The Role of Polymorphic HLA-DRβ Chain Residues in Presentation of Viral Antigens to T Cells

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Summary

The relative importance of 11 polymorphic positions in the HLA-DR7β1 chain in T cell recognition of foreign antigens was investigated using transfectants expressing mutant DR7β1 chains as APC for five rabies virus–specific T cell clones. The results indicate that multiple amino acids, located in both the β-strands and α-helix of DR7β1 in the model of a class II molecule, are involved in DR7-restricted T cell recognition of these antigens. Many of the substitutions appeared to reduce the affinity of an antigenic peptide for the mutant DR7 molecules but did not prevent binding. The heterogeneity of responses of the three G-specific T cell clones to presentation of the G11.3 peptide by several of the mutant DR7 molecules indicates that the T cell receptor (TCR) of each these clones requires a different view of the G11.3/DR7 complex and raises the possibility that the G11.3 peptide may bind to the DR7 molecule in more than one conformation.

Class II–restricted T cell recognition of foreign antigens, a central event in the normal function of the immune system, requires the formation of a trimolecular complex consisting of a class II molecule, a foreign antigenic peptide, and a TCR. This is a highly specific interaction in which the TCR recognizes a unique three-dimensional conformation of the class II–peptide complex. The demonstration of direct binding of antigenic peptides to class II molecules has greatly facilitated the current understanding of these interactions (1, 2). Amino acid differences or polymorphisms among class II molecules play a very important role in the specificity of these interactions and are the basis for class II–restricted T cell recognition. Although class II polymorphisms in general appear to be important in peptide binding and TCR interactions, the contributions of individual amino acids at polymorphic positions in these molecules are only beginning to be understood. In the murine system, several studies using mutant class II molecules generated by site-directed mutagenesis have provided insight into the contributions of individual amino acids in these molecules to restricted recognition of foreign antigens by T cells (3–8). However, similar analyses of human class II molecules have not been reported. Elucidation of the ways in which HLA class II polymorphisms influence these complex interactions will have important implications for understanding the basis for the association of certain class II alleles with specific diseases and for the development of synthetic peptide vaccines.

The recent description of a hypothetical model of MHC class II molecules (9), extrapolated from the crystal structure of a class I molecule (10, 11), has been very important in shaping the current understanding of the contribution of the class II molecule to T cell recognition. The model predicts that the antigen binding site or groove is formed by the membrane distal α_1 and β_1 domains of class II molecules that fold to form a platform of β-strands which is topped by two α-helices, one formed by a portion of the α chain and the other by a portion of the β chain. Importantly, most of the amino acids that are polymorphic among class II molecules are located in the model at positions in the β-strands and α-helices that are predicted to be important in determining peptide binding or TCR interactions.

In the studies reported here, we have investigated the role of human MHC class II β chain allelic polymorphisms in presentation of viral antigens to T cells using HLA-DR7-restricted recognition of rabies virus antigens by human T cell clones as a model system. Our strategy was to assess the relative importance of polymorphic positions in the HLA-DR7β1 chain in T cell recognition of rabies virus antigens by using site-directed mutagenesis to produce 11 mutant DR7β1 chains with single amino acid substitutions at positions that are predicted in the class II model to be involved in peptide binding or TCR interactions. Transfectants expressing these mutant DR7β1 chains with the nonpolymorphic DRα chain were used as APC for rabies virus–specific, DR7-restricted T cell clones. The data indicate that multiple residues in the β-strands and α-helix of the DR7β1 chain are involved in DR7-restricted recognition of foreign antigens.

Materials and Methods

cDNA Clones. Full-length HLA-DR α and β chain cDNA clones in the Okayama and Berg expression vector pcD (12) or pcD-SRα296 (13) were used in these studies. The DRB1*07 cDNA, which encodes the DR7β1 chain and the DRA cDNA, which encodes the DRα chain, were isolated from a DR7 library (14). The DRB1*0401 cDNA (provided by Peter Gregersen, Northshore University Hospital, Manhasset, NY), which encodes the Dw4β1 chain, was cloned from a DR4, Dw4 library (15).

Mutagenesis. The mutagenesis strategy was to produce DRB1*07 cDNAs in which specific codons had been altered by mutagenesis to introduce a codon that encodes the amino acid found at the corresponding position of the Dw4β1 chain. Eleven 17–19-mer mutagenic oligonucleotides were designed to generate these DR7β1 → Dw4β1 substitutions at positions 11, 13, 30, 37, 57, 60, 67, 70, 71, 74, and 78 of DR7β1. The oligonucleotides were synthesized by the DNA Core of the University of Iowa Diabetes and Endocrinology Research Center. Site-specific mutagenesis at positions 9 and 67 was performed on the double-stranded DRB1*07 cDNA using the gapped heteroduplex method of Barbosa et al. (16), as described by us elsewhere (17). The other mutations were performed using an in vitro mutagenesis system (Amersham Corp., Arlington Heights, IL), which is based on the method of Taylor et al. (18), as described by us elsewhere (17). After the mutagenesis and screening, mutant M13 double-stranded DNA was prepared, and aliquots were digested with BamHI or SacI and Bsu36I. The 1,300-bp BamHI fragments and the 304-bp SacI-Bsu36I fragments were recovered from agarose gels and ligated into BamHI-digested pcD vector (mutants at positions 11, 13, and 30) or pcD-SRα296 vector (mutants at positions 71 and 74), or the SacI-Bsu36I–digested pcD vector containing the remainder of the DRB1*07 cDNA (mutants at positions 37, 57, 60, 67, 70, and 78), respectively. When the BamHI cDNA fragment was cloned into the expression vector, nucleotide sequencing of DNA corresponding to the entire mature protein was performed to confirm that the sequence was identical to the DR7β1 wild-type sequence except for the nucleotide changes induced by the mutations. When the mutant SacI-Bsu36I fragment was returned to the expression vector, only this portion of the cDNA was sequenced.

