POLYMORPHIC DQα AND DQβ INTERACTIONS DICTATE HLA CLASS II DETERMINANTS OF ALLO-RECOGNITION

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T cell activation mediated by the TCR is triggered by a complex of an antigenic peptide with a cell surface protein encoded by the MHC. The specific control of the trimolecular interaction between a particular antigen, MHC molecule, and TCR follows a set of genetically programmed parameters. MHC polymorphisms regulate one of these parameters, termed MHC restriction, by which some MHC alleles form "permissive" complexes that permit T cell response, and other MHC alleles do not. Recent studies using murine class II MHC molecules (1-2) have demonstrated that the act of binding a peptide is necessary, but not always sufficient to trigger potentially reactive T cells; presumably, precise structural interactions between amino acids on the peptide and on the class II molecule itself are crucial for recognition. Structural variation among class II molecules can be critically important for this function. In the example of the H-2^{bm12} mouse, three amino acid substitutions in the class II Ia β chain distinguish the bm12 strain from the parental B6 strain, and apparently confer a wide variety of distinct immunologic characteristics, including alloreactivity against the parental B6, differential responsiveness to defined antigenic peptides, and differential susceptibility to experimentally induced myasthenia gravis (3-5).

As the bm12 mouse example illustrates, specific substitutions in class II β chains may have dramatic consequences for immune function. Among human class II molecules, both the class II α and the class II β chains in functional class II dimers potentially contribute structural variation affecting function, particularly at the HLA-DQ locus, where both α and β genes are highly polymorphic. Structural models of recognition events involving HLA class II molecules suggest that polymorphic sites on α and β chains potentially interact with each other, with peptide, and with TCR (6-7).

To evaluate the fine structural requirements of this interaction, we analyzed human T cell-MHC interactions in vitro by evaluating the effects of specific molecular substitutions within the class II HLA-DQ α and β chain components. We have previously described the use of site-directed mutagenesis on cloned human MHC genes,

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followed by retrovirus-mediated gene transfer into human B cell lines, as an experimental approach to the structural modeling of HLA class II molecules (8–9). Recently, we have generated a set of allospecific human T cell clones that distinguish among a number of closely related human MHC alleles at the HLA-DQ locus (10). In order to map the structural constraints that account for the specificity of T cell triggering in this system, we constructed a set of human B cell lines carrying an array of systematically altered HLA-DQ molecules, and tested their ability to activate a panel of human allospecific T cells. In this study, the observed patterns of activation identify specific residues on the DQβ chain, and specific polymorphic α–β chain interactions within the expressed HLA molecule, which are critical for activation.

Materials and Methods

Cell Lines and mAbs. All EBV-transformed B-lymphoblastoid cell lines (B-LCLs)[1] were homozygous lines obtained from the 9th and 10th International Histocompatibility Workshops. B-LCLs that were used for transfections were MAT, KT3, and IBW9. The HLA genotype and DQ allospecificity of these cell lines are as follows: (DQα nomenclature used is similar to that of Kwok et al. [11] and Horn et al. [12]; see also Table I) MAT is DR3, DQ2α(DQA4), DQ2β, DQw2; KT3 is DR4, DQ3α(DQA3), DQ4β, DQw4; IBW9 is DR7, DQ7α(DQA2), DQ2β, DQw2. HLA-homozygous B-LCLs that were used as postive controls for DQw3 allospecific T cell clones were PF97387, PE117, DBB, and DKβ. PF97387 is DR4, DQ3α, DQ7β(DQ3.1β), DQw7; PE117 is DR4, DQ3α, DQ8β(DQ3.2β), DQw8; DBB is DR7, DQ7α, DQ9β(DQ3.3β), DQw9; and DKB is DR9, DQ3α, DQ9β(DQ3.3β), DQw9. DQw7, DQw8, and DQw9 are all subtypes of the DQw3 family. Murine mAb used included mAb IVD12 (DQw3-specific) (13), mAb 159.1 (DQw7-specific), and 200.1 (DQw8-specific) (9).

