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Human immunodeficiency virus gp120 binding C′C″ ridge of CD4 domain 1 is also involved in interaction with class II major histocompatibility complex molecules

(HLA/AIDS/virus receptors/CD4 mutagenesis/T-cell function)

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ABSTRACT **Using site-directed mutagenesis informed by high-resolution CD4 structural data, we have investigated the role of residues of the C′C″ ridge region of human CD4 on class II major histocompatibility complex (MHC) binding. This C′C″ ridge is homologous to the CDR2 loop of an immunoglobulin variable domain and is known to contain the binding site for human immunodeficiency virus (HIV) coat glycoprotein gp120. Here we report that this region is also involved in interaction with class II MHC. Exposed positively charged residues Lys-35, Lys-46, and Arg-59 and the exposed hydrophobic residue Phe-43 contribute significantly to class II MHC binding. Moreover, mutations in the buried residues Trp-62 and Ser-49, which support the top and bottom of the C′C″ ridge, respectively, disrupt class II MHC interaction. The HIV binding region appears to involve a restricted area of the larger class II MHC binding site on CD4. Strategies of drug design aimed at interrupting CD4–HIV interaction will need to consider the extensive overlap between class II MHC and HIV gp120 binding surfaces in this region of CD4.**

CD4 is a transmembrane glycoprotein expressed on the surface of thymocytes and mature T lymphocytes (1, 2). CD4+ T lymphocytes recognize peptide antigen when presented in association with major histocompatibility complex (MHC) class II molecules and generally exhibit helper T-cell function (3–5). Cytotoxic CD4+ T cells with specificities for MHC class II molecules have also been described (6–8). CD4 interacts directly with class II MHC proteins, as shown by conjugate formation between class II MHC-expressing B cells and *CD4*-transfected fibroblasts (9, 10). Formation of conjugates depends on cell–cell adhesion mediated by CD4–class II MHC interaction. In the human, CD4 also serves as the cellular receptor for the human immunodeficiency virus (HIV) (11–14).

CD4–class II MHC interaction is critically involved in both negative and positive selection during thymic development as well as in activation of mature T lymphocytes. Elimination of CD4 function, either by administration of specific anti-CD4 or anti-Ia monoclonal antibody (mAb) during mouse ontogeny (15, 16) or by generation of animals lacking class II MHC molecules (17, 18) results in diminished CD4+ T-cell development and impairment of B-cell functions. In mature T lymphocytes, CD4 crosslinking is known to provide a costimulatory signal during T-cell activation via the T-cell receptor (TCR) (19, 20). The signaling functions of CD4 are believed to be mediated by $p56^{lck}$, a member of the src protein tyrosine kinase family, which is noncovalently associated with the cytoplasmic domain of CD4 (21, 22). Colocalization of the TCR and CD4 on the surface of stimulated T lymphocytes may facilitate the activation process by bringing the CD4-associated kinase into proximity with the TCR signaling machinery (23, 24).

CD4 is a member of the immunoglobulin (Ig) superfamily. It has a large extracellular portion (residues 1–372), a transmembrane segment (373–393), and an intracellular "tail" (394–433) (25). The extracellular part consists of four Ig variable (V)-like domains. The structure of the two N-terminal domains has been determined to 2.2 Å by x-ray crystallography (26, 27). The first and second domains are antiparallel β-barrels with the characteristic Ig connectivity. They are joined by a continuous β-strand connector, giving the molecule a rod-like structure. The first domain closely resembles a V_κ chain. There are nine β-strands, with strands A, C, C′, C″, F, and G forming one surface and strands B, D, and E forming the other. The C′C″-loop of CD4 domain 1 is larger than the corresponding loop in an Ig V domain. As a result, it protrudes noticeably from the molecular surface (26).

Previous homology-scanning mutations indicated that the interaction of CD4 with class II MHC molecules is complex, involving several parts of CD4 domains 1 and 2 (10, 28, 29). In contrast, the gp120 binding site is localized to residues near the C′C″ ridge of domain 1 (30–35). It was recently suggested that Ser-19 and Gln-89 in domain 1 and Gln-165 in domain 2 are involved in the binding of a HLA-DP molecule (29). These residues are located on the opposite side of CD4 from the gp120 binding site. Therefore, it was concluded that the interactions of CD4 with HIV gp120 and class II MHC molecules occur at physically distinct sites on different sides of the CD4 molecule (29). We have characterized the CD4 residues involved in class II MHC binding by using genetically typed B-cell lines expressing defined *HLA-DR, -DP*, and *-DQ* gene products. We report here that residues in the C′C″ region are critical for interaction with class II molecules, independent of allelic polymorphisms. In contrast to Fleury *et al.* (29), we conclude that the C′C″ ridge contains contact sites for both CD4 ligands—class II MHC and HIV gp120.