Transfection. Cells of the DAP.3 subclone of class II–negative murine L cell fibroblasts were transfected using the calcium phosphate co-precipitation method as described (19). For each transfection, 20 μg of plasmids containing the DR α chain or β chain cDNA and 1 μg of the pSV2-neo plasmid were used. Class II expression by G418-resistant L cells was analyzed using anti–class II mAb, indirect immunofluorescence, and flow cytometry, and transfectants with comparable levels of class II expression were isolated by simultaneous flow cytometric sorting and cloning as described (19). The transfectant expressing wild-type DRα:DR7β1 molecules has been described (19). Cloned transfectants were used in all studies.

Cell Proliferation Assays. The rabies virus–specific T cell clones used in this study were isolated from a CD4+, CD8− T cell line derived from a healthy male rabies vaccine recipient as described (20). Clones 1C6 (previously referred to as C6), 1C10 (C10), 1C9 (C9), and 1B8 (B8) have been described (20, 21). 2 × 10⁴ cloned T cells were incubated with various concentrations of antigen or peptides in the presence of 1 × 10⁴ transfected L cells for 3 d in each well of a 96-well microtiter plate (Costar, Cambridge, MA). The cultures were pulsed during the last 16–20 h with 1 μCi of [³H]thymidine (NEN-DuPont, Boston, MA) and harvested onto glass fiber filters. Radioactivity was measured by liquid scintillation spectroscopy and the results of the cell proliferation assays were

expressed as counts per minute. Results were calculated from the mean cpm obtained from duplicate or triplicate determinations and the SD of the mean was always <10% of the mean.

Antigens. Synthetic peptides corresponding to portions of the rabies virus glycoprotein (G)[1] and nucleoprotein (N) were prepared in Applied Biosystems or Biosearch automated synthesizers. The amino acid sequences of the synthetic peptides were deduced from the nucleotide sequences of G and N genes of the ERA and DUV6 strains. Amino acid analysis revealed that all peptides were at least 95% pure. Peptides were resuspended in PBS at 1 mg/ml before their use in tissue culture. Rabies ribonucleoprotein (RNP) complex (provided by Dr. Bernard Dietzschold, Wistar Institute, Philadelphia, PA) was purified from lysates of cells infected with the Pitman Moore strain of rabies virus as described (22). Purified RNP complexes was shown to be composed mainly of N plus small amounts (∼10%) of the phosphoprotein NS.

Results

11 mutant DR7β1 chains with single amino acid substitutions were produced by site-directed mutagenesis. In each mutant, the residue found in the wild-type DR7β1 chain was replaced by the residue found at the same position of another DRβ1 allele, Dw4β1 (Fig. 1 *a*). As shown schematically in Fig. 1 *b*, the residues at positions 11, 13, 30, and 37 are predicted, in the model of a class II molecule (9), to be located in the β-strands in the floor of the peptide binding groove with their side chains pointing up into the binding groove. The residues at positions 57, 60, 67, 71, 74, and 78 are predicted to be located on the α-helix with their side chains pointing in toward the binding groove, whereas the side chain of amino acid 70 is predicted to be pointing up and in toward the top of the binding groove. The 11 G → V, 60 S → Y, 67 I → L, and 71 R → K substitutions are conservative, and the other substitutions are nonconservative.

L cell transfectants expressing the DRα chain with the wild-type DR7β1 chain or one of the mutant DR7β1 chains were produced and cloned transfectants expressing comparable levels of DR molecules were selected from each bulk culture by simultaneous flow cytometric sorting and cloning. 12 transfectants expressing the wild-type or mutant DR7 molecules were used as APC for 5 DR7-restricted, rabies virus–specific T cell clones. T cell clones 2C5, 1C6, and 1C10 are specific for G and recognize G11.3, a 15 amino acid peptide (DALESIMTTKSVSFR) that corresponds to positions 285–299 of G. PBMC, but not L cell transfectants, are able to process and present G to clones 2C5, 1C6, and 1C10 when purified G or whole virus is used as the antigen source. Both PBMC and DR7-expressing transfectants are able to present the G11.3 peptide to these clones. Clones 1C9 and 1B8 are specific for N. L cell transfectants, as well as PBMC, are able to process and present the relevant N peptides when RNP is used as the antigen source. In addition to a naturally processed peptide of N, clone 1B8 also recognizes peptide 13D of N when presented by PBMC or transfectants. T cell

