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Immunology

Allelic polymorphism and transassociation of molecules encoded by the *HLA-DQ* subregion

(human major histocompatibility complex/gene complementation/hybrid molecules/cell-surface molecules/transplantation antigens)

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ABSTRACT **A monoclonal antibody, CC11.23, with monomorphic specificity predominantly for products of the *HLA-DQ* subregion, has been used to demonstrate primary structural variation among DQ molecules. Two cell lines of each haplotype (*DR1–7*) were radiolabeled with [^{3}H]tyrosine. α and β chains were isolated from CC11.23-reactive preparations, and their amino-terminal tyrosine sequences were determined. Each *DR* haplotype (with the exception of *DRw6*) was found to express a distinct DQ molecule with a minimum of three allelic forms of the DQ α chain and five allelic forms of the DQ β chain. At the primary structural level, the locus for the DQ β chain appears to be as polymorphic as the locus for the DR β chain. Unlike the locus for the DR α chain (which is essentially nonpolymorphic), the locus for the DQ α chain was found to be polymorphic. Comparison of DQ molecules from two different heterozygous cell lines with those from homozygous cell lines revealed that in heterozygotes, DQ α chains from either allele can associate with DQ β chains from one allele. The formation of hybrid HLA-DQ molecules by both *cis* and *trans* gene complementation, coupled with several polymorphic forms of each of the DQ subunits, considerably increases the repertoire of DQ alloantigens in heterozygotes.**

Polymorphism is a hallmark of the molecules encoded within the major histocompatibility complex (MHC) of humans and other mammals. The exact role(s) of this polymorphism is still unknown, but it may be to enhance the capacity of the immune system to respond to a wide variety of antigens. Within the human MHC, a comparison of the molecules encoded by the *HLA-DR* subregion from several different human B-cell lines shows that the molecules possess a nonpolymorphic α chain and a polymorphic β chain (1, 2). HLA-DQ[§] molecules are encoded near the *HLA-DR* subregion and are also polymorphic. This polymorphism has been suggested at several different levels by a variety of techniques (2–11). The finding that the serological markers DQw1, DQw2, and DQw3 reside on DQ molecules (8, 12–14) further strengthened early arguments that the DQw supertypic specificities represented an allelic series separate and distinct from HLA-DR.

Like DR β chains, which are highly polymorphic, DQ β chains also display allelic variation. For example, Goyert and Silver showed by two-dimensional gel electrophoresis that DQ β chains varied in electrophoretic mobility depending upon the haplotype from which the chains were derived (6). More recently, it was demonstrated at the primary structural level that DQ molecules that bear the DQw3 (MB3) determinant from *DR4* cell lines have β chains distinct from those DQ molecules that bear the DQw3 determinant in *DR5* cell lines (7).

Although the DR α chain appears to be nonpolymorphic (1), a growing body of evidence suggests that the DQ α chain is polymorphic (8–11). Comparisons of genomic and/or cDNA sequences of DQ α chain from several cell lines have demonstrated that multiple differences exist among the few haplotypes examined (9, 10).

Polymorphic variation of both the α and β chain of the DQ molecule is consistent with the extensive polymorphism exhibited by its murine counterpart, the I-A molecule. Both the α and β chains of I-A molecules display variation from one haplotype to another (15). In addition to these extensively polymorphic I-A chains, the mouse expresses a modestly polymorphic I-E α chain and a highly polymorphic I-E β chain. A similar situation appears to exist within the human counterpart of *I-E*, the *HLA-DR* subregion. Existing data are consistent with the notion that the DR α chain is invariant, or at best only modestly variant, but that the DR β chain, the DQ α chain, and the DQ β chain are polymorphic among different haplotypes.

Shortly after the demonstration of allelic polymorphism of murine class II molecules, evidence was presented for the formation of hybrid molecules in F_1 animals, providing a possible molecular mechanism for the phenomenon of gene complementation (16, 17). This *trans* gene complementation increases the alloantigen repertoire in heterozygotes. In humans, *trans* association has recently been reported for DQ molecules by two-dimensional gel electrophoresis (18).

