HLA-DR Polymorphism Affects the Interaction with CD4

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Summary

Major histocompatibility complex (MHC) class II molecules are highly polymorphic and bind peptides for presentation to $CD4^+$ T cells. Functional and adhesion assays have shown that CD4 interacts with MHC class II molecules, leading to enhanced responses of $CD4^+$ T cells after the activation of the CD4-associated tyrosine kinase $p56^{lck}$. We have addressed the possible contribution of allelic polymorphism in the interaction between CD4 and MHC class II molecules. Using mouse DAP-3–transfected cells expressing different isotypes and allelic forms of the HLA-DR molecule, we have shown in a functional assay that a hierarchy exists in the ability of class II molecules to interact with CD4. Also, the study of DR4 subtypes minimized the potential contribution of polymorphic residues of the peptide-binding groove in the interaction with CD4. Chimeras between the DR4 or DR1 molecules, which interact efficiently with CD4, and DRw53, which interacts poorly, allowed the mapping of polymorphic residues between positions β180 and 189 that can exert a dramatic influence on the interaction with CD4.

Mature T lymphocytes are divided into two major subsets. $CD8^+$ T cells recognize nominal antigens in the context of MHC class I molecules, while $CD4^+$ T cells recognize antigen bound to the MHC class II molecules (1–3). Experimental evidence obtained from a variety of functional systems has shown that the interaction between CD8 and MHC class I molecules or between CD4 and MHC class II molecules leads to significant enhancement of T cell activation, probably by recruiting CD4- and CD8-associated tyrosine kinase $p56^{lck}$ to the vicinity of the TCR complex (4, 5). Adhesion assays have further confirmed the physical association of CD8 to MHC class I (6, 7) and CD4 to class II molecules (8). These assays have also been used to determine the molecular features of the interactions between these molecules. Polymorphism in the α3 domain of class I leads to severe perturbations of class I–restricted T cell responses (9), including positive and negative selection in the thymus (10). Mutagenesis analysis of class I further demonstrated that CD8 molecules interact with a highly conserved determinant in the α3 domain of MHC class I molecules (11). Similarly, CD4 interacts with a highly conserved region in the β2 domain of MHC class II molecules (12). Interestingly, this domain in class II bears significant homology with the above-mentioned domain in class I. However, very little is known about the effect of isotypic and allelic diversity of MHC class II molecules on their interaction with CD4.

MHC class II molecules are highly polymorphic. The presence of several class II isotypes (DR, DP, and DQ) further increases their diversity. As previously mentioned, polymorphism of MHC class I molecules affects their interaction with CD8. To evaluate the effect of MHC class II polymorphisms on the interaction with CD4, we have concentrated our efforts on HLA-DR molecules. These MHC class II molecules are all composed of the same monomorphic α chain, but their β chains can be encoded by four different isotypic genes, namely, the B1, B3, B4, and B5 genes (13). The DRB1 gene is the most polymorphic, while the products of the DRB3 and DRB4 can be coexpressed with several DRB1 alleles. The DRB5 product is only coexpressed in individuals bearing the DR2 haplotype. While most of these DR molecules present Ag to $CD4^+$ T cells, the majority of class II–restricted T cell responses has been shown to be restricted by products of the BI gene (14). However, peptides presented in the context of DR2 haplotype are predominantly restricted by the product of the B5 gene (15, 16). Such differences could be attributed to variations in the affinity of CD4 for these different MHC class II molecules. Indeed, MHC class I alleles that fail to interact with CD8 are inefficient in stimulating allo-

geneic and antigen-specific responses (9, 11). The possibility of such a hierarchy in the capacity of different HLA-DR molecules to interact with CD4 was assessed in an assay that specifically isolates the CD4–class II component in the interaction between effector T cells and APC. This assay was previously used to identify residues on CD4 that are involved in the interaction with MHC class II molecules (17–19). Our results clearly indicate that allelic polymorphism and isotypic diversity of HLA-DR molecules lead to variations in their capacity to interact with CD4.

Materials and Methods

Cells and Transfectants. The generation and characterization of the 3DT52.5.8 murine T cell hybridoma and of its variant expressing the human CD4 molecule (I1B-3) have been described (19–22). The I1B-3 T cell hybridoma is maintained in culture medium consisting of RPMI 1640 supplemented with 10% FCS, 10 μM 2-ME, 2 mM L-glutamine, and 500 μg/ml of G-418 (GIBCO BRL, Gaithersburg, MD). The B4.2.3 T cell hybridoma is H-2D^d restricted and was generated by the fusion of BW1100 thymoma with lymph node cells of BALB/c mice immunized with p18 in complete Freund's adjuvant (23). The p18 peptide corresponds to residues 315–329 of the gp160 protein of the HIV-1 strain IIIB (24). The B4.2.3 T cell hybridoma is maintained in DMEM medium supplemented with 10% FCS, 10 μM 2-ME, and 2 mM of L-glutamine. The HLA-DR α chain is encoded by a full length cDNA (25). The HLA-DR β chain cDNAs correspond to the different alleles of HLA-DR previously described (26–32). The murine fibroblastic class II–negative DAP-3 cell line was transfected using calcium phosphate as previously described (33). Briefly, DAP-3 cells (3×10^5) were transfected with 10 μg of RSV.5 or RSV.3 plasmids (34) encoding the HLA-DR α and one of the different allelic or isotypic forms of the β chain, together with 5 μg of the plasmid containing the H-2D^d gene. Neomycin (G-418) or mycophenolic acid (10 μg/ml) and xanthine (100 μg/ml) selection were applied 48 h after transfection. Aseptic cell sorting using a FACStar Plus® (Becton Dickinson & Co., Cockeysville, MD) was used to obtain homogeneous populations of cells expressing comparable levels of D^d and the different HLA-DR alleles.