MATERIALS AND METHODS

Mutagenesis, Transfection, and Binding Assay. Mutagenesis of *CD4* using single-stranded DNA and oligonucleotides (18–22 bases) was performed as described (10). Mutant *CD4* was subcloned into the CDM8 vector (36), and all constructs

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Abbreviations: HIV, human immunodeficiency virus; TCR, T-cell receptor; V, variable; MHC, major histocompatibility complex; gp120, HIV coat glycoprotein; wt, wild type; mAb, monoclonal antibody.

were sequenced through the entire domain 1 coding region. The *CD4* inserts of selected constructs coding for Phe-43 → Ala, Phe-43 → Ile, and Lys-46 → Ala changes were entirely sequenced to exclude additional mutations and/or deletions. COS-1 cells were transfected and analyzed 48 h later by addition of 10^7 Raji cells as described (10). Wells were washed (2 ml of medium, five or six times), the number of rosettes containing more than five Raji cells were counted in 10 random optical fields (≈300 rosettes with wild-type (wt) CD4, 100-fold magnification), and the percent of rosettes relative to wt CD4 was determined. Cells transfected with CDM8 vector alone served as negative controls for rosette formation. Binding experiments with B-cell lines other than Raji were similarly performed. For quantitative binding assays, COS-1 cells were transfected in 10-cm Petri dishes (1.5×10^6 cells, 20 μg of DNA per dish), released after 24 h, replated onto coverslips in six-well plates (2.5×10^5 cells per well), and incubated for 24 h. Raji cells were radiolabeled as described (10). After binding and washing as above, 1 ml of 1% (vol/vol) Triton-X-100 was added to coverslips, and 800 μl of lysate was assayed in a γ counter: % specific binding = $[cpm_{mutant} - cpm_{vector}]/[cpm_{wt} - cpm_{vector}]$.

Immunofluorescence. COS-1 cells were released by rinsing at room temperature with phosphate-buffered saline containing 1 mM EDTA. Cells were washed once and subjected to indirect immunofluorescence as described (5) by using OKT4 mAb (American Type Culture Collection) at saturating concentrations and goat anti-mouse Ig labeled with fluorescein isothiocyanate (Caltag, South San Francisco, CA; 1:100 dilution). Cell-bound immunofluorescence was determined with a FACScan (Becton Dickinson) and logarithmic amplification (4-logarithmic scale). Linear equivalents of fluorescence intensity (FL) were determined by using the CONCERT30 software (Becton Dickinson). Dead cells were excluded by using propidium iodide. Relative levels of expression were calculated as follows: $[FL_{mutant} - FL_{control}]/[FL_{wt} - FL_{control}]$. Binding experiments with domain 1-specific mAbs were performed by using transient expression in COS-1 cells and confirmed in CD4-transfected MA5.8 mouse T cells by using stable expression.

MHC Typing and Typed Cell Lines. T5-1 cells were typed serologically (37). Genetic typing of Raji was performed after PCR amplification of the second exon of *HLA-DRB*, *-DQA1*, and *-DQB1* loci as described in the reference protocol for class II MHC allele typing at the 11th International Histocompatibility Workshop, Yokohama, Japan; the primers used for *HLA-DQB1* typing were as described (38). The genotype and source of homozygous typing cells have been reported (39). The mutant line 6.1.6 was derived from T5-1 and lacks all class II MHC molecules (37, 40).