The present study describes the use of monoclonal antibody CC11.23 to examine the polymorphism of HLA-DQ molecules at the primary structural level of the proteins. [^{3}H]Tyrosine-labeled DQ molecules were isolated from two homozygous cell lines of several *DR* haplotypes. A minimum of three allelic forms of HLA-DQ α chains and five allelic forms of HLA-DQ β chains was found. When examining combinations of α and β chain molecules, six of seven haplotypes examined could be distinguished from each other. These data demonstrate at the primary structural level allelic polymorphism of both the α and β chains of the HLA-DQ molecule. These observations prompted an examination of *trans* association (formation of hybrid molecules) in heterozygotes. Examination of [^{3}H]tyrosine-labeled DQ molecules from two heterozygous cell lines revealed that in heterozy-

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Abbreviation: MHC, major histocompatibility complex.

[§]According to the Ninth International Histocompatibility Workshop Nomenclature Committee, the designation HLA-DQ has been adopted to replace all earlier designations referring to the *DS/DC* subregion and its products. New provisional HLA-DQ specificities have been given the following designations to replace MB/DC specificities: DQw1 (previously MB1, DC1), DQw2 (previously MB2, DC3), and DQw3 (previously MB3, DC4).

gotes the DQ α chain from either allele can associate with the DQ β chain from one allele.

METHODS AND MATERIALS

Monoclonal Antibodies. Hybridoma CC11.23 (19) was the result of a fusion between spleen cells from mice immunized with the human B-cell line LCL721 and the mouse hybridoma line SP2/O-Ag-14. The monoclonal antibodies L203, IIIE3, and IVD12 have been described (13, 20, 21).

Lymphoblastoid B-Cell Lines. A panel of 14 *HLA-DR* homozygous cell lines was used for isolation of CC11.23-reactive molecules for structural analysis. Cell lines .45.1 (*DR1*) and .127 (*DR3*) are hemizygous, and their origins have been described (19, 22). Cell lines GM3107 (*DR2/2*), GM3161 (*DR2/2*), GM3164 (*DR4/4*), GM3105A (*DR5/5*), and GM3163 (*DR7/7*) were from the Human Genetic Mutant Cell Repository (Camden, NJ). WT49 (*DR3/3*), WT46 (*DRw6/w6*), and MANN (*DR7/7*) were part of the Ninth International Histocompatibility Workshop (Munich, FRG) (23). PRIESS (*DR4/4*) and DHI (*DR5/5*) have been described (7). LG32 (7W513) (*DRw6/w6*) (24) and MDE (8W102) (*DR1/1*) (25) were gifts from T. Ball (University of Texas Health Science Center, Dallas). The heterozygous cell lines HF6.3 (*DR4/7*) and TSK (*DR5/7*) were also provided by T. Ball.

Isolation of Radiolabeled Antigens. All cell lines were radiolabeled in culture with [^{3}H]tyrosine, lysed with 0.5% Nonidet P-40, and passed over a lentil-lectin-coupled Sepharose column to isolate a glycoprotein pool as described (20). HLA-DQ antigens were isolated by passage of the glycoprotein pool over a CC11.23 antibody affinity column. The adherent antigens were eluted with 1.5 M ammonium thiocyanate in Tris-buffered saline (0.01 M Tris/0.15 M NaCl, pH 7.5). From some of the cell lines HLA-DR antigens were isolated from a portion of the glycoprotein pool by passage over an L203 or IIIE3 antibody affinity column. Pools of eluted antigens were dialyzed against the Tris-buffered saline, and the antigens were recovered by precipitation with 10 volumes of cold acetone.

NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Affinity-purified CC11.23- and L203-reactive radiolabeled antigens were electrophoresed under reducing conditions on NaDodSO$_4$/12.5% polyacrylamide tube gels (26). The gels were fractionated into 0.05% NaDodSO$_4$ in water, and aliquots of each sample were assayed for radioactivity to determine the positions of the α- and β-chain polypeptides. Appropriate fractions were pooled and dialyzed against distilled water before amino acid sequence analysis.

Amino Acid Sequence Determination. Isolated α and β chains labeled with [^{3}H]tyrosine were sequenced on a Beckman 890C Sequencer (Beckman) as described (20). Butyl chloride fractions were evaporated by using a stream of N_2, scintillation fluid was added to each fraction, and the radioactivity of each fraction was determined with a liquid scintillation counter.