FACS® Analysis. T cell hybridomas (I1B-3) were stained with either OKT4 (anti-human CD4) and with KJ12-98 (anti-murine TCR idiotype), followed by fluorescein-coupled goat anti-mouse Igs (Becton Dickinson & Co.). DAP-3 cell lines were stained with either 34.5.8 (anti-mouse class I H-2D^d) or L-243 (mouse anti-human class II HLA-DR) antibody. Cells were analyzed on a FACScan® flow cytometer (Becton Dickinson & Co). Mean fluorescence values (M.F.V.)[1] are expressed in arbitrary units. For each fluorescence histogram, 10,000 live cells were analyzed, using a four-decade logarithmic scale. Dead cells were excluded by propidium iodide (0.5 mg/ml) gating. As a control, the cells were stained only with the FITC–goat anti-mouse Igs.

HLA-DR Nomenclature. DR1-Dw1 = DRB1 0101 (27); DR2B-Dw2 = DRB1 1501 (28); DR2A-Dw2 = DRB5 0101 (28); DRw6b III = DRB3 0201 (29); DRw53 = DRB4 0101 (30); DR4-Dw4 = DRB1 0401 (31); DR4-Dw10 = DRB1 0402 (31); DR4-Dw14 = DRB1 0404 (31); DR4-Dw15 = DRB1 0405 (31); DRw11.1 = DRB1 1101 (32).

[1] *Abbreviation used in this paper:* M.F.V., mean fluorescence value.

I1B3 Stimulation Assays. A fixed number of T cells (75×10^3) expressing the human wild-type CD4 molecule was cocultured with different DAP-3 target cells (75×10^3) expressing H-2D^d and various HLA-DR molecules. The CD4$^-$ 3DT52.5.8 T cell hybridoma was cocultured under the same conditions. The assay was performed in 200 μl of complete medium for 18 h at 37°C, 5% CO_2, in 96-well flat-bottom culture plates (Flow Laboratories, Inc., McLean, VA). Supernatants from the coculture were tested for the presence of IL-2 by their ability to support the proliferation of the IL-2–dependent cell line CTLL.2 using the hexosaminidase colorimetric assay (18, 19). A calibration curve was performed in parallel to determine the IL-2 concentration (U/ml). In previous experiments, we had demonstrated that an anti-CD4 antibody (OKT4B or L-68) could abrogate IL-2 production (19).

Growth Inhibition Assay with B4.2.3. DAP-3 cell transfectants (10^4) expressing H-2D^d alone, DRw53 alone, H-2D^d and DRw53 or H-2D^d, and DR4-Dw4 were used as APCs. DAP cells were pretreated with 50 μg/ml of mitomycin (Sigma Chemical Co., St. Louis, MO) for 45 min and then washed five times with PBS. These APC were pulsed for 4 h with 0.004, 0.02, 0.1, and 0.5 μg/ml of the p18 peptide diluted in complete DMEM without FCS and then washed three times with PBS. B4.2.3. T cell hybridomas (10^4) were then cocultured with transfected DAP cells in complete DMEM containing 10% FCS for 18 h at 37°C, 5% CO_2 in 96-well culture plates. This T cell concentration does not allow antigen presentation between themselves. After 20 h, cells were pulsed with [^{3}H]thymidine for 6 h and were then collected and counted.

Results and Discussion

Polymorphism in MHC Class II Molecules Affects the Interaction with CD4. A T cell hybridoma specific for the murine MHC class I molecule H-2D^d and dependent on the CD4–class II interaction for high levels of IL-2 production was used in these experiments. As previously demonstrated, expression of class II molecules in H-2D^{d+} APCs leads to a clear enhancement of IL-2 production by T cell hybridomas, when effector cells also express CD4 molecule (18, 19, 22, 35). Cocultures were performed between the CD4$^+$ murine T cell hybridoma (I1B-3) and transfected murine fibroblastic cells (DAP-3) expressing comparable levels of various alleles and isotypes of DR molecules together with the TCR ligand D^d (Fig. 1). DR molecules encoded by different DRB genes interact with CD4; the DRw6bIII, DRw53, and DR2A-Dw2, which are products of the DRB3, B4, and B5 genes, respectively, can trigger enhanced IL-2 production levels, as compared with DAP-3 cells expressing only H-2D^d molecules (Fig. 2 *A*). Increase in IL-2 production ranged between 3- and 17-fold (Fig. 2), when compared with control DAP-3 that express only H-2D^d. Results from Fig. 2 *A* indicate that DR4-Dw4 and DRw6B III molecules are more efficient than DRw53 and DR2A in stimulating IL-2 production by the I1B-3 T cell hybridoma. Among DRBI alleles (Fig. 2 *B*), DR4-Dw4 and DR5 are capable of stimulating high levels of IL-2 production by the T cells (14–15-fold), as compared with cells expressing D^d alone. However, coculture of CD4$^+$ T cells with DAP-3 expressing DR2B-Dw2 or DR1 constantly yielded lower levels of IL-2 (20-fold difference). Similar differences were also obtained when cocultures were carried

