Pocket 4 of the HLA-DR(α,β1*0401) Molecule Is A Major Determinant of T Cell Recognition of Peptide

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Summary

To investigate the functional roles of individual HLA-DR residues in T cell recognition, transfectants expressing wild-type or mutant DR(α, β1*0401) molecules with single amino acid substitutions at 14 polymorphic positions of the DRβ1*0401 chain or 19 positions of the DRα chain were used as antigen-presenting cells for five T cell clones specific for the influenza hemagglutinin peptide, HA307-19. Of the six polymorphic positions in the DRβ floor that were examined, mutations at only two positions eliminated T cell recognition: positions 13 (four clones) and 28 (one clone). In contrast, individual mutations at DRβ positions 70, 71, 78, and 86 on the α helix eliminated recognition by each of the clones, and mutations at positions 74 and 67 eliminated recognition by four and two clones, respectively. Most of the DRα mutations had minimal or no effect on most of the clones, although one clone was very sensitive to changes in the DRα chain, with loss of recognition in response to 10 mutants. Mutants that abrogated recognition by all of the clones were assessed for peptide binding, and only the β86 mutation drastically decreased peptide binding. Single amino acid substitutions at polymorphic positions in the central part of the DRβ α helix disrupted T cell recognition much more frequently than substitutions in the floor, suggesting that DRβ residues on the α helix make relatively greater contributions than those in the floor to the ability of the DR(α,β1*0401) molecule to present HA307-19. The data indicate that DRβ residues 13, 70, 71, 74, and 78, which are located in pocket 4 of the peptide binding site in the crystal structure of the DR1 molecule, exert a major and disproportionate influence on the outcome of T cell recognition, compared with other polymorphic residues.

The MHC molecules on the cell surface have evolved the remarkable capacity to bind and present to T lymphocytes an extremely large number of structurally diverse peptides. Recognition of the appropriate peptide–class II complexes by the antigen-specific TCR leads to CD4$^+$ T cell proliferation and a cascade of cellular immune responses. HLA class II molecules are highly polymorphic, and this structural polymorphism determines the distinct peptide-binding and antigen presentation characteristics of each molecule. However, the functional roles of individual residues in HLA class II molecules in peptide binding and T cell recognition have not been clearly defined. Elucidation of the ways in which class II residues interact with peptides and TCR may have important implications for understanding the function of the immune system, as well as for the treatment of immunologically mediated diseases.

Considerable progress has been made in the understanding of the structure of HLA class II molecules and the nature of class II–peptide interactions in recent years. Based on the crystal structure of HLA-A2 and the homologies between class I and II molecules, Brown et al. (1) proposed a structural model for class II molecules. The HLA-DR1 three-dimensional structure indicates that this model is generally accurate (2). In the initial DR1 crystal structure, the first domains of the α and β chains form an antigen-binding groove composed of two α helices that rise above a floor of β strands. Importantly, the side chains of most of the polymorphic residues in the three hypervariable regions of the DRβ chain point in toward the groove, where peptides are bound. At the level of the peptide molecule, several studies have defined residues crucial for binding to MHC and/or T cell recognition (3–6). Based on the relative spacing of such residues, it was concluded that peptide antigens were most likely to

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bind in an extended conformation. On the basis of results obtained by use of a combination of site-directed mutagenesis, direct peptide-binding studies, and single amino acid replacements in the peptide, Krieger et al. (7) described a model of peptide–class II interactions in which the peptide was bound in an NH_2 to COOH orientation and in a fully extended conformation. The recently solved structure of the complex of DR1 and an antigenic peptide of influenza hemagglutinin (HA307-19) permits precise identification of the DR1 residues that make contacts with this peptide (8) and indicates that the model of peptide–class II interactions proposed by Krieger et al. (7) is remarkably accurate. However, structural data alone cannot define the relative functional importance of each DR residue whose side chain contacts peptide or is positioned such that it may interact with TCR. Further progress in the understanding of the contribution of individual class II residues to peptide binding and T cell recognition will require integration of functional data from analysis of class II mutants with structural data from additional class II–peptide complexes and, perhaps, class II–peptide–TCR complexes. Although future determination of the structure of class II–peptide–TCR complexes may permit resolution of some of the current ambiguities in the interpretation of structure–function studies, the structure of the trimolecular complex alone will not define the relative functional importance of individual class II residues that contact TCR or peptide.

In many previous studies of the structure–function relationships of MHC class II molecules, mutagenesis has been used to produce individual or multiple interallelic substitutions between two closely related alleles that differ by a few residues (9–11). For example, the contributions of individual I-Eβ residues to T cell responses were investigated by making mutations in Eβ^k, Eβ^b, and Eβ^s at positions 29, 72, 75, and 79, which correspond to DRβ positions 28, 71, 74, and 78 (1, 9). Other studies analyzed the effects of mutations at larger numbers of positions in class II α (12) or β (7, 13) chains. However, these previous studies still did not address the functional roles of other residues predicted to contribute to peptide binding and T cell recognition, because only a limited set of substitutions was analyzed and, most importantly, the contribution of only a single chain (α or β) was investigated. In the current study, we have taken a broader approach by selecting for mutation 14 positions in the β chain and 19 positions in the α chain of a DR molecule. We selected for mutagenesis all those polymorphic DRβ positions that were predicted in the class II model to point into the peptide-binding groove: positions 9, 11, 13, 28, 30, and 37 in the floor and positions 57, 60, 67, 70, 71, 74, 78, and 86 on the α helix (1). We wondered whether the residue present at each of these polymorphic positions in the floor and α helix was equally likely to play an important role in T cell recognition of the class II–peptide complex. The DRα positions analyzed included 6 in the floor and 7 on the α helix that were predicted in the model to point into the binding groove, and 6 on outer loops. This mutagenesis approach permits assessment of the functional involvement of individual residues, as well as comparisons of the contributions of residues in the floor versus those on the α helix of each chain.

The objective of this study was to analyze the involvement of 33 individual residues of the DR(α,β1*0401) molecule in T cell recognition and peptide binding. Importantly, this molecule is associated with susceptibility to the development and severity of rheumatoid arthritis (14–16). Transfectants expressing wild-type or mutant DR(α,β1*0401) molecules were used as APC for five DR(α,β1*0401)-restricted T cell clones specific for HA307-19, a peptide of influenza hemagglutinin that can be bound and presented by multiple DR molecules. We found that mutations at six of eight positions on the β chain α helix, but only two of six positions in the β chain floor, affected T cell recognition. The α chain mutations had minimal or no effects on most of the clones. The data demonstrate that T cell recognition of the DR(α,β1*0401)-HA307-19 complex is much more sensitive to substitutions on the β chain α helix than to those in the β chain floor. In addition, DRβ residues 13, 70, 71, 74, and 78, which are located in pocket 4 in the DR1 molecule (8), exert a major and disproportionate influence on T cell recognition of the peptide/DR complex, compared with other polymorphic residues.

Materials and Methods

cDNA Clones and Site-directed Mutagenesis. cDNA clones encoding full-length HLA-DR α or β chains in the pcD expression vector (17) were used: DRA (18), DRB1*0401, DRB1*0402, DRB1*0403, DRB1*0404, DRB1*0405 (obtained from Peter Gregersen, North Shore University Hospital, Manhasset, NY) (19), and DRB1*0101 (American Type Culture Collection [ATCC], Rockville, MD) (20). Site-directed mutagenesis of the DRA and DR(B1*0401) cDNAs was performed by the PCR overlap extension method (21), and the mutants were sequenced to confirm the presence of the mutation and the absence of miscorporation as described (22).