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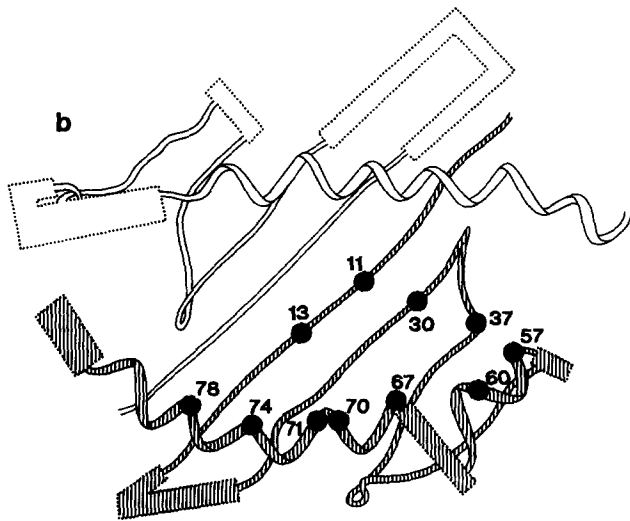| a	11	13	30	37	57	60	67	70	71	74	78
DR7β1	G	Y	L	F	V	S	I	D	R	Q	V
	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
Dw4β1	V	H	Y	Y	D	Y	L	Q	K	A	Y

Figure 1. (*a*) The amino acid substitutions (single-letter code) in mutant DR7β1 chains produced by site-directed mutagenesis. In each mutant, the residue found in the DR7β1 wild-type chain was replaced by the residue found at the same position of the Dw4β1 chain. (*b*) The locations of the substituted amino acids in the class II model of Brown et al. as described in reference 9. (Reprinted by permission from Nature 332:845, 1988, Macmillan Magazines Limited.)

proliferation data from representative experiments are presented in Tables 1 and 2, and the proliferation induced by each mutant DR7 molecule is presented as a percentage of the proliferation of the same T cell clone in response to the wild-type DR7β1 molecule and the same antigen in a reduced form in Table 3.

When the results with the three G-specific clones (2C5, 1C6, and 1C10) that recognize the G11.3 peptide are considered, several observations are noteworthy (Tables 1 and 3). First, at the lowest peptide concentration (0.01 μg/ml), 10 of the 11 substitutions in DR7β1 caused either complete elimination or significant reduction in the ability of the DR7 molecule to present the G11.3 peptide to each of the T cell clones. Only the 71 R → K mutant induced proliferation by clones 2C5 and 1C6 at levels comparable to that induced by the wild-type molecule. However, at a peptide concentration of 1 μg/ml, nine of the mutants presented peptide G11.3 to at least one of the clones as efficiently as the wild-type molecule. The 37 F → Y and 60 S → Y mutants were less efficient at presentation of G11.3 at 1 μg/ml to each of the clones. Only the position 13 and 57 mutants presented the G11.3 peptide (1.0 μg/ml) to all three clones at levels comparable to the wild-type. Some of the substitutions had very selective negative effects on presentation of G11.3 to one clone. For example, the 30 L → Y mutant presented peptide (1.0

μg/ml) very well to clones 1C10 and 1C6, but this substitution completely abrogated recognition by clone 2C5. Likewise, the position 67 mutant, with a highly conservative I → L substitution, presented peptide (1.0 μg/ml) very well to clone 1C6, moderately well to clone 1C10, and not at all to clone 2C5. The failure of the position 30 or 67 mutant to present peptide to clone 2C5 cannot be due to failure of these mutant DR7 molecules to bind the G11.3 peptide because these mutants were quite efficient at presentation of this peptide to the other clones. Therefore, the failure of a mutant DR7 molecule to present peptide G11.3 to these clones does not equate with the inability of the mutant molecule to bind the peptide. Analogous selective effects on the ability to present G11.3 were found with the position 11, 70, and 78 mutants. Therefore, these data indicate that multiple DR7β1 amino acids located in both the β-strands and α-helix in the class II model are involved in the presentation of the G11.3 peptide to these three clones.

In contrast to presentation of the G11.3 peptide, in which the peptide probably binds to class II molecules on the surface of the APC, presentation of N to clones 1C9 and 1B8 almost certainly involves internalization of RNP, the source of N in these studies, and subsequent antigen processing and binding of one or more naturally processed antigenic peptides to DR7 molecules. Although each of the N-specific T cell clones presumably recognizes a different naturally processed N peptide, the exact sequences of these peptides are unknown. Substitutions at positions 70, 71, 74, and 78 of DR7β1 eliminated the ability of the DR7 molecule to present antigen to the N-specific clones 1C9 and 1B8 (Tables 2 and 3). The substitutions at positions 11, 13, 30, 37, and 60 reduced or eliminated the ability of these mutants to present N to clone 1C9, regardless of the antigen concentration, whereas the position 11, 30, and 37 mutants presented N to clone 1B8 quite efficiently. These data suggest that these clones recognize different N peptides. The position 57 mutant presented N to both clones comparably to the wild-type molecule. Clone 1B8 also recognizes 13D, a 15 amino acid peptide that corresponds to positions 121-135 of N (ALTGGMELTRDPTVP). Eight of the DR7 mutants were unable to present the 13D peptide to clone 1B8 at any antigen concentration. The position 11 and 30 mutants induced proliferation by 1B8 that was 23 and 74%, respectively, of that induced by the wild-type at the highest peptide concentration. In contrast, the position 57 mutant induced proliferation by 1B8 that was threefold greater than that induced by the wild-type and that was similar to that obtained with RNP. Thus, the 57 V → D substitution appears to permit binding of 13D to the DR7 molecule in a conformation that is similar enough to that of the naturally processed peptide of N to permit recognition by clone 1B8. These data suggest that the naturally processed N peptide recognized by clone 1B8 and peptide 13D are not identical.