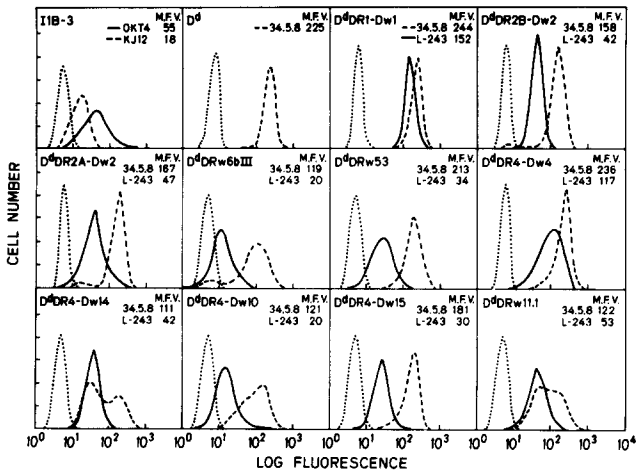


Figure 1. FACS® analysis of the 3DT52.5.8 T cell hybridoma expressing human CD4 (I1B-3) and DAP-3 cell lines expressing murine class I and human MHC class II molecules. Cells were stained with mAb OKT4 (anti-CD4), 34.5.8 (anti H-2D^d), and L-243 (anti HLA-DR).

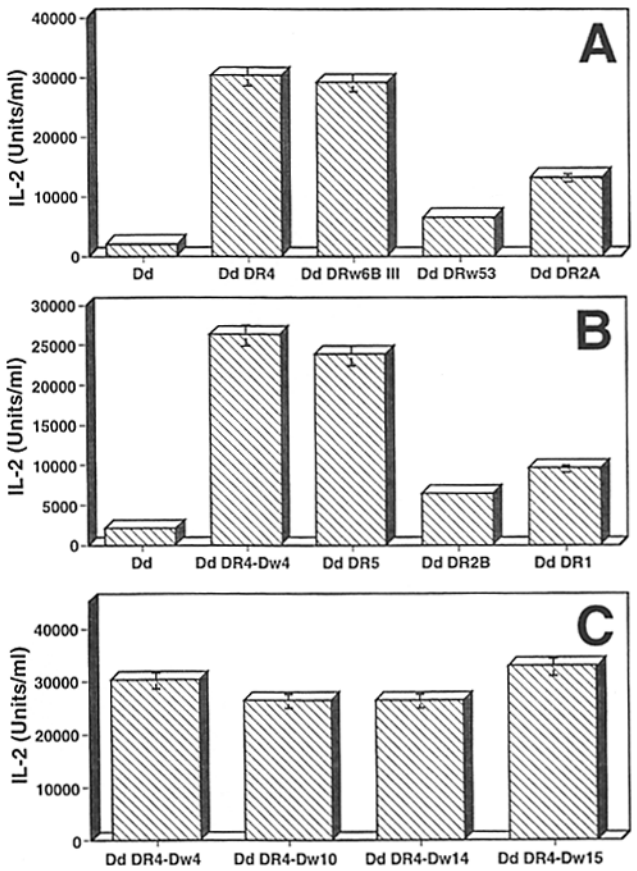
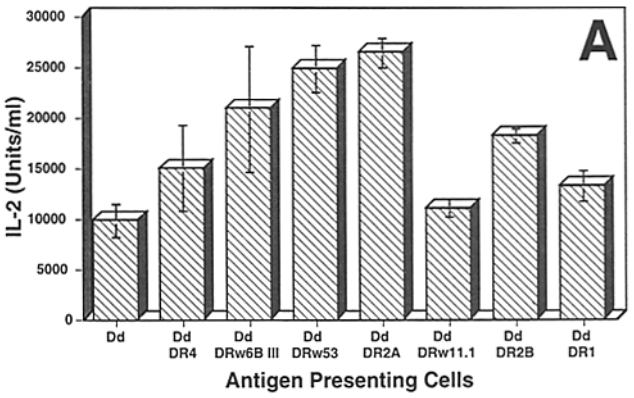