Transfection and mAbs. The mutant DRA or DRB cDNA was cotransfected with the complementary wild-type DRB or DRA cDNA into the DAP.3 subclone of class II$^-$ murine L fibroblasts as described (23). The transfectants were cloned by limiting dilution or flow cytometric sorting, and cloned transfectants were used in T cell proliferation and peptide-binding assays. The class II expression levels of the transfectants were monitored by FACS® analysis (Becton Dickinson & Co., Mountain View, CA) with the anti-DR mAb L243 (ATCC) (24), or HU-20 (obtained from Akemi Wakisaka, Hokkaido University School of Medicine, Sapporo, Japan) (25) for those transfectants whose L243 epitope was disrupted by the DRα mutations (26) (Table 1). Antibody binding to selected transfectants was also analyzed with the polymorphic anti-DR mAb AC.BM.50 (Dominique Charron, Institut Biomedical des Cordeliers, Paris, France) (27), 21r5 (Michelle Letarte, Hospital for Sick Children, Toronto, Canada) (28), and the anti-DR4 mAb GS359-13F10 (Susan Radka, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA) (29).

T Cell Clones and T Cell Proliferation Assay. Five influenza hemagglutinin peptide HA307-19–specific T cell clones were isolated from PBL of three DR(α,β1*0401)$^+$ individuals as described (7). Briefly, HA307-19–specific and DR(α,β1*0401)-restricted T cell lines were established by incubating the PBL in vitro with HA307-19, and expanded with recombinant IL-2 (Genzyme Corp., Cambridge, MA). The T cell lines were cloned by limiting dilu-

tion in the presence of IL-2 and PHA. For the T cell proliferation assays, 2×10^4 irradiated L transfectants were incubated with HA307-19 at concentrations of 0.01, 0.1, and 1.0 μM in round-bottom 96-well plates at 37°C in 5% CO_2 overnight, and then the supernatant was removed before adding 5×10^4 quiescent T cells. After incubation for 2 d, the culture was pulsed with 0.5 μCi [^{3}H]TdR (DuPont, Boston, MA) per well. After further incubation for 16 h, the cells were harvested by use of a cell harvester (Tomtec, Orange, CT), and the [^{3}H]TdR incorporation was determined by liquid scintillation counting by use of a scintillation counter (LKB-Wallac model 1205 Betaplate; LKB-Pharmacia, Finland). DAP.3 cells transfected with the neomycin resistance gene only (DAPneo) were used as a negative control. Transfectants expressing wild-type DR(α,β1*0401) at high (Dw4H), medium (Dw4M), and low (Dw4L) levels were used as positive controls.

Peptide-binding Assay. HA307-19 (PKYVKQNTLKLAT) was synthesized in the Department of Medicinal Chemistry, G. D. Searle & Co., with an automated peptide synthesizer (Applied Biosystems, Inc., Foster City, CA) with standard Boc-amino acid coupling protocols (30). The peptide resin was biotinylated by coupling with succinimidyl-6-(biotinamido) hexanoate (Pierce Chemical Co., Rockford, IL) and purified by reverse-phase HPLC (Delta-Pak C-18; Waters, Milford, MA). The composition of the peptide was verified by amino acid analysis and fast-atom bombardment mass spectroscopy. For the peptide-binding assay (31), 3×10^5 transfectants were incubated with 10 μM biotinylated HA307-19 (B-HA)[1] overnight at 37°C in a V-bottom 96-well tissue culture plate, washed three times with 1% PBS 0.02% BSA sodium azide, and incubated with streptavidin–quantum red conjugate (Sigma Chemical Co., St. Louis, MO) for 30 min. After three additional washes, the cells were analyzed on a FACScan® (Becton Dickinson & Co.). The biotinylated gp41 582-595 (B-GP) from HIV-1 envelope protein, which is presented by the DPw4.2 molecule (32), was used as a negative control. The data were collected and analyzed to obtain the mean channel fluorescence (MCF) on a linear scale for each sample by use of data analysis programs (Lysis II; Becton Dickinson & Co.). The MCF of the biotinylated peptide binding was normalized to that of L243 binding as a measure of DR expression level on each transfectant.

Dw4 Model. To better correlate the mutation data with three-dimensional structure, a homology model of DRβ1*0401 was constructed by use of the coordinates (provided by Jerry Brown, Harvard University, Cambridge, MA) of the 3.2-Å x-ray structure of DRβ1*0101 (2) as the template. The two protein sequences differ at 17 positions; there are no residue insertions or deletions. The DRβ1*0401 substitutions were imposed on the DRβ1*0101 template by following the procedures of Summers and Karplus (33, 34) using the program CHARMM (35) and parameter sets detailed elsewhere (35–38). The resulting model structure is geometrically, statistically, and energetically indistinguishable from highly refined protein crystal structures solved at high resolution. The figures of the model were created with software (InsightII version 2.20; Biosym Technologies, San Diego, CA) on a workstation (IRIS 4D/310GTX; Silicon Graphics, Mountain View, CA).

Results

To analyze the contributions of individual residues in the DR(β1*0401) and DRα chains to recognition of HA307-19 by DR(α,β1*0401)-restricted T cell clones, 23 site-specific mutants of the DR(β1*0401) chain and 22 mutants of the DRα chain were produced and expressed with the complementary wild-type DRα or DR(β1*0401) chain on transfectants that were used as APC in T cell proliferation assays. The DRβ positions selected for mutagenesis were predicted in the class II model (1) to point into the peptide-binding groove. Importantly, most of these predictions were confirmed by the DR1 structures. In the DR1 crystal structures (2, 8) and our DR(α,β1*0401) structural model, DRβ residues 9, 11, 13, 28, 30, and 37 are located in one half of the floor of the antigen-binding groove with their side chains pointing up, and residues 57, 60, 67, 70, 71, 74, 78, and 86 are located on the α helix with their side chains pointing either in toward the groove (residues 57, 60, 71, 74, 78, and 86) or up and in (residues 67 and 70) (Fig. 1 *A*). Residues in the floor with their side chains pointing up are potential peptide contact residues, whereas residues on the α helixes may be peptide and/or TCR contact residues, depending on the orientation of their side chains, the angle at which the TCR approaches the class II–peptide complex, and the bound peptide.

The substitution strategy was to change the residue in the DR(β1*0401) chain to the residue found at the corresponding positions in other DR alleles (Table 1). In most cases, two substitutions were made at each position of DR(β1*0401). The first substitution at each position in the floor, plus those at positions 60 and 78 on the α helix, were made by changing the residue to the corresponding residue found in DR(β1*0701). For the substitutions at positions 57, 67, 70, 71, 74, and 86 on the α helix, the residue of DR(β1*0401) was changed to that found in other DR4 subtypes. To gain a broader picture of the functional roles of each residue, we also constructed a second set of substitutions with physical and chemical changes different than those of the first set of substitutions. For example, the DRβ position 28 mutations include both the conservative D → E substitution from DR(β1*0701) and the nonconservative D → H substitution from DR(β1*09011). Expression of the β30 Y → R and β57 D → V mutants was not obtained.

DRα chain mutations were made at residues 7, 9, 11, 22, 24, and 31, which are located in the other half of the floor of the groove with their side chains pointing up, and at other DRα residues located on the outer loops with their side chains pointing up (residues 15, 18, and 39), away (residues 36 and 42), or down (residue 19) (8). Additional DRα residues mutated are located on the α helix with their side chains pointing into the groove (residues 65 and 72), up (residue 49), up and in (residue 55), away (residues 47 and 50), or down toward the floor (residue 66). The DRα residues were changed to the residues found at the corresponding positions in the I-Eα, DPα, DQα, or I-Aα chain, as described (39).

The five HA307-19–specific T cell clones used in this study were isolated from three DR(α,β1*0401)-positive donors, and each proliferated in response to HA307-19 presented by the DR(α,β1*0401) transfectant. Because of the DRβ sequence similarities among the DR4 subtypes and DR1, we first investigated the restriction patterns of these clones by use of

[1] *Abbreviations used in this paper:* B-GP, B-HA, biotinylated gp41 582-595 and HA307-19, respectively; MCF, mean channel fluorescence.