As shown in Table 3, the position 11, 13, 30, 57, 67, and 71 mutants induced proliferation by one or more of the T cell clones that was >120% of the proliferation induced by the wild-type at the highest antigen concentrations. In addi-

Table 1. *Responses of G-specific T Cell Clones to Peptide G11.3 Presented by Transfectants Expressing Mutant DR7β1 Chains*

APC	Peptide concentration	Proliferation by T cell clones 2C5* G11.3	1C6 G11.3	1C10 G11.3	APC	2C5 G11.3	1C6 G11.3
	$\mu g/ml$	$cpm \times 10^{-3}$				$cpm \times 10^{-3}$	
Exp. 1					Exp. 2		
DR7β1	0	0.1	0.1	0.3	DR7β1	1.0	0.2
Wild-type	0.01	19.4	3.6	4.3	Wild-type	25.6	36.1
	0.1	25.8	13.7	6.4		32.0	34.9
	1.0	22.9	17.6	4.8		23.9	38.0
DR7β1	0	0.1	0.1	0.2	DR7β1	0.2	0.2
11 G → V	0.01	ND	0.1	0.3	71 R → K	19.7	37.1
	0.1	8.0	0.2	1.1		33.3	36.1
	1.0	6.0	4.9	4.9		32.1	45.6
DR7β1	0	0.2	0.2	0.2	DR7β1	0.2	0.3
13 Y → H	0.01	1.4	0.2	0.4	74 Q → A	0.3	0.6
	0.1	19.2	5.7	5.3		0.4	15.1
	1.0	23.2	15.5	8.7		19.7	38.4
DR7β1	0	0.1	0.3	0.3	Dw4β1	0.2	0.2
30 L → Y	0.01	0.6	0.3	0.6		0.2	0.3
	0.1	0.3	5.6	5.1		0.3	0.3
	1.0	1.1	17.6	7.8		1.2	2.4
DR7β1	0	0.2	0.1	0.6			
37 F → Y	0.01	9.2	0.5	1.7			
	0.1	15.8	4.3	5.0			
	1.0	11.4	10.6	3.4			
DR7β1	0	0.1	0.3	0.7			
57 V → D	0.01	3.6	0.3	1.5			
	0.1	28.5	6.6	3.2			
	1.0	31.6	16.7	4.3			
DR7β1	0	0.1	0.1	0.6			
60 S → Y	0.1	0.5	0.0	0.8			
	0.1	8.3	0.2	1.2			
	1.0	14.7	3.2	1.0			
DR7β1	0	0.1	0.5	0.2			
67 I → L	0.01	0.1	1.0	0.3			
	0.1	0.1	8.6	2.5			
	1.0	0.6	25.0	3.1			
DR7β1	0	0.1	0.1	0.5			
70 D → Q	0.01	0.1	0.3	0.9			
	0.1	0.2	7.6	0.6			
	1.0	1.0	17.5	1.5			
DR7β1	0	0.2	0.2	0.6			
78 V → Y	0.01	0.1	0.3	0.8			
	0.1	0.7	6.2	1.4			
	1.0	7.3	15.8	2.0			
Dw4β1	0	0.1	0.3	0.9			
	0.01	0.1	0.3	0.8			
	0.1	0.1	0.3	1.4			
	1.0	0.7	0.4	1.7			

* T cell clone.

Table 2. *Responses of N-specific T Cell Clones to Rabies Virus Antigens Presented by Transfectants Expressing Mutant DR7β1 Chains*