Figure 2. Polymorphism in HLA-DR affects the interaction with CD4. (*A*) I1B-3 T cell hybridomas were stimulated with APCs expressing H-2D^d and different HLA-DR isotypes. Products of the DRB1 gene correspond to DR4-Dw4; DRB3, DRw6BIII; DRB4, DRw53; and DRB5, DR2A-Dw2. (*B*) Ability of DAP-3 cells expressing various DRB1 products to stimulate CD4$^+$ T cells. DR4-Dw4, DRw11.1, DR2B-Dw2, and DR1-Dw1. (*C*) Polymorphism in the peptide groove of the β1 domain of class II does not affect the CD4 binding. CD4$^+$ T cells were cocultured 18 h at 37°C with various DR4 subtypes. Supernatants from the coculture were tested for the presence of IL-2 by their ability to support the proliferation of the IL-2–dependent cell line CTLL.2. I1B-3 was also cocultured with DAP-3 target cells expressing only class I H-2D^d as internal control (data not shown). Results are reported as levels of IL-2 production.

out in the presence of 10-fold fewer DR4-Dw4 or DRw11.1 target cells (data not shown). Altogether, our results from cocultures indicate that DR4-Dw4, DRw6BIII, DR2A-Dw2, and DRw11.1 were the most efficient DR molecules in interacting with CD4; DR1-Dw1 and DR2B-Dw2 displayed a good reactivity; while the DRw53 isotype consistently displayed the poorest capacity to interact with CD4. Indeed, DRw53 gives reproducibly the smallest enhancement of IL-2 production, even at the highest E/T cell ratio (1:1). In all representative experiments performed, the enhancement of IL-2 production by the T cells, when stimulated with DAP D^d DRw53, always varied between 10 and 30% of the response obtained when T cells were stimulated with DAP D^d DR4-Dw4.

Polymorphism in the Peptide-binding Groove Does Not Affect the CD4–Class II Interaction. DR alleles and isotypes share the same α chain, indicating that the differences described above are determined only by polymorphic residues in the β chain. To determine whether polymorphism in the peptide groove of class II affects the interaction with CD4, DR4 subtypes were tested. All of the differences between DR4-Dw4 and the other DR4 subtypes map to residues located on the floor and the α helices of the peptide-binding groove (26). Results of a representative experiment illustrated in Fig. 2 *C* indicate that the DR4 subtypes tested (Dw4, Dw10, Dw14, and Dw15) were all very efficient in their interaction with CD4, as shown by their capacity to trigger comparable levels of IL-2 production by the T cells (14–17-fold enhancement). These results suggest that polymorphism in the antigen-binding groove of the β1 domain has little influence on the CD4–class II interaction. However, we cannot exclude at present the possible involvement of other residues of the β1 domain.

Differential Stimulation by APCs Is Not Due to Other Accessory Molecules. The differential capacity of target cells expressing the DR alleles and isotypes to stimulate CD4$^+$ T cells cannot be attributed to variations in the levels of class II or D^d on their surfaces. Indeed, DR1-Dw1, which is less efficient than DR4-Dw4, DRw11.1, or DRw6BIII in this assay, expresses the highest levels of class II and D^d molecules on its surface (Fig. 1). Controls were then carried out to eliminate the possibility that the observed differences in IL-2 production were caused by the differential expression of H-2D^d molecules or other costimulatory molecules by the APCs. The parental cell line 3DT52.5.8 (CD4$^-$) was cocultured with the same stimulator cells expressing H-2D^d and the different HLA-DR alleles and isotypes (Fig. 3 *A*) previously used to stimulate the CD4$^+$ T cells (I1B-3 hybridoma).



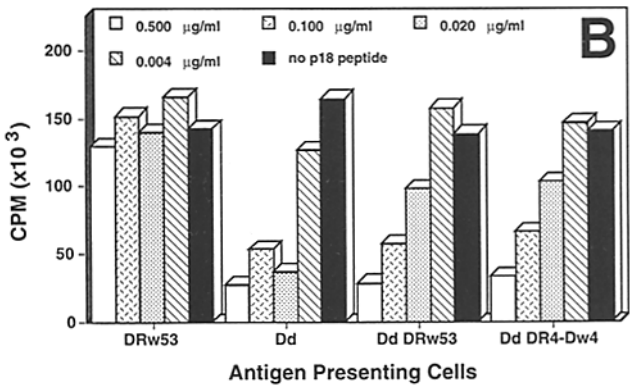


Figure 3. (*A*) Control experiments to evaluate the influence of variation in class I expression on DAP cells used to stimulate I1B-3 hybridoma. 3DT52.5.8 (CD4⁻) T cell hybridomas were cocultured with APCs expressing H-2D^d and different HLA-DR products. Results are reported as levels of IL-2 production as measured in the CTLL.2 assay. (*B*) Dose response of the B4.2.3 hybridoma to p18 peptide. The H-2D^d–restricted, p18-specific T cell hybridoma B4.2.3 cocultured with APCs responded with inhibition of its growth to APCs expressing H-2D^d pulsed with p18. Growth inhibition was measured by [^{3}H]thymidine incorporation in the B4.2.3 T cell hybridomas.