Table 1. *Site-Directed Mutagenesis of DRα and DR(β1*0401) Chains*

DR(β1*0401) chain			DRα chain		
Position	Mutation	MCF ratio*	Position	Mutation	MCF ratio
9	E → W	1.34	7	I → S	0.66
9	E → Q	1.42	9	Q → G	0.85
11	V → G	0.88	11	E → A	0.84
11	V → S	1.45	15	N → L	0.93
13	H → Y	1.80	18	Q → K	1.50
13	H → F	0.95	19	S → R	1.91
28	D → E	1.82	22	F → Y	1.23
28	D → H	1.02	24	F → H	0.84
30	Y → L	1.91	31	I → E	0.75
37	Y → F	0.81	36	M → I	1.52
37	Y → S	1.42	39	K → S	1.26
57	D → S	1.46	42	V → I	1.70
60	Y → S	1.16	47	E → L	2.18
67	L → I	0.70	47	E → R	1.39
67	L → F	1.12	49	G → A	1.64
70	Q → D	1.40	50	R → K	1.52
70	Q → R	0.79	55	E → D	1.75
71	K → E	0.84	65	V → I	1.84
71	K → R	1.50	66	D → V	1.27
74	A → E	1.73	66	D → L	1.41
74	A → Q	0.84	66	D → R	0.89
78	Y → V	0.79	72	I → V	0.91
86	G → V	1.26	Dw4H	WT	1.38
Dw4H‡	WT	1.14	Dw4M	WT	(441)
Dw4M	WT	(570)	Dw4L	WT	0.51
Dw4L	WT	0.44			

* The ratio of averaged mean channel fluorescence (MCF) of Dw4H, Dw4L, and all of the mutants to that of Dw4M. The MCF for Dw4M is given in parentheses. Data are averaged from at least three experiments.
‡ Transfectants with high (H), medium (M), and low (L) expression levels of wild-type (WT) DR (α,β1*0401).

transfectants expressing other DR molecules. The DRβ1 chains associated with the five major DR4 subtypes (DRβ1*0401, 0402, 0403, 0404, and 0405) differ only among residues on the α helix, namely, β57, β67, β70, β71, β74, and β86 (Table 2). In the first domain, DR(β1*0101) differs from DR(β1*0401) at polymorphic positions in the floor and only at β71 on the α helix. Of the five clones, three are restricted only by DR(α,β1*0401), and the other two are also cross-reactive with DR(α,β1*0405).

T cell responses to the panel of DR(β1*0401) mutant transfectants are shown in Fig. 2. Overall, the T cell clones displayed five distinct reactivity patterns. Among the DR(β1*0401) mutations made in the floor, both position 13 mutations abolished or significantly reduced the recognition of HA307-19 by four clones. The β28 D → H mutation also drastically decreased recognition by clone 3BC6.6 only, whereas the conservative D → E mutation did not significantly affect T cell recognition by any of the clones. The responses of the five clones to the β position 9, 11, 30, and 37 mutants were comparable to the responses to the wild-type transfectants.

The mutations on the α helix of DR(β1*0401) affected T cell recognition of HA307-19 much more frequently, compared with those in the floor. At least one substitution at β positions 70, 71, 78, and 86 abolished recognition by all five of the T cell clones, and at least one of the position 74 mutants eliminated recognition by four of the clones. The β70 Q → R and B86 G → V mutations had the broadest effects, resulting in abrogation of recognition by all clones. In contrast, the β70 Q → D mutant presented efficiently to two clones. The conservative β71 K → R substitution induced significant proliferation by two of the clones, but abrogated or markedly reduced responses by the other three,

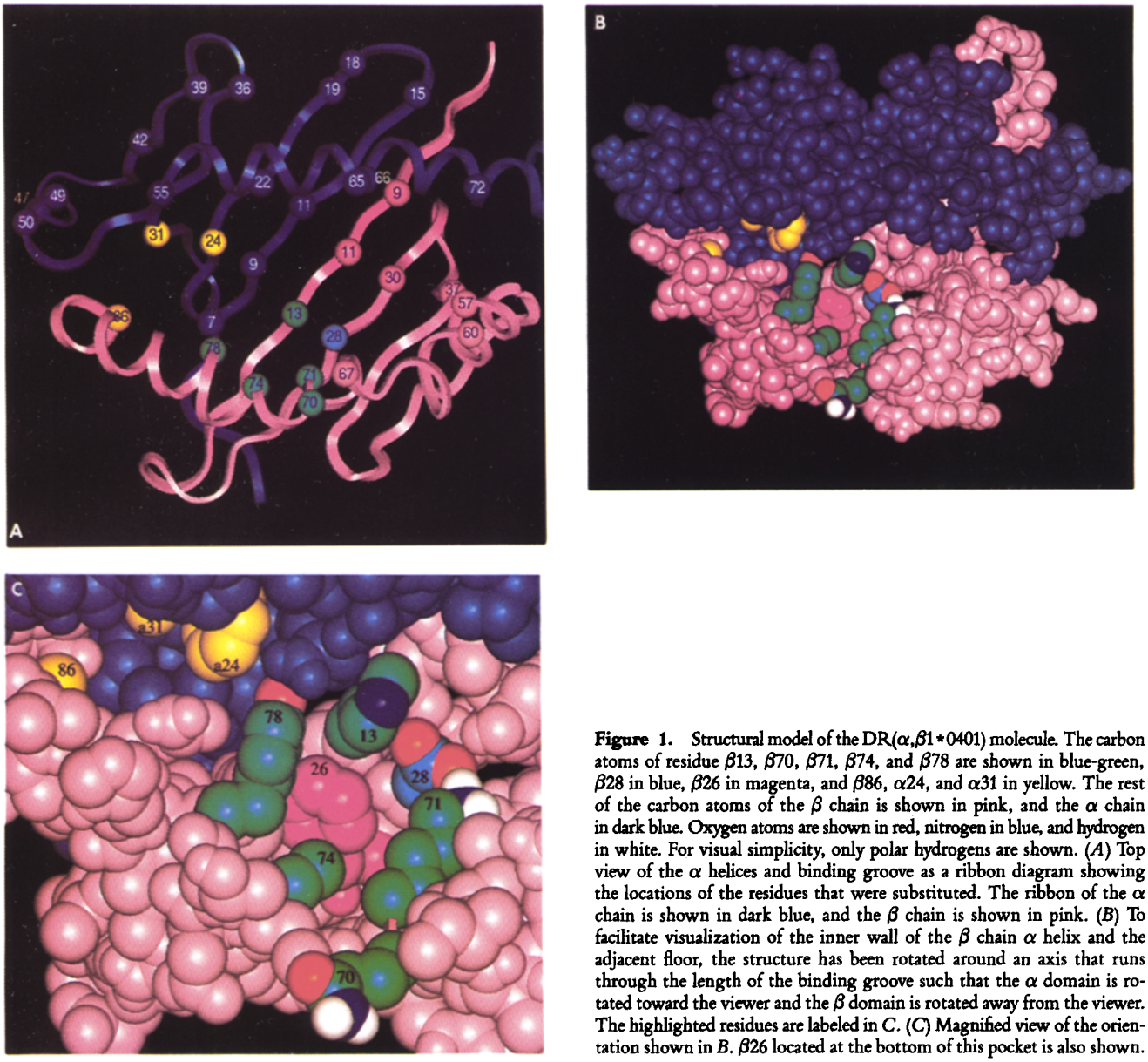

Figure 1. Structural model of the DR(α,β1*0401) molecule. The carbon atoms of residue β13, β70, β71, β74, and β78 are shown in blue-green, β28 in blue, β26 in magenta, and β86, α24, and α31 in yellow. The rest of the carbon atoms of the β chain is shown in pink, and the α chain in dark blue. Oxygen atoms are shown in red, nitrogen in blue, and hydrogen in white. For visual simplicity, only polar hydrogens are shown. (*A*) Top view of the α helices and binding groove as a ribbon diagram showing the locations of the residues that were substituted. The ribbon of the α chain is shown in dark blue, and the β chain is shown in pink. (*B*) To facilitate visualization of the inner wall of the β chain α helix and the adjacent floor, the structure has been rotated around an axis that runs through the length of the binding groove such that the α domain is rotated toward the viewer and the β domain is rotated away from the viewer. The highlighted residues are labeled in *C*. (*C*) Magnified view of the orientation shown in *B*. β26 located at the bottom of this pocket is also shown.