RESULTS

HLA-DR β Chains Are Polymorphic. Previous studies have demonstrated allelic polymorphism of DR β chains (1, 2). In this report the nature of this polymorphism has been examined by determining amino-terminal tyrosine sequences of β chains isolated from two cell lines of each *DR* haplotype (*DR1–7*). Most of these chains were isolated by using the DR-reactive monoclonal antibody L203, but a few were isolated with monoclonal antibody IIIE3. Several studies were done with both, and no differences were detected. Two cell lines of each *DR* haplotype were used to insure that any variation demonstrated represented an allelic difference and not a random point mutation. We found allelic polymorphism among the DR β chains, examining only amino-terminal tyrosine sequences (Table 1). Of the seven haplotypes examined, only two of the β chains had identical tyrosine sequences (*DR2* and *DR4*), providing evidence for a minimum of six allelic forms of DR β chains. The *DR2* and *DR4* β chains can be distinguished from one another by utilizing an additional amino acid (valine) (2).

Table 1. Amino-terminal amino acid sequences of DR β chains

DR haplotype	Position 10	13	26	30	32
DR1	—	—	—	—	Y
DR2	—	—	—	Y	Y
*DR3**	Y	—	Y	Y	—
*DR4**	—	—	—	Y	Y
*DR5**	Y	—	—	Y	Y
DRw6†	(Y)	—	—	—	—
*DR7**	—	Y	—	—	Y

*From ref. 27.
†The sequence was taken from refs. 28 and 29. The tyrosine (single-letter code Y) at position 10 was variable between the two sequences.

HLA-DQ α Chains, Unlike HLA-DR α Chains, Are Polymorphic. Just as others have demonstrated that the DR α chain is nonpolymorphic (1, 2), all DR α chains isolated in this study possessed a tyrosine at position 13 (data not shown). In contrast to these results with DR α chains, the sequence results of DQ α chains (Table 2) demonstrates the existence of multiple alleles of the locus for the DQ α-chain. While positions 16 and 19 are invariant among all of the haplotypes studied, these α chains can be placed into three groups according to the presence or absence of tyrosines at positions 11 and/or 25.

HLA-DQ β Chains Are Polymorphic. Like the DR β chains, the DQ β chains also display variable amino-terminal tyrosine residues. The amino-terminal tyrosine sequences of two DQ β chains from each *DR* haplotype (*DR1–7*) are shown in Table 3. The variable residues among the DQ β chains are 9, 26, 30, and 37. Tyrosines at positions 16 and 32 are invariant among the DQ β chains. DQ3¶ β chains are distinguished from DQ6 β chains by the absence of a tyrosine at position 37. These data demonstrate the existence of at least five allelic forms of the DQ β chain.

There Are at Least Six Structurally Distinct HLA-DQ Molecules. Matching the appropriate DQ α chain with the corresponding DQ β chain from each of the haplotypes gives rise to a *minimum* of six different DQ molecules. For example, although DQ4 and DQ5 molecules may use the same α chain (α2), they have distinct DQ β chains (β4 and β5), giving rise to two distinct DQ molecules (α2/β4 and α2/β5). Conversely, DQ1 and DQ7 molecules have distinct DQ α chains (α1 and α3), but may share the same β chain (β1), once again giving rise to two distinct DQ molecules (α1/β1 and α3/β1). It is important to appreciate that six is only a minimum estimation of the number of distinct DQ molecules. For example, although DQ1 and DQ6 molecules share similar tyrosine sequences at the amino terminus of both α and β chains, these molecules might be distinguished from one another by using additional amino acids.

***Trans* Gene Complementation Occurs Among DQ Molecules Found in *HLA-DQ* Heterozygotes.** Examination of two *DQ* heterozygous cell lines demonstrated the formation of hybrid DQ molecules within these cells. The DQw3-specific mono-

¶Although not officially designated by the recent Ninth International Workshop Nomenclature Committee, in this report allelic products of the *HLA-DQ* subregion are referred to as DQ1-7, with DQ1 representing the DQ molecule from a *DR1* cell line, DQ2 from a *DR2*, etc.