RESULTS AND DISCUSSION

Specific Cell-Based Adhesion Assay to Analyze the Interaction of Class II MHC with CD4 Mutants. The interaction between CD4 and class II MHC molecules was studied in a cell–cell adhesion assay with Raji cells, a human B-cell line known to be class II MHC positive, and COS-1 monkey cells transfected with a cDNA encoding either wt human CD4 or a CD4 variant mutated at a single amino acid residue. Previous studies have shown that B cell–COS-1 cell aggregation depends on the CD4–class II MHC interaction (10). "Rosettes" can be blocked specifically by either an anti-CD4 mAb or an anti-Ia mAb; mutant B cells lacking all class II MHC alleles fail to form such aggregates; and mock-transfected COS-1 cells do not form rosettes with B cells. This adhesion assay system has several advantages over insertion of CD4 into T-cell hybridomas derived from the same species as the antigen-presenting cells. First, CD4 is placed in a non-T-cell context. Binding is therefore independent of additional T-cell surface molecules including the TCR (23, 24). Second, the use of cell types derived from two species makes it less likely that other adhesion structures are contributing to cell–cell interaction. Third, the use of B-cell lines expressing defined HLA-DR, -DP, and -DQ molecules encoded by different alleles makes it possible to examine the effects of polymorphisms on CD4 binding.

Fig. 1*A* shows an anti-CD4 immunofluorescence histogram of wt CD4 cDNA-transfected COS-1 cells. Approximately 30% of cells transfected with wt or mutant CD4 cDNA expressed CD4 in each individual experiment. After addition of Raji B cells to wt CD4-transfected COS-1 cells, characteristic aggregates are formed (Fig. 1*B*). Clusters of B cells are observed on approximately one-third of the transfected COS-1 cells corresponding to the fraction of $CD4^+$ transfectants. B-cell binding after CD4 transfection was restricted to $CD4^+$ COS-1 cells exclusively as observed by immunofluorescence microscopy (data not shown). The formation of these clusters is specifically inhibited after the addition of the CD4-specific mAb Leu3a (Fig. 1*C*).

CD4 Point Mutants. CD4 molecules were created by site-directed mutagenesis. In general, single CD4 amino acids were replaced by alanine. Other substitutions were made to preserve or modify hydrophobicity, hydrogen bonding function or charge. The overall integrity of domain 1 in these mutants was confirmed by using a set of previously mapped mAbs directed at native structural determinants. By indirect immunofluorescence analysis, all mutants reacted with each of seven mAbs directed against domain 1 (VIT4, BL4IOT4, RFT4, MT321, Leu3a, 18T3A9, and 19Thy5D7), except for those mutants whose amino acid changes mapped to known footprints of a given antibody (26, 32, 41, 42, 45).

CD4 mutants were examined for their ability to bind to class II MHC in at least four independent experiments. Rosette formation was analyzed microscopically, and mutations were grouped according to their effects on rosette formation. The photomicrographs of B cell–COS-1 cell aggregates in Fig. 1 show the results of representative binding experiments with CD4 mutants carrying mutations that do not affect (Gln-40 → Ala) (Fig. 1*D*), partially disrupt (Lys-35 → Ala) (Fig. 1*E*), or completely disrupt (Phe-43 → Ala) (Fig. 1*F*) CD4–class II MHC interaction. The results of experiments with all mutants studied are summarized in Table 1, where each specific mutation and the location of the mutated CD4 amino acid are listed in single-letter amino acid code, which we use henceforth to identify the mutants. In every

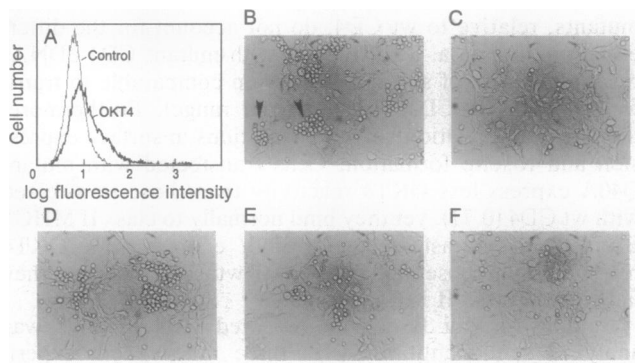

FIG. 1. Immunofluorescence of transfected COS-1 cells (*A*) and cell-binding assay of COS-1 transfectants and class II MHC^+ Raji B cells (*B–F*). COS-1 cells were transfected with wt CD4 cDNA (*A–C*) and mutants Q40A (Gln-40 → Ala) (*D*), K35A (Lys-35 → Ala) (*E*), and F43A (Phe-43 → Ala) (*F*), respectively. (*C*) Binding experiment with wt CD4 in the presence of CD4 mAb Leu3a. Cell adhesion is shown at ×160 magnification with arrowheads denoting representative clusters between COS-1 cells and B cells. Histogram is shown for 50,000 cells with negative (5REX9H5, anti-Ti) and OKT4 mAbs.