Table 2. *HLA-DR Restriction of T Cell Clones Specific for HA307-19*

	DRβ residues*						T cell recognition pattern‡				
HLA-DR	57	67	70	71	74	86	JB/Y/A2	JB/12B/A10	3BC6.6	KM1HA27	KM1HA28
DR(α,β1*0401)	D	L	Q	K	A	G	+	+	+	+	+
DR(α,β1*0402	–	I	D	E	–	V	–	–	–	–	–
DR(α,β1*0403)	–	–	–	R	E	V	–	–	–	–	–
DR(α,β1*0404)	–	–	–	R	–	V	–	–	–	–	–
DR(α,β1*0405)	S	–	–	R	–	–	–	+	+	–	–
DR(α,β1*0101)	–	–	–	R	–	–	–	–	–	–	–

* Only residues in the third hypervariable region and different among the alleles listed are shown.
‡ T cell proliferation assays were done at peptide concentrations of 0.01, 0.1, and 1.0 μM. For DR(α,β1*0401), + indicates robust proliferation, similar to that shown in Fig. 2 (Dw4H). For DR(α,β1*0405), + denotes that the dose–response curve resembles that of the prototype molecule, DR(α,β1*0401). – indicates flat dose–response curve comparable to the negative control shown in Fig. 2 (DAPneo).

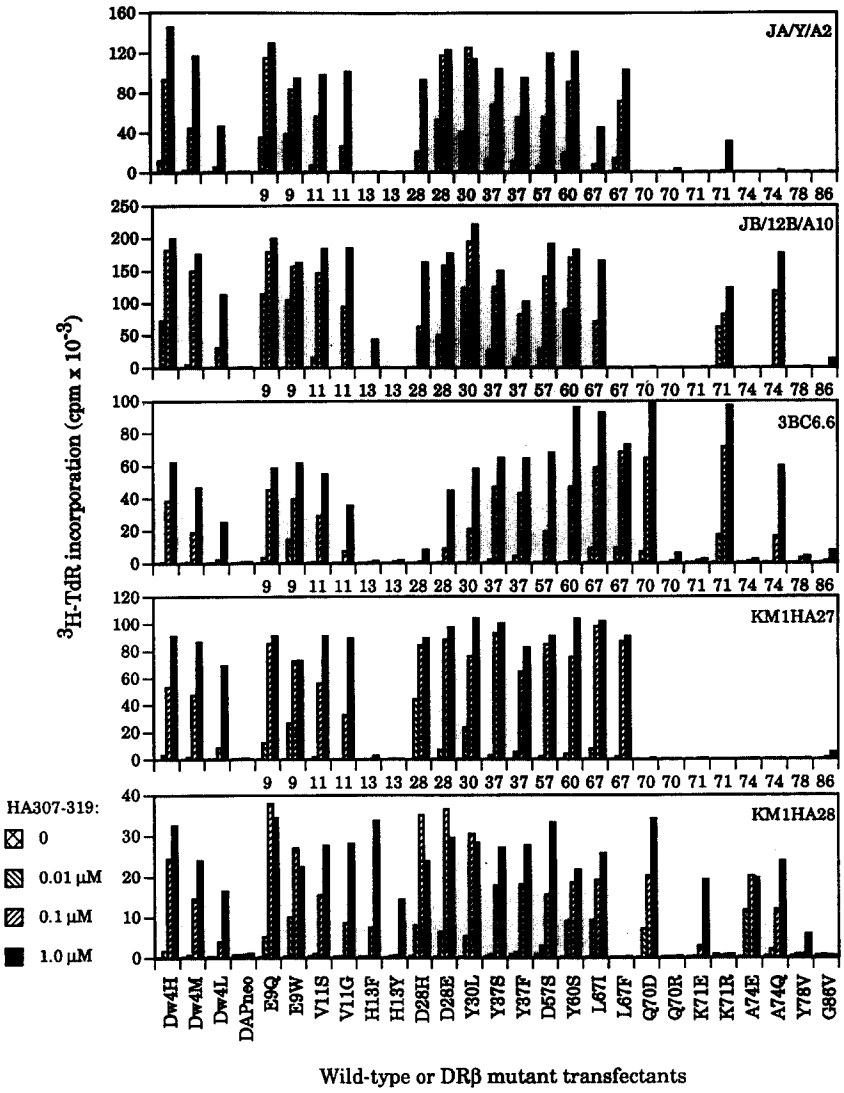

Figure 2. Effects of the DR(β1*0401) mutations on T cell recognition of HA307-19. The levels of ^{3}H[TdR] incorporation in T cell proliferation assays are shown as counts per minute on the y axis. Substitutions and/or the positions of the DR(β1*0401) chain are shown on the x axis. DAPneo, a class II$^-$ and G418-resistant L transfectant, was used as negative control. Transfectants expressing wild-type DR (α,β1*0401) at high (Dw4H), medium (Dw4M), and low (Dw4L) levels were used as positive controls.

whereas the nonconservative β71 K → E eliminated proliferation by four clones. The abilities of clones JB/12B/A10 and 3BC6.6 to recognize the β71 K → R mutant correlate well with the cross-reactivities of these clones with DR(α, β1*0405), which has arginine at β71 (Table 2). The β74 A → E and A → Q mutations eliminated the response by four and two clones, respectively. Only clone KM1HA28 gave a minimal response at the highest peptide concentration to the β78 mutant. At β67, the conservative L → I substitution reduced proliferation by only one clone, whereas the L → F substitution eliminated recognition by two clones. These data indicate that DRβ α helix residues 70, 71, 74, 78, and 86 play crucial roles in the presentation of HA307-19.

T cell responses to the DRα mutants are shown in Fig. 3. Overall, the five clones displayed five different reaction patterns with the DRα mutant panel. Recognition of HA307-19 by JB/Y/A2 and KM1HA28 was abrogated by one of the DRα chain mutations, and none of the DRα chain mutations abrogated recognition by JB/12B/A10. The single mutations at DRα positions 22, 24, and 31 and one of the multiple mutations at positions 47 and 66 abrogated or significantly decreased recognition by KM1HA27. The α11 mutation was the only one that abrogated recognition by JB/Y/A2. In contrast to the four clones described above, clone 3BC6.6 was much more sensitive to substitutions in the DRα chain; many of the DRα mutations reproducibly affected the recognition by this clone. Among the DRα mutants, the position 24 and 31 mutations abrogated or significantly decreased recognition by three clones and shifted the dose–response curve of the remaining two clones to higher peptide concentrations. In contrast to the DR(β1*0401) chain, each of the mutations made in the floor residues of DRα affected recognition of HA307-19 by at least one T cell clone.