Retrovirus-mediated Gene Transfer and Expression. The construction of the mutagenized DQ3.2 cDNAs DQ3.2m13, DQ3.2m26, DQ3.2m45, and DQ3.2m57 by site-directed mutagenesis has been described (reference 9, Fig. 1). The oligomer 5'-GGGCCGCCTGATGCCGAGTAC-3' was used to construct DQ3.2m45,57 cDNA from the DQ3.2m45 cDNA using the same protocol. All mutagenized cDNAs were shuttled into retroviral vectors that carried a neomycin phosphotransferase gene with the cDNA under the regulatory control of CMV-IE promoter. Virus-producing fibroblasts generated from the HLA retroviral vectors were used to infect B-LCLs by means of cocultivation. The detailed protocols for construction of retroviral vectors, generation of virus-producing cells, and infection of B-LCLs have been described (8).

Human T Cell Proliferation. Proliferative T lymphocytes reactive with cells expressing DQw3 were generated by in vitro priming between stimulator and responder PBMC and cloned by limiting dilution (14). Clone 1E6 was derived by priming between HLA-identical siblings who differed for the HLA-DR, DQ, DP segment of one haplotype due to intra-HLA recombination (10). Clone IE6 is specific for DR7, DQw9, Dw11 cells, and does not react with cells expressing DR9, DQw9, Dw23 (Mickelson, E., S. Masewicz, G. Nepom, and J. Hansen, manuscript submitted for publication). Clones 21J and 64B were derived from a priming between a DR4, DQw8, Dw4 homozygous stimulator cell and a DR1,4; DQw1,w7; Dw1,w4 responder cell (15). Clone 21J is specific for DQw8 and DR9, DQw9, Dw23 cells, and does not react with cells expressing DR7, DQw9, Dw11; clone 64B is specific for DQw8 cells only. Activity of each clone is blocked by anti-DQ, but not anti-DR, antibodies (Mickelson, E., et al., manuscript submitted for publication).

T cell clones were screened for specific responses by assaying 10^4 cells for proliferative activity after incubation with 2.5×10^4 irradiated (2,500 rad) stimulator cells in 0.15 ml of complete medium in V-bottomed microtiter plates for 66 h. During the final 18 h of incubation, 1 μCi of [^{3}H]thymidine (SA = 6.7 Ci/mM) was added and the activity per culture was measured as counts per minute (cpm). Stimulatory activity due to the presence of transfected class II genes is expressed as Δcpm in which the mean cpm in cultures with untransfected

[1] *Abbreviation used in this paper:* B-LCL, B-lymphoblastoid cell lines.

LCL is subtracted from the mean cpm in cultures with DNA transfected into the same LCL; all determinations were in triplicate. Significance values were determined by comparisons between LCL transfected with the wild-type DQ3.2 gene and the same LCL transfected by mutant DQ3.2 genes, performed by Student's *t*-test. For clone 64B, assays were performed in the presence of 1 U/ml IL-2.

Cytofluorometric Analysis. 5×10^5 cells were incubated with the appropriate mAb for 1 h at 4°C. The cells were then washed and incubated with FITC-labeled sheep anti-mouse Ig for 1 h at 4°C. After additional washing, cells were fixed with 2% paraformaldehyde and analyzed on a flow cytometer (No. 440; Becton Dickinson and Co., Mountain View, CA).