		Proliferation by T cell clones							
		1C9*	1B8	1B8	Antigen		1C9	1B8	1B8
APC	Antigen concentration RNP	RNP	RNP	13D	concentration 13D‡	APC	RNP	RNP	13D
	μg/ml	cpm × 10⁻³			μg		cpm × 10⁻³		
Exp. 1						Exp. 2			
DR7β1	0	0.3	0.2	0.2	0	DR7β1	0.3	0.2	0.2
Wild-type	0.56	5.2	5.1	0.1	0.1	Wild-type	5.2	7.3	1.0
	1.7	8.6	16.1	6.1	1.0		8.6	18.1	10.8
	5.0	17.0	26.1	6.5	10		17.0	36.6	36.7
DR7β1	0	0.1	0.2	0.2	0	DR7β1	0.2	0.0	0.0
11 G → V	0.56	2.1	13.0	0.1	0.1	71 R → K	0.2	0.0	0.1
	1.7	5.4	25.5	0.2	1.0		0.2	0.1	0.1
	5.0	11.5	32.4	1.5	10		0.2	0.1	0.1
DR7β1	0	0.3	0.2	0.2	0	DR7β1	0.2	0.1	0.1
13 Y → H	0.56	0.3	2.2	0.1	0.1	74 Q → A	0.2	0.1	0.1
	1.7	0.3	10.2	0.1	1.0		0.2	0.1	0.1
	5.0	0.9	19.4	0.1	10		0.2	0.3	0.1
DR7β1	0	0.2	0.2	0.2	0	Dw4β1	0.2	0.1	0.1
30 L → Y	0.56	1.7	14.2	0.1	0.1		0.2	0.1	0.1
	1.7	5.5	26.7	0.2	1.0		0.2	0.1	0.1
	5.0	10.8	28.1	4.8	10		0.2	0.1	0.1
DR7β1	0	0.3	0.4	0.3	0				
37 F → Y	0.56	0.9	11.3	0.4	0.1				
	1.7	3.2	19.7	0.3	1.0				
	5.0	3.8	22.3	0.3	10				
DR7β1	0	0.3	0.2	0.2	0				
57 V → D	0.56	4.0	6.5	8.7	0.1				
	1.7	5.7	21.1	25.4	1.0				
	5.0	14.6	25.4	20.8	10				
DR7β1	0	0.2	0.3	0.3	0				
60 S → Y	0.56	0.4	1.8	0.2	0.1				
	1.7	3.4	7.7	0.3	1.0				
	5.0	4.3	13.9	0.1	10				
DR7β1	0	0.2	0.2	0.2	0				
67 I → L	0.56	3.1	2.4	0.2	0.1				
	1.7	9.0	7.6	0.2	1.0				
	5.0	14.1	18.4	0.1	10				
DR7β1	0	0.1	0.3	0.3	0				
70 D → Q	0.56	0.2	0.3	0.2	0.1				
	1.7	0.2	0.2	0.2	1.0				
	5.0	1.7	0.4	0.1	10				
DR7β1	0	0.4	0.4	0.4	0				
78 V → Y	0.56	0.3	0.2	0.3	0.1				
	1.7	0.6	0.4	0.3	1.0				
	5.0	0.4	0.6	0.2	10				
Dw4β1	0	0.2	0.4	0.4	0				
	0.56	0.2	0.4	0.3	0.1				
	1.7	0.2	0.4	0.3	1.0				
	5.0	0.2	0.3	0.2	10				

* T cell clone.
‡ Peptide 13D.

Table 3. *Summary of T Cell Responses to Rabies Virus Antigens Presented by Transfectants Expressing Mutant DR7β1 Chains*

APC DR7β1	Proliferation of T cells*					
	2C5‡	1C6	1C10	1C9	1B8	1B8
	G11.3§	G11.3	G11.3	RNP	RNP	13D
11 G → V	+	+	+ + +	+ +	+ + + +	+
13 Y → H	+ + +	+ + +	+ + + +	−	+ +	−
30 L → Y	−	+ + +	+ + + +	+ +	+ + +	+ +
37 F → Y	+ +	+ +	+ +	+	+ + +	−
57 V → D	+ + + +	+ + +	+ + +	+ + +	+ + +	+ + + +
60 S → Y	+ +	−	+	+	+ +	−
67 I → L	−	+ + + +	+ +	+ + +	+ +	−
70 D → Q	−	+ + +	+	−	−	−
71 R → K	+ + + +	+ + +	ND	−	−	−
74 Q → A	+ + +	+ + +	ND	−	−	−
78 V → Y	+	+ + +	+	−	−	−

* The proliferation induced by each mutant DR7 molecule at the highest antigen concentration (G11.3, 1.0 μg/ml; RNP, 5.0 μg/ml; and 13D, 10 μg/ml) is shown as a percentage of the response to the wild-type DR7 molecule by the same T cell clone in the same experiment: −, <20%; +, 20-49%; + +, 50-79%; + + +, 80-120%; + + + +, >120%.
‡ T cell clone.
§ Antigen.

tion, the position 11, 30, 37, and 57 mutants induced increased proliferation of clone 1B8 at lower antigen concentrations. The position 30 and 57 mutants each induced increased proliferation by two clones, whereas the position 11, 13, 37, 67, and 71 mutants each induced increased proliferation by one clone. The T cell clones showed heterogeneity in the frequency of these increased proliferative responses when antigen was presented by the mutant DR7 molecules. Clone 1B8 showed increased proliferation in the recognition of N with four of the mutants: positions 11, 30, 37, and 57. It is notable that the responses of 1B8 with the position 11, 30, and 37 mutants were greater than twofold the proliferative response elicited by the wild-type molecule at the same antigen concentration. Three of the clones showed increased proliferation in the recognition of peptide G11.3 with one or two of the mutants: clone 1C10 with the position 13 and 30 mutants, clone 2C5 with the position 57 and 71 mutants, and clone 1C6 with the position 67 mutant. Only clone 1C9 did not manifest increased proliferation in response to any of the mutants.

To further investigate the structural constraints on peptide–class II interactions, the DR7β1 mutant transfectants were analyzed for their abilities to present other peptides that are similar to the G11.3 peptide of G to clones 2C5 and 1C6 (Table 4). Peptide G11 is a 41 amino acid peptide that was first found to contain the epitope recognized by the G-specific T cell clones (21). Peptide G11.3 is a 15 amino acid peptide whose sequence is included in the G11 peptide. The G11 and G11.3 peptides are based on the sequence of G from the ERA