The elimination of recognition by some mutations in the DRα and DR(β1*0401) chains may be due to inability of these mutants to bind the peptide. Substitutions at DRβ positions 13, 70, 71, 74, 78, and 86 and at DRα positions 24 and 31 caused major effects on the recognition of HA307-19

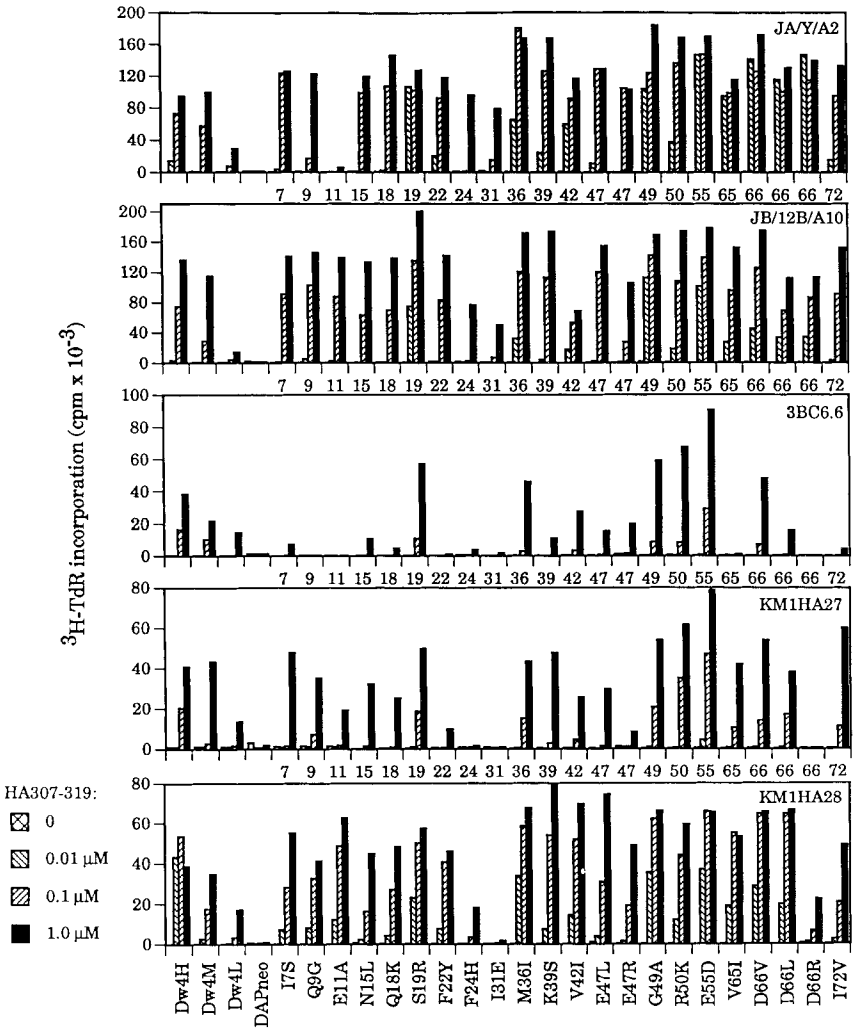

Figure 3. Effects of the DRα mutations on T cell recognition of HA307-19. Refer to Fig. 2 for legend.

by multiple T cell clones. In contrast to the β70 Q → R, 78 Y → R, 78 Y → V, and 86 G → V mutations that affected recognition by each of the five clones, the other mutants still presented the peptide efficiently to at least one T cell clone, documenting that these mutants were still able to bind the peptide. Binding of B-HA to the DR(β1*0401) 70 Q → R, 78 Y → V, and 86 G → V mutants was analyzed by flow cytometry (Fig. 4). Other mutants with major effects on recognition were also included for comparison. B-GP, which is a DP-binding peptide (32), and the DR(α, β1*0404) transfectant, which is a poor HA307-19 binder (40), were used as negative controls. The β86 G → V mutation was the only one that almost abolished the binding of HA307-19 to the DR(α, β1*0401) molecule. Interestingly, the α24 F → H substitution increased binding of the negative control peptide gp41 582-595 to the DR molecule, providing additional evidence that α24 interacts with peptides. The findings that most mutations did not prevent peptide binding are consistent with the DR1/HA peptide structure, in which hydrogen bonds between 12 nonpolymorphic and 3 polymorphic class II residues and main-chain atoms of the peptide make significant contributions to binding of the peptide (8). In this context, it is interesting to note that our mutations at α9, β57, and β71, which form such hydrogen bonds with the peptide in the DR1 structure, did not prevent peptide binding. The results of the T cell proliferation and peptide-binding studies are summarized in Table 3.

Because most of the mutants still presented the peptide efficiently to at least one T cell clone, it is unlikely that these mutants have gross conformational changes. To address the possibility that the broad effects on T cell recognition caused by the mutations at DR(β1*0401) positions 70, 78, and 86 were due to gross alteration of the DR(α,β1*0401) conformation, these mutants, as well as the DRβ position 13, 71, and 74 mutants and the DRα position 24 and 31 mutants, were analyzed with four polymorphic anti-DR mAbs. The HU-20, AC.BM.50, 21r5, and GS359-13F10 mAbs detect four distinct epitopes on DR4 molecules (41–43). All of the

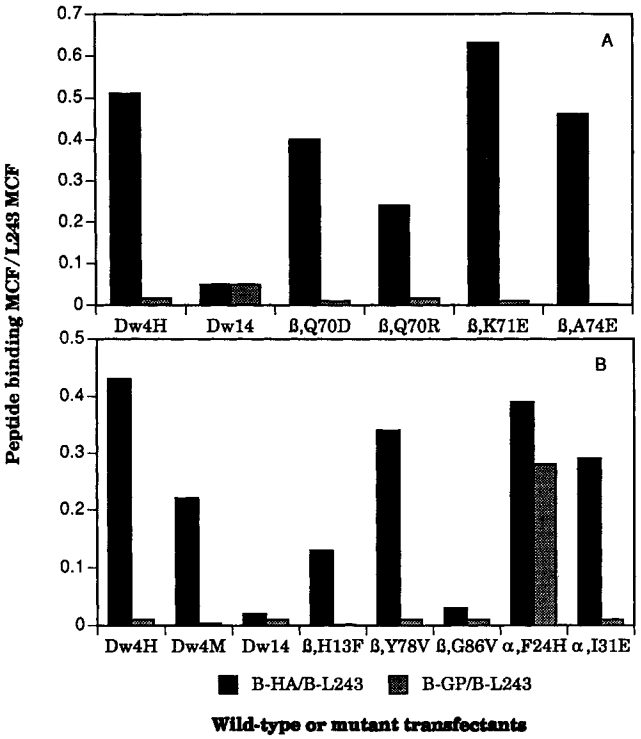

Figure 4. The binding of B-HA to transfectants expressing the wild-type DR(α,β1*0401) and the mutants. The MCF of a biotinylated peptide binding is normalized to that of L243 binding, which is shown on the y axis. The transfectants expressing DR(α,β1*0404) and B-GP were used as negative controls.

mutants still bound each of the polymorphic anti-DR mAbs at levels comparable to the wild-type DR(α,β1*0401) transfectant (data not shown), indicating that the mutations did not cause gross conformational changes in the DR(α,β1*0401) molecule.