Table 1. Effect of CD4 mutations on CD4–class II MHC interaction

Mutation	Localization of residue	Level of CD4 expression	Rosette formation
wt	—	1.00	++
S19Y	BC	1.04	+
H27A	C	1.14	++
K35A	C′	1.20	+/−
G38A	C′	1.05	++
Q40A	C′	0.71	++
F43A	C″	1.04	−
F43I	C″	0.80	−
F43L	C″	1.07	+
F43Y	C″	1.03	++
F43W	C′	1.65	++
T45A	C″	0.79	++
K46A	C″	0.90	−
P48Q	C″	0.85	++
S49V	C″D	1.13	−
N52A	C″D	1.20	++
A55V	C″D	0.82	++
D56A	D	0.90	++
R59A	D	0.99	+/−
R59K	D	1.20	++
W62Y	DE	0.68	−
Q89L	G	1.06	++
M1.1	BC	0.92	+
M1B	CC′	1.19	+/−
M8	A of domain 2	1.05	−

Binding of Raji B cells to COS-1 cells expressing CD4 mutants measured as follows: ++, rosette formation identical to wt CD4 (Fig. 1 *B* and *D*); +, modest reduction, 70–80% of rosettes compared with wt CD4; +/−, substantial reduction, 20–40% of rosettes compared with wt CD4 (Fig. 1*E*); −, complete loss, no rosette formation (Fig. 1 *C* and *F*). Expression level of mutant CD4 was compared with wt CD4. The sidechain orientation of single substitutions is exposed in all cases except for Ser-49, Ala-55, and Trp-62, and nomenclature of CD4 strands is as reported (26). All mutations were in domain 1 except for M8. Mutants M1.1 (T17E and A18S), M1B (H27T, N30F, N32D, and I34R), and M8 (G99K, S104P, and H107S) have been described and contain side chains with both exposed and buried orientations (10).

experiment, the levels of mutant CD4 expression were determined in parallel by indirect immunofluorescence with OKT4. This mAb reacts with a membrane proximal CD4 epitope far removed from the mutations created in domains 1 and 2 (41). As shown, the expression levels of individual mutants, relative to wt CD4, do not account for the differential binding data. Transfection with mutant CD4 cDNAs results in levels of surface expression comparable to transfection with wt CD4 cDNA (2-fold range). Furthermore, there is no correlation between variations in surface expression and rosette formation. Cells transfected with mutant Q40A express less OKT4 reactivity than those transfected with wt CD4 (0.71), yet they bind normally to class II MHC$^+$ B cells; cells transfected with S49V express more OKT4 reactivity than those transfected with wt CD4 (1.13), but they fail to bind class II MHC$^+$ B cells.

Binding of Raji B cells to selected CD4 mutants was analyzed semiquantitatively in three independent experiments with ^{51}Cr-labeled B lymphocytes. The result of one representative experiment is shown in Fig. 2. Consistent with the qualitative rosette formation data reported in Table 1, the F43I mutation eliminates detectable class II MHC binding. In addition, the K35A and R59A mutations both diminish binding to <30% of that observed with wt CD4. In contrast, no significant reduction is observed with the Q89L mutation (93% specific binding), and binding is only modestly affected by S19Y (77% specific binding).

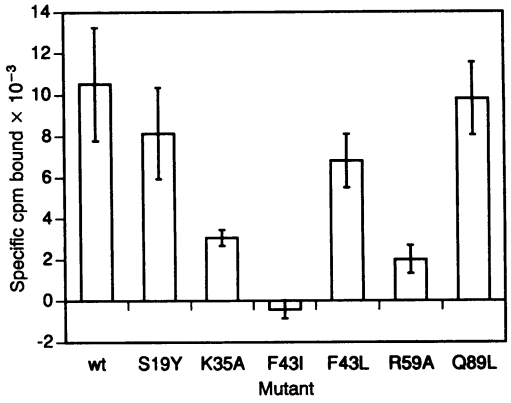

FIG. 2. Quantitation of cell binding between CD4 transfectants and B cells. Specific binding of Raji cells to selected CD4 transfectants was quantitated after ^{51}Cr-radiolabeling of Raji cells. Error bars represent standard deviation of triplicates. Nonspecific binding to vector-transfected cells resulted in 2546 ± 695 cpm, which was subtracted from experimental mean cpm. Data analysis using a mixed model of variance (44) showed that cpm obtained with the S19Y mutant in three experiments was significantly different from wt cpm ($P < 0.0001$).