Results of a representative experiment are illustrated in Fig. 3 *A* and show only a 1–2.5-fold increase in the IL-2 response for CD4⁻ T cells (3DT52.5.8), when incubated in the presence of the different APCs. These results suggest that the high IL-2 ratio obtained with the CD4⁺ T cell is due specifically to the CD4–class II interaction and not to variations in class I expression or other costimulatory molecules. Another control experiment was also performed to determine the influence of class I expression and other costimulatory molecules on APCs used in the stimulation of I1B-3. The B4.2.3 T cell hybridoma, which is specific for the HIV p18 peptide (derived from the gp160) and H-2D^d restricted, was used. The response of this T cell hybridoma is monitored by the inhibition of its growth when cocultured with APCs expressing H-2D^d and pulsed with the p18 peptide. Two stimulator cells were tested, one that gives a very high IL-2 enhancement (D^{d}DR4-Dw4) and the APC that induces the poorest IL-2 enhancement (D^{d}DRw53) (Fig. 2 *A*). Results of this experiment are illustrated in Fig. 3 *B* and show a similar ability of D^{d}DRw53 and D^{d}DR4-Dw4 to inhibit B4.2.3 growth when pulsed with the p18 peptide. This further confirms that the variations in IL-2 production induced by the different APCs are more likely to depend on the ability of these various DR alleles and isotypes to interact with the CD4 molecule.

Polymorphisms in the COOH-terminal End of the HLA-DR Chain Affect the CD4–Class II Interaction. A highly conserved sequence (^{136}QEEK^{139}), analogous to the CD8-binding site on class I (11), is found in the β2 domain of the class II β chain; this motif is part of a loop that was shown to be involved in the CD4–class II interaction (12, 36). This sequence, which is conserved amongst all class II alleles and isotypes tested in these experiments, does not explain the variations described above. Our results suggest that other residues, probably outside this loop, affect the CD4–class II interaction (Fig. 4 *a*). To identify such residues, chimeric class II molecules were generated between the β chain of DR1-Dw1 (an intermediate responder) and DRw53 (the poorest responder), or the β chain of DR4-Dw4 (a very good responder) and DRw53 (Fig. 4 *B*). Each chimera contained the first 146 residues of the DR1-Dw1 (DR1/DRw53) or DR4-Dw4 (DR4/DRw53) β chain, including the highly conserved ^{136}QEEK^{139} motif, with the COOH-terminal 91 amino

A

	179											191
DR4-DW4	S	L	T	S	P	L	T	V	E	W	R	A	R
DRw11.1	-	V	-	-	-	-	-	-	-	-	-	-	-
DRw53	-	M	M	-	-	-	-	Q	-	S	-	-	
DRw6bIII	-	V	-	-	-	-	-	-	-	S	-	-	
DR1-Dw1	-	V	-	-	-	-	-	-	-	-	-	-	
DR2A-Dw2	-	V	-	-	-	-	-	-	-	-	-	-	Q
DR2B-Dw2	-	V	-	-	-	-	-	-	-	-	-	-	-

B

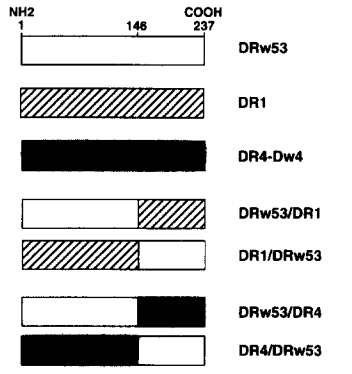


Figure 4. (*A*) Sequence comparison between the DRB alleles in the COOH-terminal portion of the second domain. This region (179–191) bears all the differences between DR1-Dw1 and DRw53 in the last 91 amino acids. Numbers refer to the mature peptides, and dashed lines represent conserved residues. The one-letter code for amino acids was used. (*B*) Schematic representation of the chimeric molecules constructed by recombination of the cDNAs encoding for the different MHC class II molecules. These constructions were transfected into DAP D^d cells and sorted using anti-human class II mAb L-243.

acids of DRw53. Reciprocal chimeras were generated by introducing the NH_2-terminal domain of DRw53 (1–146) into the membrane-proximal domain of DR1 (DRw53/DR1) or of DR4 (DRw53/DR4). These different chimeric molecules were then transfected into DAP-3 D^{d+} cells. Populations expressing homogenous levels of D^d and of DR molecules were then tested (Fig. 5).

Insertion of the COOH-terminal residues of DRw53 into the DR1 molecule led to a total loss of the capacity of DR1 to enhance IL-2 production by the $CD4^+$ T cell hybridoma (Fig. 5 *A*). The DR1/DRw53 chimeric β chain was very inefficient in its interaction with CD4. In contrast, the DRw53/DR1 chimera molecule was as efficient as DR1 (Fig. 5 *A*), indicating that polymorphisms in the last 91 amino acids of the β chain are responsible for the differences observed between DRw53 and DR1-Dw1. Similar conclusions were obtained with DR4/DRw53 and DRw53/DR4 chimeric molecules. Substitution of the first 146 amino acids of DRw53 by the corresponding DR4 residues was not sufficient to support the enhancement of IL-2 production by