Discussion

In these studies, single amino acid substitutions in the floor of DRβ1*0401 affected T cell recognition much less frequently than substitutions on the α helix, despite the fact that similar allelic substitution strategies were used for the floor and α helix. This discrepancy was surprising, based on the distribution of presumably functionally important polymorphic residue in both the β strands and the α helix of class II molecules. Similar results, with frequent effects on T cell recognition by substitutions at multiple positions on the DRβ chain α helix and infrequent effects by substitutions in the floor, were observed in studies of other DR molecules and antigen systems that used the same DRβ mutagenesis strategy: DR1-restricted recognition of pertussis toxin (13) and DR7-restricted recognition of rabies antigen and HA307-19 (7, 22). It is important to note that we have obtained similar results with DRβ mutants and T cells that recognize a promiscuous peptide, such as HA307-19, and a monogamous peptide, such as p30-42 of pertussis toxin. However, in these earlier studies, only one substitution was made at β floor positions 9, 11, 13, 28, 30, and 37. Because the nature of the substitution may affect the results, it is possible that the

Table 3. *Summary of Effects of DR(α,β1*0401) Mutants on T Cell Proliferation and Peptide Binding**

Position	Accessibility‡	Mutation	T cell proliferation§					Peptide binding‖
			JB/Y/A2	JB/12B/A10	3BC6.6	KM1HA27	KM1HA28	
β13	p	H → F	−	±	−	−	+	+
β13		H → Y	−	−	−	−	+	+
β70	p,t	Q → D	−	−	+	−	+	+
β70		Q → R	−	−	−	−	−	+
β71	p,t	K → E	−	−	−	−	+	+
β71		K → R	±	+	+	−	−	nd
β74	p	A → E	−	−	−	−	+	+
β74		A → Q	−	+	+	−	+	nd
β78	p	Y → V	−	−	−	−	±	+
β86	p	G → V	−	−	−	−	−	−
α24	p	F → H	+	+	−	−	±	+
α31	p	I → E	+	±	−	−	−	+

* Data shown in reduced form are from experiments shown in Figs. 2–4.
‡ The accessibility of a DR residue to peptide and TCR contacts is based on the DR1/HA307-19 structure (8). *p*, peptide contact; *t*, potential TCR contact.
§ +, Proliferation levels at peptide concentration 1.0 μM >30% of that with wild-type Dw4H; ±, proliferation <30%; −, proliferation close to that with the negative control (DAPneo).
‖ +, HA307-319 binding levels >30% of that with the wild-type Dw4H; −, binding levels close to that with the negative controls.
nd, not done.

paucity of effects on T cell recognition by substitutions in the floor in these previous studies was because the substitutions were too conservative. To address this possibility, two substitutions with distinct size and/or charge characteristics were introduced at five of the six positions in the floor of DRβ1*0401. However, only mutations at β floor residues 13 and 28 were found to affect T cell recognition.

What is the basis for the finding that T cell recognition was not affected by substitutions in the DRβ1*0401 chain at four of the six polymorphic residues whose side chains point up from the floor into the peptide binding groove? One possibility is that the substitutions at positions 9, 11, 30, and 37, which affected recognition by none of the clones, resulted in less substantial size, charge, and/or chemical characteristic changes than those made at positions 13, 28, 67, 70, 71, 74, 78, and 86, which eliminated T cell recognition by one or more clones. This explanation seems unlikely because at least one substitution at each of positions 9, 11, 30, and 37 represented a very significant change. For example, the position 9 E → W substitution replaced the negatively charged side chain of glutamic acid with the bulkier, nonpolar side chain of tryptophan, and the position 11 V → S and V → G substitutions replaced the nonpolar side chain of valine with the polar side chain of serine or the much smaller glycine. The absence of effects by the position 9, 11, 30, and 37 substitutions are in contrast to the marked effect of the highly conservative α helix β71 K → R substitution on three clones. Another possibility is that the side chains of residues 9, 11, 30, and 37 in the wild-type chain and/or the substituted residues do not contact the peptide. The absence of effects by substitutions at DRβ1*0401 residues 30 and 37 is consistent with the findings that these residues are not in contact with either the endogenous peptides or HA307-19 in the DR1 structures (2, 8) and suggests that the same is true when HA307-19 is bound to DR4. In contrast, however, the side chains of DRβ1*0101 residues 9 and 11 are in contact with the HA307-19 in the DR1 structure. The third possibility is that DRβ1*0401 residues 9, 11, 30, and/or 37 do make contacts with bound HA307-19, but the substitutions at these positions have little or no functional consequences because these interactions have no effect on the determinants recognized by the TCR.

Despite attempts to gather evidence to the contrary, we have consistently found the surprising result that T cell recognition of multiple DR–peptide complexes is more sensitive to interallelic substitutions on the α helix than to analogous substitutions in the floor, suggesting that, as a group, residues at polymorphic positions in the floor make lesser contributions to T cell recognition of a specific peptide than those on the α helix. These results underscore the necessity to complement structural data with in-depth functional analyses, in order to fully appreciate the relative functional roles of the different interactions revealed by crystallographic analysis. Polymorphic β residues in the floor are clearly important in the function of class II molecules. We envision that, in many cases, their contributions are operative more in determining the range of peptides that can be bound by a given molecule than in influencing the outcome of recognition of a peptide that has already been selected for binding, as in our analytical system. However, an example in which a class II β chain residue in the floor determined peptide binding has been reported (9).

What are the mechanisms by which the substitutions at DR(β1*0401) positions 13, 28, 67, 70, 71, 74, 78, and 86 eliminated T cell recognition? Depending on the location of the residue, the possibilities include (*a*) disruption of a critical TCR contact, (*b*) prevention of peptide binding, (*c*) change in the conformation of the bound peptide, or (*d*) change in the conformation of the DR(α,β1*0401) molecule. Each of the mutants except β86 G → V still bound the peptide, documenting that most residues other than β86 do not individually determine whether the peptide does or does not bind. The mutant molecules do not appear to have grossly altered conformations, as judged by the ability of each mutant to bind several mAbs and by the fact that each mutant, except 70 Q → R, 71 K → E, 78 Y → V, and 86 G → V, presented antigen efficiently to at least one clone.

The position 86 mutation probably affected T cell recognition by causing a marked decrease in the ability of the molecule to bind the peptide. These findings are consistent with previous reports showing that the dimorphism at position 86 of the DRβ chain is critical in peptide binding and T cell recognition (7, 40, 44–46). On the other hand, the natural dimorphism at β position 86 does not seem to be the only factor that determines the binding of HA307-19 to DR molecules. For example, DR(α,β1*1301) and DR(α,β1*1401) bind HA307-19 at high levels but have valine at position 86, and DR(α,β1*09011), which has glycine at position 86, binds HA307-19 poorly (47). These data suggest that polymorphic residues in other regions, in addition to several conserved residues (8), also contribute to the binding of HA307-19 by some molecules. In the DR1/HA307-19 structure, residues in a deep pocket in the peptide-binding site, consisting of β86 and α24, α31, α32, α43, β85, β89, and β90, contact tyrosine 309 of the peptide (8). This pocket is likely to be similar between DR(α,β1*0101) and DR(α,β1*0401), because their sequences in this region are identical. Interestingly, mutations at DRα positions 24 and 31 had major effects on T cell recognition but not on HA307-19 binding. Although these DRα residues were not essential for HA307-19 anchoring, they are likely to contribute to the conformation of the pocket and/or the anchored peptide. The spatial relationships of DRβ86, α24, and α31 are highlighted in Fig. 1, *B* and *C*.

Residue β70 played a crucial role in recognition by each of the clones studied. In the DR1/HA307-19 structure, DRβ1*0101 residue 70 glutamine contacts peptide residue 312 (glutamine) and is exposed such that it might also interact with TCR (8). In our DR(α,β1*0401) model, the side chain of β70 glutamine points up and in over the peptide-binding groove such that it might contact both peptide and TCR. In addition, antibody-binding data suggest that the side chain of DRβ1*0401 residue 70 is solvent accessible; the 70 Q→D mutation in β1*0401 eliminated binding of two mAbs but did not affect binding of several others (48). In the initial DR1 structure and our DR(α,β1*0401) model,

residue 67 is located at the highest point of the β chain α helix, making it a prominent feature of the top surface of the molecule. In contrast, residue β67 contacts peptide leucine 315 and is exposed for possible TCR interaction in the DR1/HA307-19 structure. Therefore, DRβ1*0401 residues 67 and 70 likely affected T cell recognition in our studies by altering peptide conformation and/or a critical TCR contact.