strain of rabies virus. Three single amino acid substitution variants of the G11.3 peptide were produced based on the sequence of G from the Duvenhage 6 (DUV6) strain. G-specific T cell clones 2C5 and 1C6 were previously shown to recognize ERA strain virus but not DUV6 strain (20 and our unpublished data). Peptide G11.5 contains a S → T substitution at the position that corresponds to amino acid 289 of G, G11.6 has a M → I substitution at position 291, and G11.7 contains a V → I substitution at position 296. It is important to note that the G11.5 and G11.7 peptides were presented efficiently at 0.01 to 1.0 μg/ml to clones 2C5 and 1C6 by both DR7+ PBMC (data not shown) and the transfectant expressing the wild-type DR7 molecule. Peptide G11.6 is not recognized by clone 1C6 at any concentration, whereas half-maximal proliferation of clone 2C5 in response to G11.6 presented by the wild-type DR7 molecule (PBMC or transfectant) occurred at 100-fold higher concentration of G11.6 than was required for G11.3, G11.5, or G11.7 (data not shown). The proliferation data for clones 2C5 and 1C6 in response to presentation of these peptides by the position 11, 13, and 60 DR7β1 mutants are shown in Table 4. Only the position 57 mutant presented G11.6 to clone 2C5 (data not shown). For the position 30, 37, 57, 67, 70, and 78 DR7β1 mutants, the results with the presentation of the G11, G11.5, and G11.7 peptides to clone 2C5 and the G11.5 and G11.7 peptides to clone 1C6 were comparable to those found with the presentation of G11.3 to these clones by each of the mutants in Table 1 (data not shown). However, there was considerable heterogeneity in the ability of the position 11, 13, and 60

Table 4. *T Cell Responses to Peptides of Rabies Virus Glycoprotein Presented by Transfectants Expressing Mutant DR7β1 Chains*

Peptides

283	323
G11	CLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLM
G11.3	DALESIMTTKSVSFR
G11.5	DALETIMTTKSVSFR
G11.6	DALESI I TTKSVSFR
G11.7	DALESIMTTKS I SFR

Proliferation by T cell clones[*]

APC	Peptide concentration	2C5[‡]				1C6		
		G11[§]	G11.3	G11.5	G11.7	G11.3	G11.5	G11.7
	μs/ml							
11 G → V	0.01	118	ND	2	1	2	28	25
	0.1	63	31	1	7	2	51	55
	1.0	91	26	2	74	28	86	92
13 Y → H	0.01	2	7	2	3	7	71	57
	0.1	3	74	1	47	42	82	67
	1.0	4	101	33	112	88	98	86
60 S → Y	0.01	19	3	2	2	1	15	6
	0.1	31	32	3	41	2	58	57
	1.0	38	64	46	87	18	91	88

[*] Proliferation induced by each transfectant expressing a mutant DR7β1 chain is expressed as a percentage of the proliferation induced in the same experiment by the transfectant expressing the DR7β1 wild-type molecule.
[‡] T cell clone.
[§] Peptide.

DR7β1 mutants to present the different peptides. Although the G11.3 peptide sequence is contained within the G11 sequence, G11 and G11.3 were recognized differently by clone 2C5 in the context of the position 11 and 13 DR7β1 mutants. It is important to note that the molar dose–response curves for presentation of G11 and G11.3 by the wild-type DR7 molecule to clone 2C5 are indistinguishable (data not shown). Peptide G11 was presented very efficiently by the 11 G → V mutant, whereas the presentation of G11.3 by this mutant was considerably diminished compared with the wild-type. In contrast, the 13 Y → H mutant, which presented the G11.3 peptide (1.0 μg/ml) equivalently to the wild-type, was unable to present the G11 peptide to clone 2C5. The additional COOH-terminal amino acids in G11, compared with G11.3, may interfere with binding of this peptide to the position 13 mutant, but on the other hand, may enhance binding to the position 11 mutant.

Comparison of the abilities of the mutant DR7 molecules to present the G11.3, G11.5, and G11.7 peptides that differ only by single amino acid substitutions revealed several interesting findings (Table 4). Clone 1C6, which proliferated at only 28% of that induced by the wild-type molecule in response to the G11.3 peptide (1 μg/ml) presented by the

11 G → V mutant, responded to peptides G11.5 and G11.7 presented by this mutant at 86 and 92% of that induced by the wild-type molecule, respectively. The 60 S → Y mutant had a similar pattern with clone 1C6: negligible presentation of G11.3 and very efficient presentation of G11.5 and G11.7. The 13 Y → H mutant presented both the G11.3 and G11.7 peptides (1 μg/ml) to clone 2C5 as efficiently as the wild-type molecule. However, the ability of the position 13 mutant to present peptide G11.5 to clone 2C5 was significantly reduced. In contrast, all three peptides were presented efficiently by the position 13 mutant to clone 1C6 at peptide concentrations of 1 μg/ml.

Discussion

The data presented here indicate that multiple amino acids, located in both the β-strands and α-helix of DR7β1 in the class II model (9), are involved in DR7-restricted T cell recognition of rabies virus antigens. When transfectants expressing the mutant DR7 molecules were used as APC, each of the five rabies virus–specific T cell clones had a different reactivity pattern, emphasizing the diversity of the DR7 molecule/antigenic peptide/TCR trimolecular complexes involved.