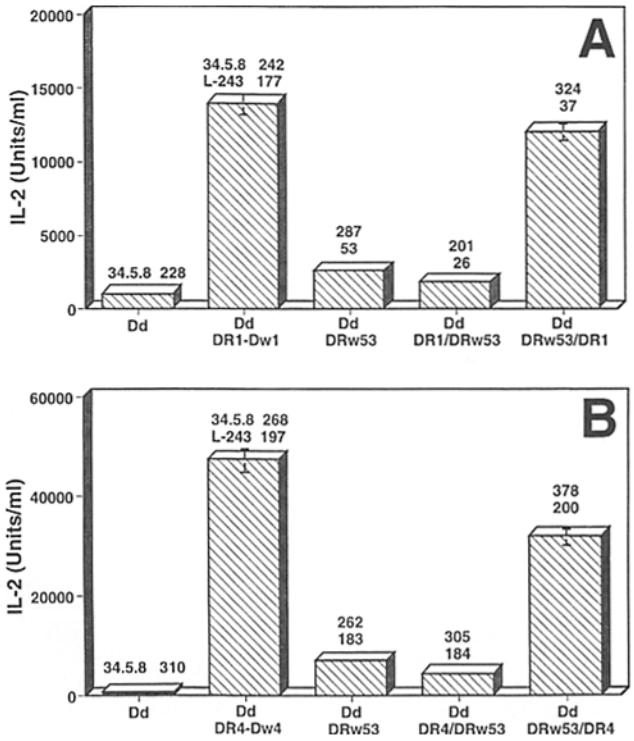


Figure 5. Polymorphism in the COOH-terminal domain of the β2 domain of class II molecules affects CD4 binding. (*A*) I1B-3 was cocultured 18 h at 37°C with APCs expressing H-$2D^d$ and class II chimera molecules DR1/DRw53 or DRw53/DR1. APCs expressing the wild-type DR1 or DRw53 with H-$2D^d$ were used as controls. (*B*) I1B-3 was cocultured as previously described with APCs expressing H-$2D^d$ and chimera molecules DR4/DRw53 or DRw53/DR4. DAP-3 cells expressing H-$2D^d$ and DRw53 or DR4-Dw4 were used as controls. Results are reported as levels of IL-2 production. Numbers that appear at the top of each bar histogram correspond to the M.F.V. for class I D^d (*upper number*) and class II expression (*lower number*). Cell surface expression of class I was determined with 34.5.8 mAb and class II with L-243 mAb.

I1B-3 (Fig. 5 *B*). On the other hand, introducing the carboxy-terminal residues of DR4 into DRw53 fully restored the capacity of DRw53 to interact with CD4. Results obtained with both chimeric molecules indicate that polymorphic residues located in the last 91 amino acids of the β chain of class II molecule play a role in the CD4 interaction. Sequence alignments of the 91 COOH-terminal residues indicate the presence of only four polymorphic amino acids between DR4-Dw4, DR1-Dw1, and DRw53. These residues are located between positions 179 and 191 of the β chain (Fig. 4 *A*). The differences in DRw53 include two methionines, which replace the highly conserved valine and threonine residues at positions 180 and 181. However, the DR4-Dw4 has a leucine instead of a valine, which is a very conservative substitution. Two highly conserved charged residues in DR and other class II molecules, glutamic acid 187 (E187) and arginine 189 (R189), are substituted by glutamine and serine residues, respectively, in DRw53. However, the amino acid S189 is also present in DRw6bIII, which interacts efficiently with CD4 in the assay described above. There are other differences in the amino acid sequences between DR molecules. However, sequence alignments between DRw53 (poor responder) and DR4 (good responder) did not show any other differences outside of this region. This observation limits the contribution of the polymorphism to the three residues M180, M181, and Q187.

According to the DR1 class II structure (37), the stretch of residues that includes the highly conserved ^{136}QEEK^{139} motif on the β2 domain of the β chain is contained in a loop between two β strands. Several of these residues are highly exposed to solvent. Moreover, localization on the class II structure of the two methionines at positions 180 and 181 in DRw53 (Fig. 6, *A* and *C*) mapped to a loop adjacent to the highly conserved QEEK motif. These two methionines are not located on the same side of the β2 domain as the ^{136}QEEK^{139} motif or the CD4-binding site on class II (residues β 137–148), but rather on the opposite side of the β2 domain of MHC class II molecules (Fig. 6). Residue 181, however, is highly conserved amongst other DR alleles (Fig. 4 *A*) and is highly exposed to solvent (Fig. 6).

How do residues 180 and/or 181 contribute in CD4 binding? In Fig. 6, we can see that only residue 181 has a prominent side chain (Fig. 6 *A*, *pink residue on left side*) and pointing out and that is ~20 Å further up (diagonal angle) from the side chain of residue 137. Residue 180 shows a side chain pointing inside the class II, suggesting that it is most likely unable to interact with CD4. Based on these observations, we can postulate that CD4 might contact a single heterodimer class II through residue 181 on one side of the β2 domain and residues from the loop 136–148 on the other side (see models in Fig. 7). However, if we consider residues that are in the same plan as residues 181, only residues 136–139 are properly located. This leads us to postulate that CD4 probably interacts with residues 181 and the loop 136–139 (Fig. 6). The possibility that two CD4 molecules interact with one class II molecule also exists. One CD4 molecule would interact with residue 181, and another CD4 would interact with the loop 136–148. The DR1 class II molecules have been