Results

Characterization of HLA-DQ3.2 Expression and Recognition Patterns. We chose the HLA-DQ3.2 (DQw8) molecule as the focus of this study for several reasons: The HLA-DQ3.2 gene is prevalent in the population, present in ~18% of Caucasians. HLA-DQ3.2 is a member of a closely related family of DQw3-associated alleles that have distinct genetic and immunologic properties: The HLA-DQ3.2 gene is strongly associated with HLA-DR4; the HLA-DQ3.3 gene, which is strongly associated with HLA-DR9 and DR7, differs from DQ3.2 by only one amino acid substitution (Fig. 1). The HLA-DQ3.1 gene, which differs from the DQ3.2 by four amino acid substitutions in the first domain, is associated with HLA-DR4, DR5, and DR8. On different DR4 haplotypes, which in some cases may carry a DQ3.2 gene and in other cases a DQ3.1 gene, these DQ allelic differences are presumed to account for major functional differences, including a significantly different risk of disease susceptibility for diabetes mellitus (16–21).

cDNA from the DQ3.2 gene was inserted into a retroviral expression vector and transfected into human B cell lines with different endogenous DQα genes. We chose recipient cell lines representing three of the major four families of DQα alleles (Table I), termed DQ2α, 3α, and 7α (11, 12, 22–23). Reactivity patterns of these transfected human B-LCLs with a panel of DQ-reactive mAbs and T cell-allospecific clones are shown in Table II.

Figure 1

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DQ8 gene (specificity)
                          10        20        30        40        50        60        70        80
DQ3.2 (DQw8)      RDSPEDFVYQFKGMCYFTNGTERVRLVTRYIYNREEYARFDSDVGVYRAVTPLGPPAAEYWNSQKEVLERTRAELDTVCRHNY
DQ3.3 (DQw9)      ----------------------------------------------------------D---------------------------
DQ3.1 (DQw7)      ----------A----------Y----------------E----------D---------------------------
3.2m13            ----------A-----------------------------------------------------------------------
3.2m26            ----------Y-----------------------------------------------------------------------
3.2m45            ----------------------------------------E-----------------------------------------
3.2m57            ----------------------------------------------------------D---------------------------
3.2m45,57         ----------------------------------------E----------D---------------------------
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FIGURE 1. First domain amino acid sequences for the DQβ alleles and site-directed substitutions used in this study.

TABLE I

Specific DQα Alleles Associated with Distinct DQ and DR Specificities

DQα cluster (synonym)*	Associated HLA Specificities	
	DQ	DR
DQ1α (DQA1, DQA1*01)	DQw1	DR1, DR2, DR6, (DR8, DR5)
DQ2α (DQA4, DQA1*05)	DQw2	DR3
	DQw3	DR5 (DR6, DR8)
DQ3α (DQA3, DQA1*03)	DQw3	DR4, DR9
DQ7α (DQA2, DQA1*02)	DQw2, DQw3	DR7

* DQα alleles cluster into the four major families shown; synonyms that have also been used in the literature (12) are indicated, along with proposed WHO nomenclature revisions. HLA-DR specificities in parentheses represent rare linkage patterns. DQ1α polypeptides do not form stable bimolecular dimers with DQ2β or DQ3β polypeptides (11), and were therefore not tested in this study.

Each of three transfected B-LCLs expressed an intact HLA class II dimer on their cell surface containing the DQ3.2β chain, as detected by indirect immunofluorescence with anti-DQw3 mAb IVD12 and anti-DQw3.2 mAb GS200.1, indicating the presence of the 3.2β polypeptide in all lines, apparently complexed to endogenous α chains (8). Among the T cell clones tested, three different anti-DQw3-specific clones, with different fine specificities distinguishing DQw7, DQw8, and DQw9, also reacted with the transfected B-LCL panel. None of these T cells responded to sham-infected LCL that had been transfected with a control vector (not shown). However, some combinations of DQα chains complexed with the DQ3.2β chain were sufficient to stimulate each clone: Expression of the DQ3.2β chain in DQ7α$^+$ cells stimulated only clone 1E6, not clones 21J or 64B, and reciprocally the DQ3.2β chain expressed in DQ2α$^+$ or 3α$^+$ cells stimulated only clones 21J or 64B, but not clone 1E6. For both clones 21J and 64B, the homologous 3α/3.2β dimer stimulated proliferation better than the 2α/3.2β dimer (Table II).