Mutations in the C′C″ Ridge Affect Class II MHC Binding. The mutations we have studied here that affect class II MHC binding involve the following residues on or near the C′C″ ridge: Lys-35, Phe-43, Lys-46, Ser-49, Arg-59, and Trp-62 (Table 1, Fig. 2). Fig. 3 shows the location of these residues within the two domain CD4 structure (Fig. 3*A*) and the orientation of their side chains (Fig. 3*B*). The exposed residues Lys-35, Phe-43, Lys-46, and Arg-59 represent potential contact sites for class II MHC. The positively charged lysine and arginine residues may mediate electrostatic interactions with suitably placed negatively charged side chains on the class II MHC molecule. In contrast to R59A, the R59K mutation does not affect class II MHC interaction, indicating that substitution of another positively charged side chain at that position is tolerated.

The exposed hydrophobic Phe-43 is an unusual structural feature of CD4 (26). This residue appears to be critical in class II MHC binding because the F43A and F43I mutations eliminate the interaction (Table 1, Fig. 2). The requirement for a large hydrophobic side chain at this position is confirmed by the observation that binding of F43Y and F43W mutants to class II MHC is equivalent to that of wt CD4. We note that the F43L mutation is fairly well tolerated, while F43I is not (Fig. 2, 65% vs. 0% of specific binding). Partial reduction in adhesion of B cells to a CD4 cDNA-transfected hybridoma containing F43L was also observed by others (43). Leucine, like phenylalanine, has a γ-branched side chain and therefore is more structurally related to phenylalanine than isoleucine with its β-branched side chain. These results strongly suggest that Phe-43 in CD4 interacts with a specific hydrophobic pocket in class II MHC and that the shape of the side chain is important in addition to its hydrophobicity.

The mutant CD4 molecules S49V and W62Y were also unable to bind to class II MHC (Table 1). These two residues are buried in the hydrophobic core of CD4 domain 1 (see Fig. 3*B*). The changes in buried residues were chosen to perturb conformation at the "top" and "bottom" of the C′C″ ridge. Trp-62 is a strut that supports the projecting C′C″ turn; Ser-49 inserts into the C″D corner. The W62Y mutant maintains a hydrogen bonding function of this residue but shifts the position of the potential donor by 2 Å. The S49V mutant eliminates the ability of the residue to interact with a buried water molecule. The preservation of CD4 mAb-binding [S49V, all mAbs mentioned above; W62Y, all except 18T3A9 and 19Thy5D7 (45)] indicates that S49V and W62Y introduce

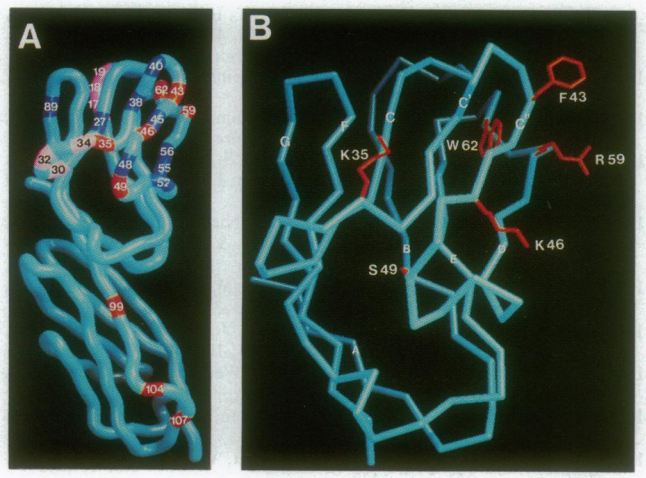

FIG. 3. (*A*) Representation of the polypeptide backbone CD4, domains 1 and 2. Residues mutated in this work are shown in contrasting colors and identified by sequence number. The color scheme is as follows: blue, no effect (++ according to Table 1); purple, modest reduction (+); pink and red, substantial or complete loss of rosette formation (+/− and −). (*B*) Representation of the polypeptide backbone of CD4 domain 1, with labeled side chains of residues in the C′C″ ridge that, following substitution, resulted in substantial or complete loss of rosette formation. Note that side chains of Ser-49 and Trp-62 lie within the cage defined by the backbone, whereas the side chains of Lys-35, Phe-43, Lys-46, and Arg-59 project outward.