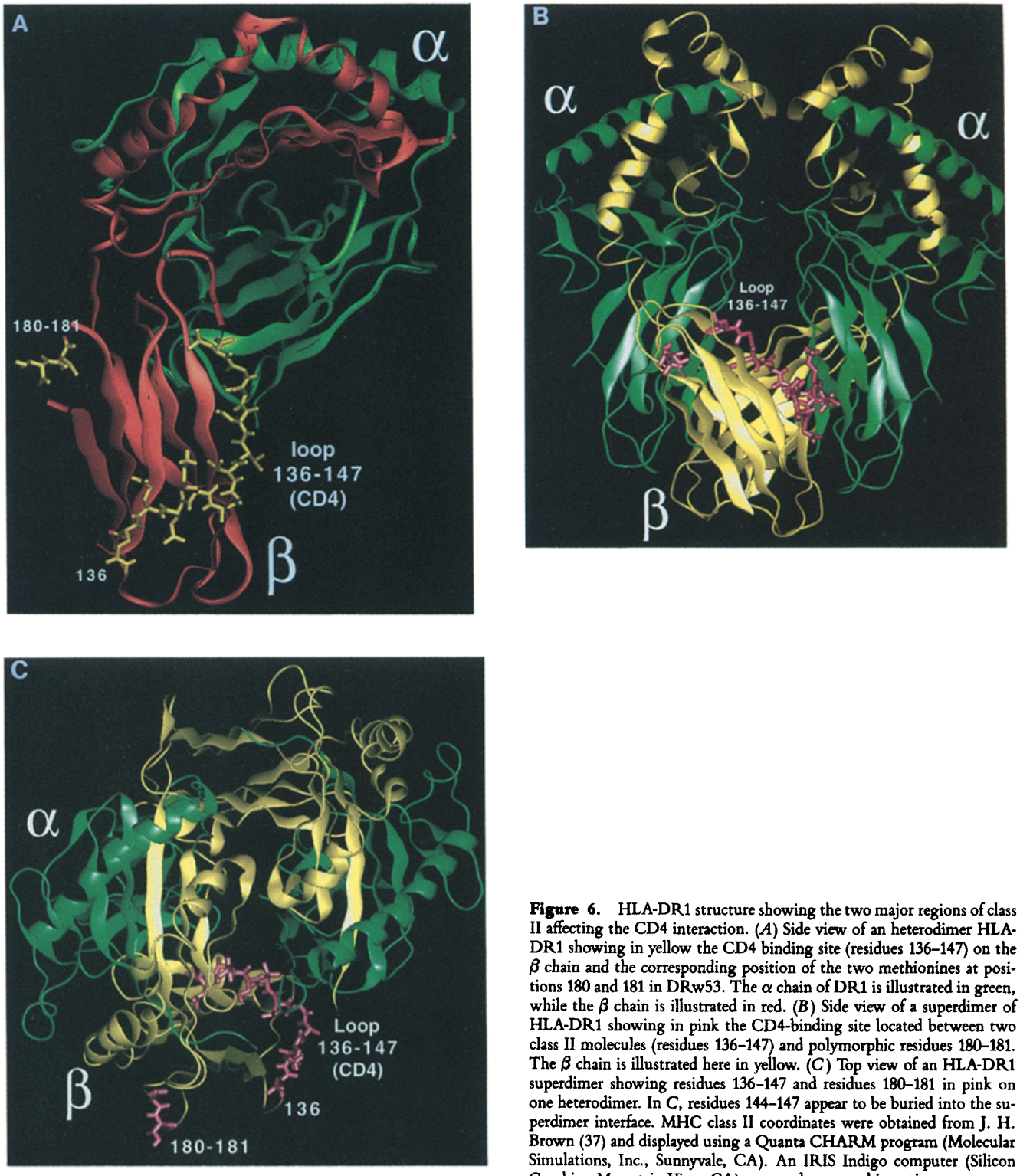


Figure 6. HLA-DR1 structure showing the two major regions of class II affecting the CD4 interaction. (*A*) Side view of an heterodimer HLA-DR1 showing in yellow the CD4 binding site (residues 136–147) on the β chain and the corresponding position of the two methionines at positions 180 and 181 in DRw53. The α chain of DR1 is illustrated in green, while the β chain is illustrated in red. (*B*) Side view of a superdimer of HLA-DR1 showing in pink the CD4-binding site located between two class II molecules (residues 136–147) and polymorphic residues 180–181. The β chain is illustrated here in yellow. (*C*) Top view of an HLA-DR1 superdimer showing residues 136–147 and residues 180–181 in pink on one heterodimer. In *C*, residues 144–147 appear to be buried into the superdimer interface. MHC class II coordinates were obtained from J. H. Brown (37) and displayed using a Quanta CHARM program (Molecular Simulations, Inc., Sunnyvale, CA). An IRIS Indigo computer (Silicon Graphics, Mountain View, CA) was used as a graphic station to generate class II pictures.

