, as demonstrated by the absence of an association of the "Dw10" HVR-DRw6a molecule with PV. Class II–mediated events may be only one of several critical steps that lead to autoimmune cell destruction and the clinical manifestations of disease. However, it is now apparent that selective inhibition of class II molecules associated with susceptibility may be sufficient to prevent disease.

New therapeutic strategies can be envisaged. (i) It has been demonstrated in both induced and spontaneous animal models of autoimmunity that antibodies to class II molecules can prevent or reverse disease (*47*). (ii) The autoimmune response may be facilitated by a defect in class II–mediated T cell suppressor mechanisms. It may be possible to stimulate disease-protective T suppressor cells by using peptides that bind class II molecules and create epitopes that are recognized by T suppressor cell receptors. (iii) There is evidence from animal models of autoimmune disease that differences in class II molecule expression may be important (*41*). Therefore, manipulation of the levels of class II molecules in vivo with lymphokines, such as interferon-γ or antibodies or antagonists to interferon-γ (*48*) may influence disease onset. Finally, it may be possible to design peptides that bind strongly to certain class II molecules but are not recognized by T cells.

The use of any of these approaches in IDDM requires the identification of susceptible individuals before they become diabetic. Class II genes are clearly the most useful markers for susceptibility at present. In addition, other susceptibility genes and possible environmental factors must be identified to improve detection of susceptible individuals. The identification of autoantigens will greatly enhance the development of potential therapeutic strategies. Given what is currently known about the structure and function of HLA class II molecules, the necessary components for the design of successful therapeutic interventions may already be at hand.

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