Table 2. Amino terminal amino acid sequences of CC11.23-reactive α chains

Cell lines	Haplotype		Position			
	DR	*DQ*	11	16	19	25
.45.1	*1*	*w1*	—	Y	Y	Y
MDE			—	Y	Y	Y
3107	*2*	*w1*	—	Y	Y	Y
3161			—	Y	Y	Y
.127	*3*	*w2*	Y	Y	Y	Y
WT49			Y	Y	Y	Y
PRIESS	*4*	*w3*	Y	Y	Y	Y
3164			Y	Y	Y	Y
3105A	*5*	*w3*	Y	Y	Y	Y
DHI			Y	Y	Y	Y
WT46*	*w6*	*w1*	—	Y	Y	Y
LG32			—	Y	Y	Y
MANN	*7*	*w2*	Y	Y	Y	—
3163			Y	Y	Y	—

Y, single-letter code for tyrosine.
*WT46 has been recently typed *DRw13* (a *DRw6* split)

clonal antibody IVD12 (13), which by electrophoretic immunoblotting analysis reacts with isolated DQ β chains from DQw3-positive cells (S. Radka, personal communication), was used to isolate [^{3}H]tyrosine-labeled DQ molecules from two heterozygous cell lines, HF6.3 (*DR4/7*) and TSK (*DR5/7*). Both of these cell lines were typed as *DQw2/DQw3* heterozygotes. Since the amino-terminal tyrosine sequences of DQ4 and DQ5 α chains are distinct from DQ7 α chains (DQ4 and DQ5 α chains possess a tyrosine at position 25, whereas the DQ7 α chain does not), it was possible (by subjecting the separated chains of IVD12-reactive molecules to amino-terminal amino acid sequence analysis) to compare the results between homozygous and heterozygous cell lines in order to test directly for *trans* complementation (Fig. 1). In these experiments the amount of [^{3}H]tyrosine at position 25 of the DQ α chains was ≈50% of the amount expected in comparison to positions 11, 16, and 19, which are invariant between DQ4 (or 5) and DQ7 α chains. When β chains were sequenced, the tyrosine yield at position 26 (*DR5/7*) was exactly as predicted from the repetitive yield line and was identical in sequence to that seen in *DR4/4* or *DR5/5* homozygotes. These data demonstrate at the primary structural level that both the DQ4 (or 5) and the DQ7 α chains are found associated with the DQ4 (or 5) β chain in these heterozygotes.

Table 3. Amino-terminal amino acid sequences of CC11.23-reactive ω chains

Cell lines	Haplotype		Position					
	DR	*DQ*	9	16	26	30	32	37
.45.1	*1*	*w1*	Y	Y	—	—	Y	ND
MDE			Y	Y	—	—	Y	
3107	*2*	*w1*	—	Y	—	—	Y	Y
3161			—	Y	—	—	Y	Y
.127	*3*	*w2*	Y	Y	—	—	Y	—
WT49			Y	Y	—	—	Y	—
PRIESS	*4*	*w3*	Y	Y	—	Y	Y	ND
3164			Y	Y	—	Y	Y	
3105A	*5*	*w3*	Y	Y	Y	Y	Y	ND
DHI			Y	Y	Y	Y	Y	
WT46*	*w6*	*w1*	Y	Y	—	—	Y	Y
LG32			Y	Y	—	—	Y	Y
MANN	*7*	*w2*	Y	Y	—	—	Y	ND
3163			Y	Y	—	—	Y	

ND, not determined. Y, single-letter code for tyrosine.
*See footnote, Table 2.