shown by Brown et al. to crystallize as a dimer of heterodimers (superdimer) (37). In this context, there are two CD4-binding sites per class II superdimer. However, in the superdimer structure, not all residues from the stretch 136–148 are accessible to CD4 (Fig. 6 *B*). In fact, residues 144–148 are buried between two class II molecules, leaving only residues 136–143 accessible to CD4. Konig et al. have reported that residues 137 and 140 of the class II β chain are the most critical for the interaction with CD4 (12). The importance of E137 was further confirmed by Cammarrota et al. (36), who

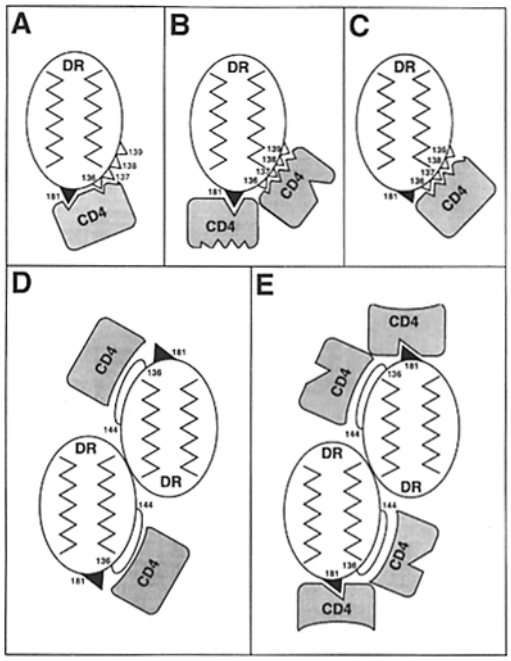


Figure 7. Possible interactions between CD4 and class II molecules. (*A*) One face of the CD4 molecules is engaged with two binding sites on the β2 domain of a class II molecule. These binding sites might involve residues 181, 136, and 137 because they are on the same plan. In this model, residues 138–148 are not in the same plan as residue 181. (*B*) One CD4 molecule makes contact through one of its faces with residue 181, while another CD4 molecule binds to the loop 136–144 through its opposite face. Only residues 136–139 are illustrated. (*C*) CD4 binds to the loop formed of residues 136–144 of the β2 domain of class II but comes in close proximity to residue 181, whose side chain could modulate CD4 docking. (*D*) In the superdimer, among residues 136–148 from the CD4-binding site on the β2 domain of class II, only residues 136–144 are easily accessible to CD4. Residues 145–148 are buried inside the superdimer structure. CD4 would bind to the loop 136–144, and the side chain of residue 181 could affect CD4 binding. (*E*) Two CD4 molecules would bind on each side of the superdimer. One CD4 is engaged with the loop 136–144, and the other CD4 contacts a second binding site on the β that includes residue 181.

showed that a peptide spanning residues 138–152 is severely impaired in its capacity to bind soluble CD4. In the superdimer, amino acid T181 on DR1 is still very exposed to solvent (Fig. 6, *B* and *C*). A single CD4 molecule can still interact with residue 181 and the loop 136–139. However, we do not know if CD4 establishes contact with these two binding sites through the same face. Alternatively, it is possible that two CD4 molecules could interact with each class II molecule. Several reports in the literature have suggested the presence of two class II–binding sites on CD4 (19, 38, 39).

According to the observations described above, it is more likely that the CD4-binding site on class II would implicate residues 181 and some residues of the loop 136–143, most likely E137 and V140 (12). A single CD4 molecule can accomodate this surface area, but residues extending farther than position 139 can hardly make contact with CD4. It is also possible that residues 180–181 are not directly implicated in CD4 binding, but may rather influence the docking between CD4 and MHC class II molecules, thereby influencing the affinity of CD4 for its ligand. The two methionines at positions 180 and 181 in DRw53 instead of a valine and a threonine might have an effect on CD4 binding because of the presence of a longer side chain. The introduction by mutagenesis of residues with short nonpolar side chains at these positions might help to determine the real contribution of residues 180 and 181 in CD4 binding. Finally, it is possible that the orientation of these residues could differ from one DR structure to another. Mutation on residue 187 should also help to answer this question. Unfortunately, the class II crystal structure does not include residue 187. It would have been interesting to see whether it maps to the same face as residues 180–181 and loop 136–147.

Our experiments clearly demonstrate important differences between alleles and isotypes of DR molecules in their ability to interact functionally with the CD4 molecule. Results have been obtained with natural MHC class II molecules known to be efficiently expressed at the cell surface and to present Ag to T cells. An important region of the DR β chain implicated in the interaction with CD4 has been identified between residues 179 and 190. Other polymorphic residues in DR β chains can affect the interaction with CD4. Indeed, the low response of DR2B-Dw2 might be explained by R133L or V142M substitutions, since R133 and V142 are highly conserved in all other DR alleles. Efficient interaction with CD4 could thus contribute to the predominance of certain DR alleles or isotypes in antigen-restricted responses. Interestingly, such a correlation exists for DR2A and DR2B. The DR2A molecule, which interacts better than DR2B with CD4 (Fig. 2, *A* and *B*), predominates over DR2B in presentation of natural antigens to T cells (16). The effect of such polymorphism on the selection of the T cell repertoire remains to be determined.

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