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

By Robert W. Karr,* Wei-yuan Yu,* Rebecca Watts,* Karin S. Evans,† and Esteban Celis‡

From the *Department of Veterans Affairs Medical Center and the Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242; and †T Cell Sciences, Cambridge, Massachusetts 02139

Summary

The relative importance of 11 polymorphic positions in the HLA-DRβ1 chain in T cell recognition of foreign antigens was investigated using transfectants expressing mutant DRβ1 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 DRβ1 in the model of a class II molecule, are involved in DRβ-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 of 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 α1 and β1 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-DRβ-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-DRβ1 chain in T cell recognition of rabies virus antigens by using site-directed mutagenesis to produce 11 mutant DRβ1 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 DRβ1 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 DRβ1 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 DRβ1 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 DRβ1 → Dw4β1 substitutions at positions 11, 13, 30, 37, 57, 60, 67, 70, 71, 74, and 78 of DRβ1. 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 gap-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 DRβ1 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 by 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:DRβ1 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 irradiated 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) 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 DRβ1 chains with single amino acid substitutions were produced by site-directed mutagenesis. In each mutant, the residue found in the wild-type DRβ1 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, 61 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 DRβ1 chain or one of the mutant DRβ1 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 DRβ7 molecules were used as APC for 5 DRβ1-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 to clones 2C5, 1C6, and 1C10 when purified G or whole virus is used as the antigen source. Both PBMC and DRβ7-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

1 Abbreviations used in this paper: G, glycoprotein; N, nucleoprotein; RNP, ribonucleoprotein.
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 DR7 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 DR7/81 Chains

| APC | Peptide concentration | 2C5* | 1C6 | 1C10 | 2C5 | 1C6 |
|-----|-----------------------|------|-----|------|-----|-----|
|     |                       | G11.3| G11.3| G11.3| APC | G11.3| G11.3|

| Exp. 1 | µg/ml | cpm x 10^{-3} | Exp. 2 | cpm x 10^{-3} |
|--------|-------|----------------|--------|----------------|
| DR7/81 | 0     | 0.1            | DR7/81 | 1.0            |
|        | 0.01  | 19.4           | 1C6    | 3.6            | 2C5  | 4.3  |
|        | 0.1   | 25.8           |        | 13.7           | 1C10 | 6.4  |
|        | 1.0   | 22.9           |        | 17.6           | APC  | 4.8  |
| DR7/81 | 0     | 0.1            | DR7/81 | 0.2            |
|        | 0.01  | ND             | 11 G → V | 0.1            |
|        | 0.1   | 8.0            | 71 R → K | 0.3            |
|        | 1.0   | 6.0            |        | 9.9            | 74 Q → A | 4.9 |
| DR7/81 | 0     | 0.2            | DR7/81 | 0.2            |
|        | 13 Y → H | 0.01 | 1.4 | 0.2 | 0.4 | 74 Q → A | 0.3 |
|        | 1.0   | 23.2           |        | 15.5           | 15.1 |
| DR7/81 | 0     | 0.1            | Dw4/81 | 0.2            |
|        | 0.01  | 0.6            |        | 0.3            | 0.6  |
|        | 0.1   | 0.3            |        | 5.6            | 0.3  |
|        | 1.0   | 1.1            |        | 17.6           | 1.2  |
| DR7/81 | 0     | 0.2            | DR7/81 | 0.2            |
|        | 37 F → Y | 0.01 | 9.2 | 0.5 | 1.7 | 30 L → Y | 0.1 |
|        | 0.1   | 15.8           |        | 4.3            | 0.6  |
|        | 1.0   | 11.4           |        | 10.6           | 1.7  |
| DR7/81 | 0     | 0.1            | DR7/81 | 0.2            |
|        | 57 V → D | 0.01 | 3.6 | 0.3 | 1.5 | 78 V → Y | 0.1 |
|        | 0.1   | 28.5           |        | 6.6            | 0.8  |
|        | 1.0   | 31.6           |        | 16.7           | 3.2  |
| DR7/81 | 0     | 0.1            | DR7/81 | 0.2            |
|        | 60 S → Y | 0.01 | 0.5 | 0.0 | 0.8 | 70 D → Q | 0.1 |
|        | 0.1   | 8.3            |        | 0.2            | 1.2  |
|        | 1.0   | 14.7           |        | 3.2            | 1.0  |
| DR7/81 | 0     | 0.1            | DR7/81 | 0.2            |
|        | 67 I → L | 0.01 | 0.1 | 1.0 | 0.3 |
|        | 0.1   | 0.1            |        | 8.6            | 0.3  |
|        | 1.0   | 0.6            |        | 25.0           | 0.5  |
| DR7/81 | 0     | 0.1            | DR7/81 | 0.2            |
|        | 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  |
| DR7/81 | 0     | 0.2            | DR7/81 | 0.2            |
|        | 78 V → Y | 0.01 | 0.1 | 0.3 | 0.8 |
|        | 0.1   | 0.7            |        | 6.2            | 0.6  |
|        | 1.0   | 7.3            |        | 15.8           | 1.4  |
| Dw4/81 | 0     | 0.1            | Dw4/81 | 0.2            |
|        | 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