only local perturbations. The exposed residues we have altered also probe the "top" (Phe-43 and Arg-59) and the "bottom" (Lys-35 and -46) of the ridge (Fig. 3*B*). Both groups affect class II MHC interactions. Thus, the simplest conclusion is that the entire C′C″ ridge has a role in the adhesion interaction with class II MHC molecules.

Clayton *et al.* (10) have reported strong effects from changes in the CC′ (mutant M1B), and C″D corners of domain 1 as well as from changes in the A strand of domain 2 (mutant M8), which lies on the same side of the CD4 molecule. Those studies also identified significant but weaker effects from changes in the BC loop of domain 1 (mutant M1.1), the E strand of domain 1, and the CC′ turn of domain 2. The effects of mutants M1.1, M1B, and M8 are confirmed independently here (Table 1). Fig. 3*A* shows the location of these residues in the first and second domain of CD4. Thus, other parts of domains 1 and 2 are involved in the interaction with class II molecules. Reports by Bowman *et al.* (43) and Fleury *et al.* (29) also showed interference from changes in strands C′ and C″ of domain 1. These papers demonstrate negative effects of proline substitutions at either position 40 (ref. 43) or 45 (ref. 29), both of which are expected to perturb the C′C″ corner by destroying the geometry or by eliminating a β-sheet hydrogen bond. By contrast, T45A, which should not interrupt the β-ribbon, is without effect on class II MHC binding (Table 1).

Mutations of CD4 Affecting Class II MHC Binding Are Essentially Independent of MHC Polymorphism. To discern whether the importance of the exposed charged residues, Lys-35 and -46, and the exposed hydrophobic residue, Phe-43, was restricted to the particular MHC alleles expressed on Raji cells, an additional set of experiments was performed with genotypically unrelated B lymphocytes. CD4 transfectants expressing K35A, K46A, or F43I mutations were partially or completely unable to bind to each of five additional homozygous and one heterozygous B lymphoblastoid cell lines of defined and distinct *HLA-DR*, *-DP*, and *-DQ* genotypes (Table 2). Binding to class II-expressing homozygous B cells by cells transfected with Q89L mutant was equivalent to that of the wt CD4 transfectants. A modest but definite decrease in B-cell binding was observed with the neighboring S19Y mutant, however.

Overlap Between Class II MHC and HIV gp120 Binding Sites. Data from a number of laboratories provide strong evidence that the HIV gp120 binding site on CD4 involves the C′C″ ridge (26). We have recently investigated in greater detail the binding of HIV gp120 to CD4, utilizing the panel of mutants reported here, by both equilibrium and kinetic analysis (45). These studies show that the binding site on CD4 appears to be a surface region of 900 Å^2 on the C″ edge of the first domain. This site contains the exposed hydrophobic residue, Phe-43, in the C′C″ loop and four positively charged residues, Lys-29, -35, -46, and Arg-59 on the C, C′, C″ and D strands, respectively. Replacement of Phe-43 with alanine or isoleucine reduces affinity for gp120 by a factor of >500. The four positively charged sidechains each make significant contributions (7- to 50-fold). Thus, it is particularly striking that Phe-43, Lys-35, Lys-46, and Arg-59 also influence CD4-class II binding. Indeed, these data suggest that there may be a chemical similarity in the nature of the contacts between gp120 and CD4 and class II MHC and CD4. HIV may have usurped a component of the class II MHC binding site to prevent helper T-cell activation and thereby to evade the immune response.