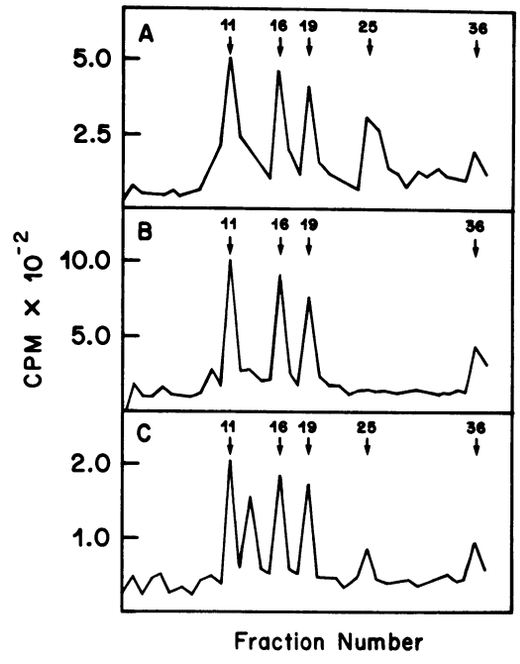

FIG. 1. *Trans* association of HLA-DQ molecules. Amino-terminal tyrosine sequences of HLA-DQ α chains are presented. (*A*) α chain of the DQ molecule isolated from a *DR5* homozygous cell line with CC11.23. The tyrosines found at positions 11, 16, 19, 25, and 36 are characteristic of DQ α chains isolated from *DR5* homozygous cell lines studied thus far. (*B*) α chain of the DQ molecule isolated from a *DR7* homozygous cell line with CC11.23. The tyrosines found at positions 11, 16, 19, and 36 are characteristic of DQ α chains isolated from *DR7* homozygous cell lines studied thus far. (*C*) α chain of the DQ molecule isolated from a *DR5/7* heterozygous cell line with the DQw3 (anti-MB3) monoclonal antibody IVD12. The peak at position 25 represents ≈50% of the expected yield. The peak at position 13 represents the presence of a tyrosine in position 13 of the DR α chain and is commonly seen in IVD12-reactive molecules from *DR5* cell lines (30). As there are no additional tyrosines in the amino-terminal portion of the DR α chain, their presence does not interfere with interpreting the data.

DISCUSSION

This study documents primary structural variation among both the α and β polypeptide chains of *HLA-DQ* subregion-encoded molecules. The variation correlates with the *DR* haplotype and, thus, represents allelic polymorphism. This study was possible because of the availability of a monoclonal antibody, CC11.23, which recognizes DQ molecules in all human lymphoblastoid B-cell lines studied thus far. Like many of the *HLA-D* region-specific monoclonal antibodies described to date, CC11.23, in several circumstances, also recognizes other HLA-D products in addition to DQ molecules. For example, in some cell lines CC11.23 is reactive with a population of molecules consisting of 10–50% DR molecules and 50–90% DQ molecules. These two populations of molecules are easily distinguishable from one another because of the differences in positions of their amino terminal tyrosine residues in both their α and β chains (31).

A rather surprising finding of this study was the ease of demonstrating allelic polymorphism by examining only the amino termini of DQ molecules with a single amino acid. It should be noted that by using this same amino acid, tyrosine, it has been possible not only to distinguish each of the different subregion products [i.e., α and β chains of DP(SB), DQ, and DR (31)] but also most of the alleles at the locus for the DR β-chain (Table 1). The ability to show a considerable amount of allelic polymorphism examining only amino terminal tyrosine positions may have important implications

about the residues comprising the allodeterminants of a given haplotype. Neither the allelic polymorphism of the HLA-DQ β chains nor the polymorphism seen in DR β chains would be detected using most other amino acids in amino-terminal sequencing. This hypothesis that tyrosine residues near the amino terminus may contribute to allospecificity may be used to explain why very few, if any, DRw6-specific alloantisera exist. From examination of the available primary structural data of the DRw6 β chains (see Table 1), it is obvious that these β chains are distinct in that they have only one, or lack altogether, amino-terminal tyrosines. Likewise, in the murine system, comparison of the amino-terminal amino acid sequences of I-A β chains from strain *b* mice with strain *d* mice reveals only 5 amino acid differences in their amino-terminal 50 residues; 4 of these 5 differences involve tyrosine substitutions (32). Thus, it is possible that tyrosine residues in the amino termini of class II molecules are important in generating allodeterminants.

A minimum of three allelic forms of HLA-DQ α chains and five allelic forms of HLA-DQ β chains (Tables 2 and 3) have been documented at the primary structural level. It is important to appreciate that, based on the limited sequence analysis presented here, only a minimum number of allelic products can be predicted. Perhaps each haplotype will possess distinct α chains as well as distinct β chains that have not been apparent serologically. Combination of an appropriate α chain with one of the five β chains gives rise to a distinct DQ molecule in six out of the seven haplotypes examined. This finding is consistent with the data of Goyert and Silver (6), who used two-dimensional gel electrophoresis to define six distinct β chains isolated from haplotypes *DR1–7* (excluding *DRw6*).