The side chains of β residues 13, 28, 71, 74, and 78 point into the peptide-binding groove in the DR1 structures and the Dw4 model (Fig. 1, *B* and *C*). The side chains of DRβ residues 13, 70, 71, 74, and 78, as well as α9, form a shallow pocket, referred to as pocket 4, which is occupied by peptide residue 312 glutamine in the DR1/HA307-19 structure (8). In addition, DRβ residue 28 makes contact with peptide residue 315 in the DR1/HA307-19 structure. The fact that DRβ1*0401 and 0101 differ only at positions 13 and 71 within pocket 4 suggests that the shape of this pocket in DRβ1*0401 is likely to be similar to that found in DRβ1*0101. In the DR1/HA307-19 structure (8), residue 13 is located in the floor of the peptide-binding groove below the peptide, indicating that it is not accessible to TCR contact. The side chains of residues 74 and 78 point down toward the peptide-binding groove and contact the peptide, but they were not felt to be exposed for potential TCR contact (8). In the context of these structural data, our findings that substitutions at DRβ1*0401 positions 13, 74, and 78 eliminated recognition by multiple clones, whereas peptide binding was preserved, indicate that these mutations most likely affected T cell recognition by altering the conformation of the bound peptide instead of by altering TCR contacts. Because β71 is also exposed in the DR1/HA307-19 structure, substitutions at this position may affect T cell recognition by altering peptide conformation and/or a TCR contact. The likelihood that the mutations at all or some of DRβ1*0401 residues 13, 28, 70, 71, 74, and 78 affected T cell recognition by altering peptide conformation are consistent with recent studies that indicate that TCR recognition of the class II–peptide complex is primarily determined by the TCR CDR3–peptide interaction (49). Taken together, the functional and structural data indicate that DRβ1*0401 residues 13, 70, 71, 74, and 78 in pocket 4 contribute to a functional unit that has a disproportionately large influence on the outcome of T cell recognition, compared with the other polymorphic positions. In the context of the functional data, the proximity and arrangement of residues 13, 70, 71, 74, and 78 in the Dw4 model are impressive (Fig. 1, *B* and *C*).

The DRα mutations had minimal or no effects on three of the clones, an intermediate effect on one clone, and widespread, significant effects on one clone. The DRα substitutions at positions 22, 42, 49, 50, 55, 65, and 72 are relatively conservative, which may contribute to the lack of effects on T cell recognition with these DRα mutants. Mutations at α24 and 31, which, as noted above, are located in the β86 pocket, affected the largest number of clones. The sensitivity of clone 3BC6.6 to 11 mutations scattered throughout DRα is noteworthy, especially in contrast to the reactivity patterns of the other four clones to the DRα mutants and the localized effects of the DR(β1*0401) mutants on 3BC6.6. Proliferation of 3BC6.6 was eliminated or reduced by each of the 6 mutations in the floor of DRα. Because these mutations did not prevent peptide binding, they probably caused changes in peptide conformation to which 3BC6.6 is exquisitely sensitive but which are tolerated by most of the other clones. Interestingly, mutations at α positions 15, 18, and 39 that affected recognition by this clone are located on outer loops away from the peptide-binding groove, raising the possibility that the area of contact of the TCR with class II may, in some cases, extend beyond the α helices.

Our findings that substitutions at multiple positions on the DRβ chain α helix frequently eliminated T cell recognition are consistent with previous studies in murine (11, 50, 51) and human (52, 53) systems that found that β chain α helix residues, especially those in the central portion of the helix, are important for recognition. However, the current study adds the important perspective that these DRβ chain α helix residues influence T cell recognition far more frequently than other highly polymorphic residues in the same chain. These residues, especially those in pocket 4, appear to be uniquely positioned to influence features of the class II–peptide complex that are required for T cell recognition by affecting peptide conformation and/or providing critical TCR contacts. Influences on peptide conformation by these β chain α helix residues may more frequently affect T cell recognition than those on the floor because the conformational effects are more pronounced at the surface of the peptide contacted by TCR.

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References

1. 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.).* 332:845–850.
2. Brown, J.H., T.S. Jardetzky, J.C. Gorga, L.J. Stern, R.G. Urban, J.L. Strominger, and D.C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature (Lond.).* 364:33–39.
3. Sette, A., S. Buus, S. Colon, J.A. Smith, C. Miles, and H.M. Grey. 1987. Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. *Nature (Lond.).* 328:395–399.
4. Sette, A., S. Buus, E. Appella, J.A. Smith, R. Chesnut, C. Miles, S.M. Colon, and H.M. Grey. 1989. Prediction of major histocompatibility complex binding regions of protein antigens by sequence pattern analysis. *Proc. Natl. Acad. Sci. USA.* 86: 3296–3300.
5. O'Sullivan, D., T. Arrhenius, J. Sidney, M.F. Del-Guercio, M. Albertson, M. Wall, C. Oseroff, S. Southwood, S.M. Colon, F.C.A. Gaeta, et al. 1991. On the interaction of promiscuous antigenic peptides with different DR alleles. Identification of common structural motifs. *J. Immunol.* 147:2663–2669.
6. Sette, A., J. Sidney, C. Oseroff, M.-F. del Guercio, S. Southwood, T. Arrhenius, M.F. Powell, S.M. Colon, F.C. Gaeta, and H.M. Grey. 1993. HLA DR4w4-binding motifs illustrate the biochemical basis of degeneracy and specificity in peptide-DR interactions. *J. Immunol.* 151:3163–3170.
7. Krieger, J.I., R.W. Karr, H.M. Grey, W.-Y. Yu, D. O'Sullivan, L. Batovsky, Z.L. Zheng, S.M. Colon, F.C. Gaeta, J. Sidney, et al. 1991. Single amino acid changes in DR and antigen define residues critical for peptide-MHC binding and T cell recognition. *J. Immunol.* 146:2331–2340.
8. Stern, L.J., J.H. Brown, T.S. Jardetzky, J.C. Gorga, R.G. Urban, J.L. Strominger, and D.C. Wiley. 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature (Lond.).* 368:215–221.
9. Racioppi, L., F. Ronchese, R. Schwartz, and R. Germain. 1991. The molecular basis of class II MHC allelic control of T cell responses. *J. Immunol.* 147:3718–3727.
10. Rosloniec, E.F., K.S. Beard, and J.H. Freed. 1993. Functional analysis of the antigen binding region of an MHC class II molecule. *Mol. Immunol.* 30:491–501.
11. Ronchese, F., M. Brown, and R. Germain. 1987. Structure-function analysis of the A^{bm12} mutation using site-directed mutagenesis and DNA-mediated gene transfer. *J. Immunol.* 139:629–638.
12. Dellabona, P., J. Peccoud, J. Kappler, P. Marrack, C. Benoist, and D. Mathis. 1990. Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell.* 62:1115–1121.
13. Olson, R.R., M.T. De Magistris, A. Di Tommaso, and R.W. Karr. 1992. Mutations in the third, but not the first or second, hypervariable regions of DR(β1*0101) eliminate DR1-restricted recognition of a pertussis toxin peptide. *J. Immunol.* 148: 2703–2708.
14. Stastny, P. 1978. Association of the B-cell alloantigen DRw4 and rheumatoid arthritis. *N. Engl. J. Med.* 298:869–871.
15. Weyand, C.M., K.C. Hicok, D.L. Conn, and J.J. Goronzy. 1992. The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. *Ann. Intern. Med.* 117:801–806.
16. Weyand, C.M., C. Xie, and J.J. Goronzy. 1992. Homozygosity for the HLA-DRB1 allele selects for extraarticular manifestations in rheumatoid arthritis. *J. Clin. Invest.* 89:2033–2039.
17. Okayama, H., and P. Berg. 1993. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. *Mol. Cell. Biol.* 3:280–289.
18. Karr, R.W., P.K. Gregersen, F. Obata, D. Goldberg, J. Maccari, C. Alber, and J. Silver. 1986. Analysis of DRβ and DQβ chain cDNA clones from a DR7 haplotype. *J. Immunol.* 137: 2886–2890.
19. Gregersen, P.K., M. Shen, Q.-L. Song, P. Merryman, S. Degar, T. Seki, J. Maccari, D. Goldberg, H. Murphy, J. Schwenzer, et al. 1986. Molecular diversity of HLA-DR4 haplotypes. *Proc. Natl. Acad. Sci. USA.* 83:2642–2646.
20. Tonnelle, C., R. DeMars, and E.O. Long. 1985. DOβ: a new β chain gene in HLA-D with a distinct regulation of expression. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2839–2847.
21. Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen, and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene.* 77:51–60.
22. Karr, R.W., W. Yu, R. Watts, K. Evans, and E. Celis. 1990. The role of polymorphic HLA-DRβ chain residues in presentation of viral antigens to T cells. *J. Exp. Med.* 172:273–283.
23. 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.* 141:2158–2164.
24. Lampson, L.A., and R. Levy. 1980. Two populations of Ia-like molecules on a human B cell line. *J. Immunol.* 125:293–299.
25. Ishikawa, N., M. Kasahara, H. Ikeda, K. Ogasawara, S. Hawkin, T. Takenouchi, A. Wakisaka, Y. Kikichi, and M. Aizawa. 1985. Demonstration of structural polymorphism among MB3 light chains by two-dimensional gel electrophoresis. *J. Immunol.* 134:417–422.
26. Fu, X.-T., and R.W. Karr. 1994. HLA-DRα chain residues located on the outer loops are involved in nonpolymorphic and polymorphic antibody binding epitopes. *Hum. Immunol.* 39: 253–260.
27. Piatier-Tonneau, D., M. Viguier, P. Debre, and D.J. Charron. 1984. Idiotypic relationships of 43 monoclonal antibodies to MHC class II antigens. *Dis. Markers.* 2:93–100.
28. Addis, J.B.L., R. Tisch, J.A. Falk, and M. Letarte. 1982. The analysis with monoclonal antibodies of the heterogeneity of Ia glycoproteins on chronic lymphocytic leukemia cells. *J. Immunol.* 129:2033–2039.
29. Radka, S.F., D.B. Amos, L.J. Quackenbush, and P. Cresswell. 1984. HLA-DR7-specific monoclonal antibodies and chimpanzee anti-DR7 serum detect different epitopes on the same molecule. *Immunogenetics.* 19:63–76.
30. Gross, E., and J. Meienhofer. 1979. The Peptides. Academic Press, New York. 484 pp.
31. Busch, R., G. Strang, K. Howland, and J. Rothbard. 1990. Degenerate binding of immunogenic peptides to HLA-DR proteins on B cell surfaces. *Int. Immunol.* 2:443–451.
32. Hammond, S.A., E. Obah, P. Stanhope, C.R. Monell, M. Strand, F.M. Robbins, W.B. Bias, R.W. Karr, S. Koenig, and R.F. Siliciano. 1991. Characterization of a conserved T cell epitope in HIV-1 gp41 recognized by vaccine-induced human cytolytic T cells. *J. Immunol.* 146:1470–1477.
33. Summers, N.L., and M. Karplus. 1991. Modeling of side chains, loops, and insertions in proteins. *Methods Enzymol.* 202A: 156–204.
34. Summers, N.L. 1989. Monsanto researchers apply CAMD methods in globular protein research. *Chemical Design & Au-*