The effects of some substitutions were highly selective. For example, at the highest antigen concentrations, the position 13 mutant induced proliferation by the three G-specific and one of the N-specific T cell clones that was 75% or greater of that induced by the wild-type molecule, but was unable to present N to clone 1C9 at any antigen concentration. The position 60 substitution had the most negative effect on the ability of the DR7 molecule to present antigens to the clones. The conservative 71 R → K substitution had little effect on the recognition of the G peptide G11.3 by clones 2C5 and 1C6, but it completely eliminated recognition of N by clones 1C9 and 1B8. Taken together, the results are consistent with the predictions of the class II model that the amino acids analyzed are important in peptide binding or TCR interactions.

Three aspects of the experimental system used in these studies should be emphasized. First, the amino acids substituted in the DR7β1 chain were those found at the same positions in another DRβ1 chain, Dw4β1. Second, the transfectants expressing wild-type or mutant DR7 molecules that were used as APC in these studies expressed comparable levels of DR7 molecules based on indirect immunofluorescence and flow cytometry. Finally, the molecules containing the mutant DR7β1 chains did not seem to have grossly altered conformations. 10 of the 11 mutant DR7 molecules induced proliferation by one or more of the rabies virus–specific T cell clones that was equivalent to that induced by the wild-type molecule, and the position 60 mutant induced proliferation by two of the clones that was >50% of that induced by the wild-type. Moreover, in an allospecific system, each of the mutant molecules was able to induce proliferation by at least one DR7-allospecific clone that was comparable to that induced by the wild-type molecule (Rosen-Bronson, S., W. Yu, and R. W. Karr, manuscript submitted for publication). Therefore, this experimental system permits a reasonable analysis of the structure-function relationships of DR7 molecules.

In the class II model, the amino acids at positions 11, 13, 30, and 37 in the β-strands on the floor of the peptide binding groove and the amino acids at positions 57, 60, 67, 71, 74, and 78 along the α-helix are predicted to be involved in peptide binding (9). However, because of their positions, it is also possible that some or all of these residues on the α-helix interact directly with TCR. Substitutions at each of these positions, except position 57, in the DR7β1 chain reduced or eliminated the ability of each mutant molecule to present antigen to at least one of the T cell clones, indicating that the amino acids at these positions in the wild-type DR7β1 chain contribute to antigen-specific T cell recognition. In a previous study by Mellins et al. (23), a substitution in the DR3β1 chain at position 9, which is also predicted to be located on a β-strand and to be involved in peptide binding, caused loss of DR3-restricted antigen presentation. Based on the class II model, the predicted functional role of the amino acid at position 70 is less clear. The orientation of the side chain of the amino acid at position 70 is predicted to be up and in toward the top of the peptide binding site. This predicted upward orientation is in contrast to most of the

other putative peptide binding residues on the α-helix, whose side chains are predicted to point in toward the site. Therefore, this residue is a strong candidate for direct interaction with TCR or antibody. However, because of the predicted orientation of the side chain, it is also possible that, in some cases, residue 70 is involved in peptide binding. Indeed, the dose-response with the position 70 mutant in the presentation of the G11.3 peptide to clone 1C6 suggests that position 70 is involved in peptide binding in this case. If residue 70 were involved solely in direct TCR interaction in the recognition of G11.3 by 1C6, a dose-related increase in the ability of the molecule to present antigen would not be expected. The position 70 substitution, however, eliminated or significantly reduced the ability of the DR7 molecule to present antigen to the other four clones. The position 70 substitution had a similar negative effect on DR7 allorecognition by five of six clones analyzed (Rosen-Bronson, S., W. Yu, and R. W. Karr, manuscript submitted for publication). Therefore, Asp at position 70 of DR7β1 appears to play a central role in DR7-restricted T cell recognition.

The results with the DR7β1 57 V → D mutant warrant comment in the context of predictions of the class II model. In many murine and human class II β chains, the Asp at position 57 is predicted to form a salt bridge with Arg at position 76 of class II α chains (9). Because the wild-type DR7β1 chain has Val 57, the wild-type DR7 molecule probably does not have a salt bridge at the right end of the peptide binding groove. The predicted presence of a salt bridge in the 57 V → D mutant might be expected to cause a significant change in the conformation of this mutant DR7 molecule, compared with the wild-type DR7 molecule. Surprisingly, however, the position 57 mutant efficiently presented antigen to each of the G- or N-specific clones at the highest antigen concentrations. These results suggest that the 57 V → D substitution has not caused a major conformational change in the molecule and that the reduced stimulation of the G-specific clones at 0.01 μg/ml of the G11.3 peptide may reflect subtle changes in peptide affinity, as noted below. These data may also be interpreted to indicate that the substituted Asp 57 in the mutant DR7β1 chain does not participate in the formation of a salt bridge, perhaps because of structural constraints imposed by sequences elsewhere in the chain.