The amino acid sequences that have been reported for the DQ molecules bearing the supertypic specificities DQw1 (12) and DQw3 (13) through use of monoclonal antibodies Genox 3.53 and IVD12, respectively, are identical to the sequences reported here using antibody CC11.23. We recently have shown that the DQw3-bearing DQ molecule isolated from two homozygous *DR4* lines was distinct from the DQw3-bearing DQ molecule from two homozygous *DR5* lines (7). The present study extends these findings by suggesting that each of the DQ supertypic specifities (i.e., DQw1, DQw2, and DQw3) can be further subdivided into at least two distinct subsets based on their polymorphic DQ β chains. For example, the DQw3 supertypic specificity resides on both the DQ4 and DQ5 molecules, yet these molecules have different β chains.

A growing body of evidence at both the DNA and protein level suggests that there are two DQ-like molecules in at least some cell lines (10, 30, 33). In none of the sequences reported in this study was there any evidence that the two DQ molecules from a single cell line differed in the positions of their amino-terminal tyrosines. This kind of difference would be detected in our analysis by a change in the repetitive yield of the amino acid sequence from one tyrosine residue to the next as compared to the expected repetitive yield obtained for an internal standard. At least three explanations for this finding are readily apparent. First, CC11.23 may recognize in all haplotypes two DQ molecules that are invariant in their amino-terminal tyrosine residues. This idea is consistent with a previous study with CC11.23, which suggested that this antibody recognizes two DQ molecules that did not differ in their amino-terminal tyrosine sequences (30). This also supports the hypothesis presented above that particular amino-terminal tyrosine residues may be important in forming allodeterminants. Second, CC11.23 may only recognize two DQ molecules in certain haplotypes, and in these haplotypes the tyrosines are invariant. Third, CC11.23 may only recognize a single DQ product in each cell line, and the previous finding with CC11.23, performed in a mutant cell line, might represent an artifact of the system studied.

Allelic polymorphism within the murine *I-A* subregion is extensive, and to date at least 20 distinct allotypes have been mapped to the *I-A* subregion serologically (15). The apparent lack of DQ-specific alloantisera in humans does not exclude the possibility that the human *HLA-DQ* subregion will be at least as polymorphic as the mouse *I-A* subregion. Thus far, any DQ allele-specific alloantisera that exist would probably have been designated as DR-specific alloantisera. In fact, recent evidence suggests that such DQ alloantisera do exist, either as solely DQ-specific sera or in conjunction with DR-specific alloantisera (34). Such DQ allele-specific alloantisera should be important reagents in studying large groups of cell lines, normal individuals, and patients, allowing clearer interpretations regarding linkage relationships between the *HLA-DR* and *HLA-DQ* subregions and, in particular, closer correlations of disease associations with one or the other gene product.

Finally, these techniques for demonstrating allelic polymorphism of DQ α and β chains have provided an approach to study the formation of hybrid molecules in heterozygotes, confirming the earlier findings in the mouse (16, 17) and human (18) that *trans* gene complementation can occur in at least certain combinations. In this study the DQ7 α chain has been shown to associate with either the DQ4 or DQ5 β chains to form a hybrid heterodimer. The implications of these findings, although uncertain at present, could be important in terms of responsiveness to particular antigens and/or susceptibility to certain diseases. For example, insulin-dependent (type I) diabetes mellitus is a disease with complex *HLA-D* region associations. The disease is associated with two *DR* alleles: *DR3* (relative risk of 3.3) and *DR4* (relative risk of 6.4). In homozygous individuals the relative risk increases to 10 for *DR3* and 16 for *DR4*. Interestingly, the highest relative risk is found for individuals who are *DR3/4* heterozygous (relative risk of 33) (35). If the increased susceptibility to insulin-dependent diabetes mellitus is, in fact, more closely related to alleles of *HLA-DQ* rather than alleles of *HLA-DR*, the formation of hybrid DQ molecules in *DR3/4* heterozygotes and the attendant implications of these particular heterodimers in the immune response process might be a plausible explanation for the increased relative risk for *DR3/4* heterozygotes.

Many questions remain concerning the broader implications of *trans* gene complementation in the *HLA-D* region; nonetheless, these studies demonstrate that, in addition to the serologically and biochemically defined MB supertypic specificities and the electrophorectic variation previously seen among DQ molecules, there are primary structural differences that distinguish a minimum of six distinct DQ molecules. This high level of polymorphism has important implications not only for disease susceptibilities and tissue transplantation but also for the evolution of the MHC itself.

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