Contributions of β Chain Epitopes to T Cell Recognition. Fig. 1 summarizes the amino acid sequences of the polymorphic first domain from DQβ chains encoding the DQw7,

TABLE II

Serologic and Cellular Recognition Patterns among the DQW3-Related Family of HLA Class II Dimers

	Transfected B-LCL			DQw3 HTC		
	DQ2α/3.2β	DQ3α/3.2β	DQ7α/3.2β	DQw7 (3.1β)	DQw8 (3.2β)	DQw9 (3.3β)
mAbs						
IVD12 (DQw3)	+	+	+	+	+	+
200.1 (DQw8)	+	+	+	−	+	+
159.1 (DQw7)	−	−	−	+	−	−
T cell clones*						
21J	18.5	35.1	0.7	0	27.9	39.6‡
64B	8.1	27.7	1.3	6.4	24.8	5.6‡
1E6	0.7	0	12.2	0	0.2	16.0§

* Δcpm (stimulator cell transfected LCL − untransfected control LCL) × 10^{-3}.
‡ DQw9 on DR9 (DQ3α) haplotypes only.
§ DQw9 on DR7 (DQ7α) haplotypes only.

DQw8, and DQw9 specificities. Also shown in Fig. 1 are the corresponding residues encoded by a panel of DQβ analogs, which we constructed using site-directed mutagenesis of the DQβ3.2 cDNA. Mutations were introduced at key polymorphic residues corresponding to the sites which distinguish among the DQw7, w8, and w9 specificities. In one case (i.e., m45,57) more than one residue was substituted by site-directed mutagenesis. Each of these β chain genes were introduced into B-LCL with an endogenous DQ3α gene and tested for stimulation of the alloreactive T cell panel (Table III). Since the 3α/3.2β dimer mimics the normal linkage pattern for the DQ3.2 gene, this experiment was designed to focus on the contribution of the substitutions within the β chain on T cell recognition. Clone 64B, allospecific for DQw8 (i.e., 3α/3.2β), had diminished reactivity to transfectants with substitutions at codons 13, 26, or 57 of the DQ3.2β chain. In contrast, none of these substitutions altered the reactivity of clone 21J, which has a slightly broader reactivity pattern (i.e., DQw8 + DQw9), and none reconstituted the activity of clone 1E6.

DQα Chain Contributions to T Cell Reactivity Patterns. When this same panel of mutagenized β genes was introduced into different B-LCL for DQβ expression with heterologous DQα chains, interactions between DQα chains and specific β chain substitutions became apparent. The DQ3.2m26 β chain (which had failed to stimulate clone 64B when paired with a DQ3α chain), nevertheless stimulated clone 64B when paired with a DQ2α chain (Table IV). This activity contrasted markedly with the diminished stimulatory activity of transfected DQ3.2m13, m45, and m57, none of which stimulated the 64B clone when expressed in the DQ2α cells. In contrast, DQ3.2m26, as well as DQ3.2m45 and DQ3.2m57, failed to stimulate clone 21J when expressed in DQ2α$^+$ cells, even though they were entirely competent for stimulation in the DQ3α cells. Interestingly, DQ3.2m26 also was sufficient to stimulate clone 64B in the context of DQ7α. With DQ7α, however, but not with DQ2α, mutations at codon 57 also reconstituted stimulatory activity for clone 64B. Clone 21J did not react with any of the DQ7α associated dimers, and clone IE6 reacted with all, although with diminished reactivity on dimers containing DQ3.2m45 or DQ3.2m57.

mAb-Determined Epitopes Associated with DQw3 Are Primarily β Chain Associated. Several murine anti-DQw3-associated mAbs were tested by indirect immunofluorescence for reactivity against each of the DQα and β chain combinations reported here. In all cases, reactivity patterns for each mAb were consistent with previously