tomations News. 4(1):12–17.
35. Brooks, B.R., R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, and M. Karplus. 1983. CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Computer Chemistry.* 4:187–217.
36. States, D.J. 1983. Nuclear magnetic resonance and theoretical studies of the refolding of bovine pancreatic trypsin inhibitor. Ph.D. thesis. Harvard University, Cambridge, MA. 414 pp.
37. Bruccoleri, R.E., M. Karplus, and A. McCammon. 1986. The hinge-bending mode of a lysozyme–inhibitor complex. *Biopolymers.* 25:1767–1802.
38. Reiher, W.E., III. 1985. Theoretical studies of hydrogen bonding. Ph.D. thesis. Harvard University, Cambridge, MA. 423 pp.
39. Panina-Bordignon, P., X.-T. Fu, A. Lanzavecchia, and R.W. Karr. 1992. Identification of HLA-DRα chain residues critical for binding of the toxic shock syndrome toxin superantigen. *J. Exp. Med.* 176:1779–1784.
40. Busch, R., C.M. Hill, J.D. Hayball, J.R. Lamb, and J.B. Rothbard. 1991. Effect of natural polymorphism at residue 86 of the HLA-DR β chain on peptide binding. *J. Immunol.* 147:1292–1298.
41. Fu, X.-T., W.-Y. Yu, C. Alber, C. Benson, R. Watts, H. Nordwig, J.P. Johnson, R.W. Knowles, and R.W. Karr. 1992. Identification of residues involved in polymorphic antibody binding epitopes on HLA-DR molecules. *Hum. Immunol.* 33:47–56.
42. Klohe, E., X.-T. Fu, M. Ballas, and R.W. Karr. 1993. HLA-DRβ chain residues that are predicted to be located in the floor of the peptide binding groove contribute to antibody binding epitopes. *Hum. Immunol.* 37:51–58.
43. Maurer, D., and J. Gorski. 1991. Transfer of polymorphic monoclonal antibody epitopes to the first and second domains of HLA-DR β-chains by site-directed mutagenesis. *J. Immunol.* 146:621–626.
44. Newton-Nash, D.K., and D.D. Eckels. 1993. Differential effect of polymorphism at HLA-DR1 β-chain position 85 and 86 on binding and recognition of DR1-restricted antigenic peptides. *J. Immunol.* 150:1813–1821.
45. Demotz, S., C. Barbey, G. Carradin, A. Amoroso, and A. Lanzavecchia. 1993. The set of naturally processed peptides displayed by DR molecules is tuned by polymorphism of residue 86. *Eur. J. Immunol.* 23:425–432.
46. Marshall, K.W., A.F. Liu, J. Canales, B. Perahia, B. Jorgensen, R.D. Gantzos, B. Aguilar, B. Devaux, and J.B. Rothbard. 1994. Role of the polymorphic residues in HLA-DR molecules in allele-specific binding of peptide ligands. *J. Immunol.* 152: 4946–4957.
47. Geluk, A., W. Bloemhoff, R.R.P. De Vries, and T.H.M. Ottenhoff. 1992. Binding of a major T cell epitope of mycobacteria to a specific pocket within HLA-DRw17 (DR3) molecules. *Eur. J. Immunol.* 22:107–113.
48. Drover, S., W.H. Marshall, G.T. Nepom, and R.W. Karr. 1994. Amino acids in the groove influence on antibody-defined, disease-associated HLA-DR epitope. *Scand. J. Immunol.* 39: 539–550.
49. Ehrich, E.W., B. Devaux, E.P. Rock, J.L. Jorgensen, M.M. Davis, and Y.-H. Chien. 1993. T cell receptor interaction with peptide/major histocompatibility complex (MHC) and superantigen/MHC ligands is dominated by antigen. *J. Exp. Med.* 178:713–722.
50. 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^k polymorphisms define a functionally dominant region for the presentation of hen egg lysozyme peptides. *J. Immunol.* 143: 50–58.
51. Lee, J.M., D.J. McKean, and T.H. Watts. 1991. Functional mapping of MHC class II polymorphic residues. *J. Immunol.* 146:2952–2959.
52. Barber, L.D., B. Bal, J.R. Lamb, R.E. O'Hehir, J. Yendle, R.J.T. Hancock, and R.I. Lechler. 1991. Contribution of T-cell receptor-contacting and peptide-binding residues of the class II molecule HLA-DR4 Dw10 to serologic and antigen-specific T-cell recognition. *Hum. Immunol.* 32:110–118.
53. Coppin, H.L., P. Carmichael, G. Lombardi, F.E. L'Faqihi, R. Salter, P. Parham, R.I. Lechler, and C. de Preval. 1993. Position 71 in the α helix of the DRβ domain is predicted to influence peptide binding and plays a central role in allorecognition. *Eur. J. Immunol.* 23:343–349.