The present results lead to different conclusions from those of others, who have found that deletion of the C″ strand of CD4 (29) or extensive mutation of the C′C″ corner (28) has little if any effect on class II MHC binding. One of the papers (29) does report that certain changes in the C″ ridge (at positions 45 and 47) strongly inhibit both adhesion and activation, but based on their results with the C″ deletion mutant, the authors conclude that the C′C″ ridge of domain 1 is not involved in class II MHC binding. Because our

Table 2. Analysis of CD4 mutations on rosette formation of class II MHC-typed lymphoblastoid B cells

B-cell line	Genotype of class II *HLA* locus				Rosette formation of cells					
						CD4 mutants				
	DRB1	*DQA*	*DQB*	*DP*	wt	S19Y	Q89L	F43I	K35A	K46A
Raji	*0301/1001*	*0101/0501*	*0201/0501*	ND	++	+	++	−	+/−	−
Boleth	*0401*	*0301*	*0302*	*0401*	++	+/−	++	−	ND	ND
BM-15	*1102*	*0501*	*0301*	*0201*	++	+	++	−	+/−	−
TEM	*1401*	*0101*	*0503*	*0401*	++	++	++	−	+/−	+/−
Pitout	*07*	*0201*	*0201*	*0401*	++	+	++	−	−	−
DKB	*0901*	*0301*	*0303*	*0401*	++	+/−	++	−	−	−
T5-1*	*01/03*		*01/02*	X/*04*†	++	+	+	−	ND	−

Analysis of class II MHC-typed human B-cell lines for binding to CD4 mutants, measured by rosette formation as in Table 1. Raji and T5-1 are heterozygous B-cell lines, and the remaining B-cell lines are homozygous typing cells. ND, not done. Line 6.1.6 is a mutant of cell line T5-1 lacking expression of all class II MHC molecules that failed to bind wt or mutant CD4 transfectants (not shown and refs. 10, 37, and 40).
*T5-1 assignments were made by serotyping.
†X, detection of only one specificity.

studies utilize B lymphocytes expressing different *HLA-DR*, *-DP*, and *-DQ* alleles and because changes in several residues of the C′C″ ridge result in loss of detectable class II MHC binding, we believe that this ridge must be generally important in the class II interaction.

Fleury *et al.* (29) report that class II MHC binding is prevented by mutations at CD4 positions 19, 89, and 165, which lie on the side of CD4 opposite to the C′C″ ridge (see Fig. 3*A*), and they focus on this region in interpreting their results. In the binding assay used here, the Q89L mutant is essentially unaltered in B-cell adhesion, but the S19Y mutant is slightly affected. It is possible that CD4 residues 19 and 89 are crucial for binding of CD4 to the particular HLA-DP molecule studied by Fleury *et al.* (29) but less important for other MHC proteins.

Implications. It is clear from our own data and those of others that the region of class II MHC interaction is more extensive than the HIV gp120 site, even though class II MHC binding is apparently of substantially lower affinity (10, 28, 29). Of the mutations studied here that lie outside the HIV gp120 binding site, G99K, S104P, and H107S on the A strand of CD4 domain 2 (M8 mutant) lie on the same side of the CD4 molecule as the C′C″ ridge (Fig. 3*A*), potentially consistent with a simple, extended contact surface. This site forms part of the notch between domains 1 and 2 into which class II MHC might bind. In contrast, Ser-19 lies on the opposite side of CD4 domain 1 as do residues Thr-17 and Ala-18 of the M1.1 mutant and Ser-132, Pro-133, and Asn-137 of the M11 mutant, as well as Gln-165. These residues also affect class II MHC binding (Table 1; ref. 10). We believe it unlikely that these mutations can have an indirect effect, for example by a subtle but distinct distortion of the CD4 structure elsewhere, undetected by the mAbs we tested. Rather, we suggest that the site of interaction of CD4 and class II MHC extends from the C′C″ ridge on one margin, across the region between domains 1 and 2 on the BED (B–E–D) face of domain 1, and around to the side of CD4 opposite the C′C″ ridge (residues 17–19). Parts of domain 2 are also contacted. We note that the class I MHC molecule has a deep notch between its two nonpolymorphic Ig-like domains and its antigen-presenting "platform" (46). If class II MHC molecules have a similar structure (47), then they could indeed be complementary to the complex CD4 surface we have just described. We cannot rule out the additional possibility that CD4 oligomerization may be needed to form a functional MHC binding site. A single site might then be created by juxtaposition of opposite sides of two CD4 molecules, or one surface of CD4 might be involved in a dimer interface and the opposite surface might be the actual interface interacting with class II molecules. Whatever the explanation, the data presented here indicate that the CD4 binding site for gp120 is contained in the larger class II MHC interaction site on CD4.

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