| APC    | Antigen concentration (RNP) | 1C9* | 1B8 | 1B8 | 13D | Antigen concentration (13D) | APC   |
|--------|-----------------------------|------|-----|-----|-----|-----------------------------|-------|
|        | µg/ml | cpm x 10^-3 | µg   | cpm x 10^-3 | µg |
| Exp. 1 |       |             |      |             |    |
| DR781  | 0     | 0.3         | 0.2  | 0.2         | 0  |
| Wild-type | 0.56 | 5.2         | 5.1  | 0.1         | 0.1 | 5.6 | 5.2 | 5.1 | 0.1 | 0.1 | 5.6 |
|         | 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 |
| DR781  | 0     | 0.1         | 0.2  | 0.2         | 0  |
| 11 G → V | 0.56 | 2.1         | 2.2  | 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 |
| DR781  | 0     | 0.3         | 0.2  | 0.2         | 0  |
| 13 Y → H | 0.56 | 0.3         | 10.2 | 0.1         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 0.9         | 19.4 | 0.1         | 10 | 0.2 | 0.3 | 0.1 |
| DR781  | 0     | 0.2         | 0.2  | 0.2         | 0  |
| 30 L → Y | 0.56 | 1.7         | 14.2 | 0.1         | 0.1 | 2.0 | 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 |
| DR781  | 0     | 0.3         | 0.4  | 0.3         | 0  |
| 37 F → Y | 0.56 | 0.9         | 11.3 | 0.4         | 0.1 | 74 Q → A | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 3.2         | 19.7 | 0.3         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 3.8         | 22.3 | 0.3         | 10 | 0.2 | 0.3 | 0.1 |
| DR781  | 0     | 0.3         | 0.2  | 0.2         | 0  |
| 57 V → D | 0.56 | 4.0         | 0.6  | 8.7         | 0.1 | 74 Q → A | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 4.0         | 21.1 | 25.4         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 14.6        | 25.4 | 20.8         | 10 | 0.2 | 0.1 | 0.1 |
| DR781  | 0     | 0.2         | 0.3  | 0.3         | 0  |
| 60 S → Y | 0.56 | 0.4         | 1.8  | 0.2         | 0.1 | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 3.4         | 7.7  | 0.3         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 4.3         | 13.9 | 0.1         | 10 | 0.2 | 0.1 | 0.1 |
| DR781  | 0     | 0.2         | 0.2  | 0.2         | 0  |
| 67 I → L | 0.56 | 0.3         | 1.8  | 0.2         | 0.1 | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 9.0         | 7.6  | 0.2         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 14.1        | 18.4 | 0.1         | 10 | 0.2 | 0.1 | 0.1 |
| DR781  | 0     | 0.1         | 0.3  | 0.3         | 0  |
| 70 D → Q | 0.56 | 0.2         | 0.3  | 0.2         | 0.1 | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 0.2         | 0.2  | 0.2         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 1.7         | 0.4  | 0.1         | 10 | 0.2 | 0.1 | 0.1 |
| DR781  | 0     | 0.4         | 0.4  | 0.4         | 0  |
| 78 V → Y | 0.56 | 0.3         | 0.2  | 0.3         | 0.1 | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 0.6         | 0.4  | 0.3         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 0.6         | 0.6  | 0.2         | 10 | 0.2 | 0.1 | 0.1 |
| Dw4β1  | 0     | 0.2         | 0.4  | 0.4         | 0  |
| 0.56   | 0.2   | 0.4         | 0.3  | 0.1         | 0.1 | 0.2 | 0.1 | 0.1 |
|         | 1.7   | 0.2         | 0.4  | 0.3         | 1.0 | 0.2 | 0.1 | 0.1 |
|         | 5.0   | 0.2         | 0.3  | 0.2         | 10 | 0.2 | 0.1 | 0.1 |