TABLE III

T Cell Recognition of Polymorphic DQβ Residues in Homologous DQ3α Dimers

T cell clones*	DQβ (DQw8) wild-type 3.2	β gene substitutions				
		m13	m26	m45	m57	m45.57
21J	35.1	38.9	39.1	38.1	41.8	38.3
64B	27.7	5.6‡	1.5‡	21.5	5.6‡	2.8‡
1E6	0	0.4	0	0	0	NT

* Δcpm (transfected stimulator LCL − control LCL) × 10^{-3}; background cpm (uninfected control LCL) were 520 cpm (21J), 4,563 cpm (64B), and 1,130 cpm (IE6).

‡ $p < 0.001$, Student *t*-test, compared to the wild-type 3.2β transfectant.

TABLE IV

Recognition of Polymorphic DQβ Residues in Heterologous DQ2α and DQ7α Dimers

α chain	T cell clones*	DQβ wild-type 3.2	β gene substitutions m13	m26	m45	m57	m45.57
	21J	18.5	17.3	0.7‡	0.4‡	0.1‡	0.1‡
2α	64B	8.1	1.8‡	10.3	0‡	1.3‡	0‡
	1E6	0.7	1.0	0.7	0.2	0.2	NT
	21J	0.7	0.2	1.3	0.2	0.5	0.4
7α	64B	1.3	0.4	9.8§	0.8	6.8§	7.6§
	1E6	12.2	14.8	20.9	6.8	6.5	18.1
mAb							
IVD12 (DQw3)		+	+	+	+	+	+
200.1 (DQw8)		+	+	+	−	+	−
159.1 (DQw7)		−	−	−	+	−	+

* Δcpm × 10^{-3}; background cpm (uninfected control LCL) were 334 cpm (21J), 8,205 cpm (64B) and 353 cpm (1E6) with DQ2α, and 341 cpm (21J)J, 1,737 cpm (64B) and 377 cpm (1E6) with DQ7α.

‡ $p < 0.001$, compared with 3.2β wild-type transfectant.

§ $p < 0.01$.

reported specificities regardless of the associated DQα chain. These reactivity patterns are summarized in Table IV. The presence of a glutamic acid residue at codon 45 of the DQ3.2 molecule accounts for the epitope corresponding to DQw7, recognized by mAb 159.1, which was previously termed "TA10," or "DQw3.1" (24, 25). Reciprocally, presence of a glycine at this position corresponds to the DQw8 specificity, previously designated DQw3.2, recognized by mAb 200.1 and IIB3 (1, 9, 18). These epitopes, as well as the broad DQw3 epitope recognized by mAbs IVD12, 17.15, and 100.1, remained intact on the panel of β chains transfectants associated with either DQ2α, 3α, or 7α, and therefore appeared to be "blind" to the α chain contribution.

Discussion

The extreme genetic polymorphism of the human MHC translates into a diverse array of polymorphic class I and class II cell surface molecules. Since class II HLA molecules are composed of dimers of α and β chains, each of which may be polymorphic, there is a potential element of bimolecular interaction in the expressed structure. Particularly in heterozygous individuals, where *trans*-associated class II dimers form (26), there is the potential for an identical gene product, such as the DQ3.2 β chain, to contribute to different recognition elements based on dimerization with different DQα chains. When this level of structural and functional heterogeneity is augmented by structural polymorphisms within the α and β chains themselves, there is potential for an extremely wide degree of variation among different, even haploidentical, individuals. To begin to understand some of the structural parameters and constraints on this diversity, we constructed a panel of 18 transfectants, expressing combinations of β chains and α chains that distinguish the expressed DQ molecules of the DQw3-related family. Site-directed mutagenesis was used to introduce β chain substitutions at single codons, and the resulting mutagenized DQβ

genes were expressed in human B cell lines to form stable DQ dimers with endogenous DQ2α, 3α, and 7α chains.