One striking finding with presentation of the G11.3 peptide to the three G-specific clones is that, at the highest peptide concentration, many of the mutant DR7 molecules induced proliferation by one or more clones equivalent to that induced by the wild-type molecule, whereas at the lowest peptide concentration, these mutant molecules were unable to induce significant proliferation by the same clones. This phenomenon was observed with the substitutions at positions 11, 13, 30, 57, 67, 70, 74, and 78. For example, at a G11.3 concentration of 1.0 μg/ml, the position 13 mutant induced proliferation by clones 1C10, 2C5, and 1C6 that was 182, 101, and 88% of that induced by the wild-type DR7 molecule, respectively. In contrast, at a peptide concentration of 0.01 μg/ml, this mutant induced proliferation by these clones that was only 9, 7, and 7% of that induced by the

wild-type, respectively. These data suggest that each of the substitutions at positions 11, 13, 30, 57, 67, 70, 74, and 78 has reduced the affinity of G11.3 for the mutant DR7 molecules. Presumably, on both wild-type and mutant DR7 molecules, the G11.3 peptide must displace endogenous or exogenous peptides that are already bound to the DR7 molecule when G11.3 is added to the proliferation assay. At a low peptide concentration, G11.3 may not compete effectively for binding because of the decreased affinity due to the substitutions in DR7β1, resulting in the inability of these mutant molecules to present the peptide to any of the T cell clones. However, at high peptide concentration, the equilibrium may be shifted in favor of G11.3 binding, in spite of the reduced affinity. In addition, the substitutions may also affect the affinities of the peptides already bound to DR7 molecules, perhaps making their displacements more difficult. It is noteworthy that this marked contrast between inability to present antigen at low peptide concentration and presentation equivalent to the wild-type molecule at high peptide concentration was noted only with the recognition of the G11.3 peptide by the three G-specific clones; this phenomenon was not noted with presentation of N by the mutant molecules.

Considerable heterogeneity of responses of the three G-specific T cell clones to presentation of the G11.3 peptide by several of the mutant DR7 molecules was observed. For example, at a peptide concentration of 1.0 μg/ml, the position 30 mutant induced proliferation by clones 1C6 and 1C10 that was equivalent to or greater than that induced by the wild-type molecule but was unable to present G11.3 to clone 2C5 at any peptide concentration. Similar heterogeneous responses of these T cell clones were observed with the position 11, 67, 70, and 78 mutants. This heterogeneity of T cell response patterns indicates that at least two, and probably three, distinct T cell recognition determinants are generated by the G11.3 peptide and the DR7 molecule. Therefore, these three T cell clones appear to have different fine specificities for the same peptide/class II complex and to be differentially sensitive to several of the DR7β1 mutations. These results may be interpreted to indicate that the G11.3 peptide binds to the DR7 molecule in multiple conformations. The possibility that one peptide may bind to a class II molecule in more than one conformation has previously been suggested based on studies in the murine system (24, 25). In this construct, a given substitution in DR7β1 might alter or prevent the binding of G11.3 in one conformation, while having no effect on the binding of G11.3 in a second conformation. An alternative interpretation is that each TCR requires a different view of the G11.3/DR7 complex in which the peptide is bound in one conformation at a single site (26, 27). In this interpretation, a substitution in DR7β1 altered the conformation of the G11.3/DR7 complex required by the clone that does not recognize the complex, whereas the conformations required by the other clones were maintained. There-

fore, we envision that some antigenic peptides may bind to a given class II molecule in only one conformation at a single site, while others may bind in multiple conformations to the same molecule. It is important to note that the results indicate that the G11.3 peptide was bound to each of the mutant DR7 molecules; therefore, measurement of G11.3 binding to mutant DR7 molecules in a direct binding assay would not distinguish between these two interpretations.

The data with the three G peptides that differ by single amino acid substitutions offer some insights into the complex interactions that are required for the formation of a productive complex of class II, antigenic peptide, and TCR. As noted previously, each of these peptides was presented efficiently to clones 2C5 and 1C6 by the wild-type DR7 molecule. The position 13 mutant presented the G11.3, G11.5, and G11.7 peptides to clone 1C6 but presented only the G11.3 and G11.7 peptides to clone 2C5. Therefore, the combination of Tyr at position 289 of the peptide and the 13 Y → H substitution in DR7β1 prevented recognition by clone 2C5. The contrast between the efficient presentation of the G11.5 and G11.7 peptides to clone 1C6 by the position 11 and 60 mutants and the markedly reduced ability of these mutants to present G11.3 to this clone is also informative. The other seven mutants tested as APC for presentation of these peptides to 1C6 were each able to present all three peptides. In regard to the peptide, these data indicate that it is recognized with Ser at position 289 (G11.7) or with Val at position 296 (G11.5), but not when the peptide has both Ser 289 and Val 296 (G11.3). Therefore, the combination of Ser 289 and Val 296 in the peptide and either the 11 G → V or 60 S → Y substitution in DR7β1 prevents recognition of G11.3 by 1C6. This could result from the prevention of G11.3 binding by the substitutions, or if binding does occur, the inability of 1C6 to recognize the resulting DR7/G11.5 complexes.

The results of these studies emphasize the complexity of the interactions of class II molecules, antigenic peptides, and TCR and suggest that the diversity of these interactions is greater than previously appreciated. Because of this apparent complexity and the limitations of the methods of analysis, the results of studies such as these are often difficult to interpret. However, as the understanding of MHC-restricted, antigen-specific T cell recognition continues to be refined, the possibility that the same antigenic peptide is bound to a specific MHC molecule in more than one conformation must be considered. This diversity of the same peptide/MHC complex may be related to the observed heterogeneity of TCRs that recognize the same complex. Ultimately, determination of the three-dimensional structures of multiple MHC molecule/peptide/TCR complexes will be required to resolve these issues. Based on the results presented here, the trimolecular complexes of DR7/G11.3 peptide and the TCR of clone 2C5 or clone 1C6 would be excellent candidates for these studies.

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