* T cell clone.
† Peptide 13D.
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, 60, 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 3. Summary of T Cell Responses to Rabies Virus Antigens Presented by Transfectants Expressing Mutant DR7β1 Chains**

| APC | 2C5 | 1C6 | 1C10 | 1C9 | 1B8 | 1B8 |
|-----|-----|-----|------|-----|-----|-----|
| DR7β1 | 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.
Table 4.  T Cell Responses to Peptides of Rabies Virus Glycoprotein Presented by Transfectants Expressing Mutant DR7β1 Chains

| Peptides | 283 | 323 |
|----------|-----|-----|
| G11      | CLDALESIMTTKSVSFRRLSHLRKLVPFGKAYTIFNKLTM |
| G11.3    | DALESIMTTKSVSFRR |
| G11.5    | DALETIMTTKSVSFRR |
| G11.6    | DALESI1TTKSVSFRR |
| G11.7    | DALESIMTTKSIIFR |

Proliferation by T cell clones*

| APC       | Peptide concentration | G11 | G11.3 | G11.5 | G11.7 |
|-----------|-----------------------|-----|-------|-------|-------|
| 11 G → V  | 0.01                  | 118 | ND    | 2     | 1     |
|           | 0.1                   | 63  | 31    | 1     | 7     |
|           | 1.0                   | 91  | 26    | 2     | 74    |
| 13 Y → H  | 0.01                  | 2   | 7     | 2     | 3     |
|           | 0.1                   | 3   | 74    | 1     | 47    |
|           | 1.0                   | 4   | 101   | 33    | 112   |
| 60 S → Y  | 0.01                  | 19  | 3     | 2     | 2     |
|           | 0.1                   | 31  | 32    | 3     | 41    |
|           | 1.0                   | 38  | 64    | 46    | 87    |

* Proliferation induced by each transfectant expressing a mutant DR7 β1 chain is expressed as a percentage of the proliferation induced in the same experiment by the transfectant expressing the DR7 β1 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 DR7 β1 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 DR7 β1 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 allore cognition 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
conformations of the G11.3/DR7 complex required by the clone interpretation, a substitution in DR701 altered the conformation of the G11.3/DR7 complex in which the peptide is native. The 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. This would mean that the 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.

We thank Vickie Weidner and Sherry Brown for preparation of the manuscript; and Charles Lutz, Sandra Rosen-Bronson, Andrea Sant, Jim Miller, and Peter Gregersen for helpful discussions.
References

1. Babbitt, B.P., P.M. Allen, G. Matsueda, E. Haber, and E.R. Unanue. 1985. Binding of immunogenic peptides to la histocompatibility molecules. Nature (Lond.) 317:359.

2. Buus, S., A. Sette, S.M. Colon, D.M. Jenis, and H.M. Grey. 1986. Isolation and characterization of antigen-Ia complexes involved in T cell recognition. Cell. 47:1071.

3. Ronchese, F., M.A. Brown, and R.N. Germain. 1987. Structure-function analysis of the A3m'12 mutation using site-directed mutagenesis and DNA-mediated gene transfer. J. Immunol. 139:629.

4. Davis, C.B., J.M. Buerstedde, D.J. McKean, P.P. Jones, H.O. McDevitt, and D.C. Wraith. 1989. The role of polymorphic I-A\(^β\) chain residues in presentation of a peptide from myelin basic protein. J. Exp. Med. 169:2239.

5. Davis, C.B., D.J. Mitchell, D.C. Wraith, J.A. Todd, S.S. Zamvil, H.O. McDevitt, L. Steinman, and P.P. Jones. 1989. Polymorphic residues on the I-A\(^β\) chain modulate the stimulation of T cell clones specific for the N-terminal peptide of the autoantigen myelin basic protein. J. Immunol. 143:2083.

6. Norton, F.L., C.B. Davis, P.P. Jones, and J.W. Goodman. 1989. Arsonate-specific murine T cell clones: V. antigen presentation by L cells transfected with normal and mutant class II genes. J. Immunol. 143:446.

7. Reske-Kunz, A.B., D. Landais, J. Peccoud, C. Benoist, and D. Mathis. 1989. Functional sites on the A\(α\)-chain: Polymorphic residues involved in antigen presentation to insulin-specific, A\(\alpha\):A\(\beta\)-restricted T cells. J. Immunol. 143:1472.

8. Rosloniec, E.F., L.J. Vitez, B.N. Beck, J.M. Buerstedde, D.J. McKean, C. Benoist, D. Mathis, and J.H. Freed. 1989. I-A\(^α\) polymorphisms define a functionally dominant region for the presentation of hen egg lysozyme peptides. J. Immunol. 143:50.

9. Brown, J.H., T. Jardetzky, M.A. Saper, B. Samraoui, P.J. Bjorkman, and D.C. Wiley. 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature (Lond.) 332:845.

10. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. Nature (Lond.) 329:506.

11. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature (Lond.) 329:512.

12. Okayama, H., and P. Berg. 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. Mol. Cell. Biol. 3:280.

13. Takeda, Y., M. Seiki, J.-I. Fujisawa, P. Hoy, K. Yokota, K.-I. Arai, M. Yoshida, and N. Arai. 1988. SRaI promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T cell leukemia virus type 1 long terminal repeat. Mol. Cell. Biol. 8:466.

14. Karr, R.W., P.K. Gregersen, F. Obata, D. Goldberg, J. Mac- cari, C. Alber, and J. Silver. 1986. Analysis of DR\(\beta\) and DQ\(\beta\) chain cDNA clones from a DR7 haplotype. J. Immunol. 137:2886.

15. Gregersen, P.K., M. Shen, Q.-L. Song, P. Merryman, S. Degar, T. Seki, J. Maccari, D. Goldberg, H. Murphy, J. Schwenzer, C.Y. Wang, R.J. Winchester, G.T. Nepom, and J. Silver. 1986. Molecular diversity of HLA-DR4 haplotypes. Proc. Natl. Acad. Sci. USA. 83:2642.

16. Barbosa, J.A., J. Santos-Aguado, S.J. Mentzer, J.L. Strominger, S.J. Burakoff, and P.A. Biro. 1987. Site-directed mutagenesis of class I HLA genes. Role of glycosylation in surface expression and functional recognition. J. Exp. Med. 166:1329.

17. Yu, W.-Y., R. Watts, and R.W. Karr. 1990. Identification of amino acids in HLA-DR4\(\alpha\) and DR3\(\beta\) chains that are involved in antibody binding epitopes using site-directed mutagenesis and DNA-mediated gene transfer. Hum. Immunol. 27:122.

18. Taylor, J.W., J. Ott, and F. Eckstein. 1985. The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA. Nucleic Acids Res. 13:8765.

19. Klohe, E.P., R. Watts, M. Bahl, C. Alber, W.-Y. Yu, R. Anderson, J. Silver, P.K. Gregersen, and R.W. Karr. 1988. Analysis of the molecular specificities of anti-class II monoclonal antibodies by using L cell transfectants expressing HLA class II molecules. J. Immunol. 141:12158.

20. Celis, E., O. Dewei, B. Dietzschold, and H. Koprowski. 1988. Recognition of rabies and rabies-related viruses by T cells derived from human vaccine recipients. J. Virol. 62:3128.

21. Celis, E., R.W. Karr, B. Dietzschold, W.H. Wunner, and H. Koprowski. 1988. Genetic restriction and fine specificity of human T cell clones reactive with rabies virus. J. Immunol. 141:2721.

22. Schneider, L.G., B. Dietzschold, R.E. Dierks, W. Mathaues, P.J. Enzman and K. Strohmaier. 1973. Rabies group specific ribonucleoprotein antigen and a test system for grouping and typing rabidoviruses. J. Virol. 11:748.

23. Mellins, E., B. Arb, P. Ochs, H. Erlich, and D. Pious. 1988. A single amino acid substitution in the human histocompatibility leucocyte antigen DR3 \(\beta\) chain selectively alters antigen presentation. J. Exp. Med. 168:1531.

24. Allen, P.M., G.R. Matsueda, E. Haber, and E.R. Unanue. 1985. Specificity of the T cell receptor: Two different determinants are generated by the same peptide and the I-A\(\alpha\) molecule. J. Immunol. 135:368.

This work was supported by the Department of Veterans Affairs Medical Research Funds and National Institutes of Health grants AI27214, AI24138, and DK25295.

Address correspondence to Dr. Robert W. Karr, Department of Internal Medicine, University of Iowa College of Medicine, 540A EMRB, Iowa City, IA 52242.

Received for publication 5 February 1990.
25. Bhayani, H., and Y. Paterson. 1989. Analysis of peptide binding patterns in different major histocompatibility complex/T cell receptor complexes using pigeon cytochrome c-specific T cell hybridomas. J. Exp. Med. 170:1609.

26. Cease, K.B., I. Berkower, J. York-Jolley, and J.A. Berzofsky. 1986. T cell clones specific for an amphipathic α-helical region of sperm whale myoglobin show differing fine specificities for synthetic peptides. J. Exp. Med. 164:1779.

27. Guillet, J.G., M.Z. Lai, T.J. Briner, J.A. Smith, and M.L. Gelfter. 1986. Interaction of peptide antigens and class II major histocompatibility antigens. Nature. 324:260.