In these experiments, the stimulatory capacity of a particular class II dimer in assays of alloreactive T cell clone stimulation was dependent on α-β interactions. This dependence on complex epitopes for T cell recognition contrasted with the serologically defined epitopes studied, which were determined by the β chain alone. Interactions between class II α and β chains have been inferred in the past by experiments in which T cells are apparently restricted to heterozygotes, implying the possibility of a *trans*-associated class II dimer (27, 28) and by structural studies in which *trans*-associated α and β chains do (26) or do not (11, 29–30) pair and form stable dimers. We directly demonstrate the potential for such interactions to dictate T cell activation, illustrated by variation in recognition of DQ3.2-related epitopes.

The recent structural elucidation of the three-dimensional structure of an HLA class I molecule offers the opportunity for considerable insight into the precise molecular contributions to these interactions. In the HLA-A2 class I crystallographic structural analysis (31), and in the hypothetical class II model based on homologies between class I and class II genes (6), several sites for potential interaction between α and β chains occur. In addition, both α and β chains have direct and indirect contact points with the predicted antigen (peptide) binding site of the class II molecule. Allorecognition is most likely a composite of clonal events, some of which involve direct triggering of allospecific TCR by polymorphic class II molecules and some of which require a peptide bound by the class II molecule. In this sense, the clonal reactivity patterns analyzed in our studies represent discrete examples of anti-DQw3 allorecognition events. By comparing our results to the class II structural model, these examples suggest interpretations for class II-TCR interactions that involve both direct and peptide-dependent activation.

In the context of this molecular model, the amino acid substitutions introduced by mutagenesis in our experiments highlight potential sites of molecular interactions. Residues 26 and 57 showed evidence of direct or indirect interaction with DQα chains: Recognition by clone 64B was dependent on the presence of specific DQβ3.2 residues at codons 13, 26, and 57, when paired with a DQ3α chain. Substitution at each of those positions resulted in loss of activity. However, recognition by clone 64B of the substitution at residue 26 could be reconstituted in the context of DQ2α or DQ7α. This argues against a model in which clone 64B recognizes two epitopes (i.e., at codon 26 on the β chain and an additional polymorphic site on DQ3α) and argues for a model in which there is some conformational synergy between polymorphisms on the α chain and codon 26 on the β chain. This synergy between residue 26 and the α chain may well be due to indirect molecular interactions, possibly involving peptide binding by the class II molecule, since residue 26 is predicted to lie on the opposite side of the postulated peptide binding groove from the α chain helix, in current models of class II structure.

Substitution of an aspartic acid at codon 57 showed a similar α/β interaction effect, in that the DQ7α/3.2m57β dimer was stimulatory for clone 64B whereas the wild-type DQ7α/DQ3.2β dimer was not. Furthermore, a complex role for residue 57 in α chain interactions was suggested by results with clone 1E6. Reactivity of clone 1E6 was heavily dependent upon an appropriate DQα chain, since stimulation was only seen with DQ7α. While all DQβ chains tested stimulated clone 1E6 in DQ7α^+

cells, substitutions at codons 57 and 45 had reduced stimulatory activity. Again, this is more likely to be related to conformational effects of α/β interaction rather than on the contribution of a specific β chain epitope, since the "double mutant" with substitutions at both codons 45 and 57 reconstituted clone 1E6 activity. A role for residue 57 in α/β interactions has previously been postulated, based on the structural model mentioned above, in which an aspartic acid at position 57 would potentially form a stable "salt bridge" with an invariant arginine at residue 79 of the α chain. Since the codon 57 substitutions in our studies generated an aspartic acid replacement for an alanine, such a potential direct α/β interaction could account for the observed structural synergy.

With clone 64B, but not 21J or 1E6, substitution at DQβ codon 13 also interfered with stimulatory activity, indicating a more limited potential for this residue contributing to key epitopes. Although speculative, the postulated placement of residue 13 in the class II model in the "floor" of the peptide binding site might suggest that activity of alloreactive clone 64B is particularly subject to influence by bound peptides. In contrast, substitution at DQβ codon 45 (postulated to be "outside" the peptide contact regions) was responsible for the gain and loss of the major serologically defined allospecificities (DQw7, DQw8) associated with these DQβ molecules, and also contributed to some, but not all, of the T cell–defined epitopes.

Although the T cell recognition epitopes illustrated in this study are complex, it is apparent that single residue substitutions in most cases are sufficient to alter or abolish reactivity. This suggests that fairly simple structural modifications of class II molecules may have profound functional effects. In previous studies, mutagenesis of the HLA-A2 molecule has been used to map sites of potential interaction with antigen or cytolytic T cells; in these studies, as in ours, single amino acid substitutions are in some cases sufficient to alter T cell reactivity (32). Limited studies of murine class II molecules, based on selection of functionally defective class II variants, have similarly implicated specific potential sites of interaction critical for interactions with antigen and/or T cells (5, 33). In addition, a recent report demonstrated that a number of discrete Aβ substitutions heavily influenced by Aα polymorphisms contributed to murine allorecognition, analogous to the studies reported here (34).

Our studies of T cell clones that recognize DQw3-related specificities indicate both α chain and β chain contributions to specificity. Furthermore, all three clones studied have major differences in their fine specificity, even though 64B and 21J were clones derived from the same individual during the same in vitro priming experiment (Mickelson, E., et al., manuscript submitted for publication). Although we analyzed a small sample, our results indicate that the T cell response, even to fairly similar epitopes, is likely to be diverse. The relative contributions of HLA class II α chain and β chain polymorphisms to T cell recognition, and consequently to an in vivo immune response, are likely to be extremely heterogeneous.

The DQ3.2β gene used as a model in these studies has been implicated as the most likely candidate for an HLA-associated disease susceptibility gene in type I diabetes (IDDM) (17, 35). Individuals who carry a DQ3.2 gene are more than eightfold more likely of developing IDDM than individuals without a DQ3.2 gene. Although the mechanisms whereby the 3.2 gene is associated with IDDM are not known, they are assumed to be related either to an antigen-presenting function associated with autoimmunity or to a role in T cell repertoire selection during thymic develop-

ment. In any event, since the DQ3.1 gene is not associated with IDDM, a number of investigators have speculated on the potential functional relevance of residues at codons 13, 26, 45, and 57, which distinguish DQ3.2 from DQ3.1 (9, 12, 20, 36–39). From our studies, it is apparent that the distinction between DQ3.2 and DQ3.1, as recognized by the immune system, depends on an interaction between multiple sites on the β chain, with contributions from the α chain as well. Since single residue substitutions can abolish or restore T cell recognition as demonstrated in these studies, it is conceivable that single residue substitutions can be critical for disease pathogenesis. However, detailed molecular modeling with class II structural mutants, putative peptide antigens, and candidate T cell clones will be necessary in order to identify the constellation of interacting factors that coincide to trigger HLA-associated pathogenic events in IDDM.

Summary

18 transfected cell lines were generated that expressed distinct DQ molecules related to the serologically defined HLA-DQw3 specificity. These transfectants were constructed using site-directed mutagenesis to introduce nucleotide substitutions into DQ3.2β cDNA, followed by retrovirus-mediated gene expression of the mutagenized genes in human B cell lines with different endogenous DQα chains. The capacity of particular class II dimers to stimulate alloreactive T cell clones was investigated. T cell activation was found to be dependent on both DQα and DQβ chains. In some cases, single amino acid substitutions at codons 13, 26, 45, or 57 of the DQβ chain were sufficient to dramatically alter T cell reactivity; T cell recognition of these substitutions, however, was strongly influenced by the α chain polymorphisms present in the stimulatory class II dimer. Both gain and loss of major serologic and cellular specificities associated with specific DQw3$^+$ alleles were observed with a limited array of site-directed substitutions.

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