Involvement of Both Major Histocompatibility Complex Class II α and β Chains in CD4 Function Indicates a Role for Ordered Oligomerization in T Cell Activation

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Summary

CD4 is a membrane glycoprotein on T lymphocytes that binds to the same peptide:major histocompatibility complex (MHC) class II molecule recognized by the antigen-specific receptor (TCR), thereby stabilizing interactions between the TCR and peptide:MHC class II complexes and promoting the localization of the *src* family tyrosine kinase $p56^{lck}$ into the receptor complex. Previous studies identified a solvent-exposed loop on the class II $\beta 2$ domain necessary for binding to CD4 and for eliciting CD4 coreceptor activity. Here, we demonstrate that a second surface-exposed segment of class II is also critical for CD4 function. This site is in the $\alpha 2$ domain, positioned in single class II heterodimers in such a way that it cannot simultaneously interact with the same CD4 molecule as the $\beta 2$ site. The ability of mutations at either site to diminish CD4 function therefore indicates that specifically organized CD4 and/or MHC class II oligomers play a critical role in coreceptor-dependent T cell activation.

CD4 is an integral membrane glycoprotein that functions as a coreceptor for α/β TCRs specific for MHC class II molecule:peptide complexes (1–3). It is able to bind to MHC class II proteins expressed on cell surfaces (4, 5) and can interact simultaneously with the TCR and MHC class II during T cell interactions with antigen-presenting cells (6–9), stabilizing interactions between these molecules. In addition, CD4 promotes the localization of the *src* family tyrosine kinase $p56^{lck}$ in the receptor complex (10, 11). These two functions of CD4 regulate this molecule's contribution to antigen-specific thymic selection (12) and peripheral T cell activation (13, 14). In T cells restricted by MHC class I molecules, CD8 performs functions similar to those of CD4 in MHC class II–restricted T cells (15, 16).

MHC class II glycoproteins that interact with CD4 are heterodimers consisting of two non-covalently associated integral membrane polypeptide chains, α and β (17, 18). Both chains fold to form two distinct protein domains, with the highly polymorphic $\alpha 1$ and $\beta 1$ domains combining to form a single membrane-distal antigen-binding region (19, 20). Conversely, the membrane-proximal domains are relatively nonpolymorphic and consist of typical immunoglobulin-like domains that are frequently involved in receptor:counter-receptor interactions between hematopoietic cells.

Previous studies identified a solvent-exposed loop on the membrane-proximal $\beta 2$ domain of class II molecules that is necessary for binding to CD4 and for eliciting CD4 coreceptor activity (21, 22). Here, we demonstrate that a second surface-exposed segment of the class II heterodimer located in the $\alpha 2$ domain is also critical for CD4 function. This site is positioned in such a way that it does not appear able to simultaneously interact with the same CD4 molecule as the $\beta 2$ site. These results impact on our understanding of coreceptor-dependent TCR complex formation during T cell activation, indicating the importance of organized CD4 and/or MHC class II oligomerization. The class II $\beta 2$ and $\alpha 2$ regions that interact with CD4 are brought next to one another in the recently described crystallographic structure of the class II "dimer of dimers" (20), suggesting an intriguing molecular model for this oligomerization event.

Materials and Methods

Cells. DO.11.10 cells (23) were transduced with cDNA expression vectors encoding the human CD4, mouse CD4, or mouse CD8α coreceptors and selected as described (22). The 171.3 T cell hybridoma lines were kindly provided by Drs. Glaichenhaus and Shastri (24). All hybridomas were grown in RPMI 1640 containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10 mM Hepes, 50 μM 2-mercaptoethanol, and 250 μg/ml of the neomycin analogue G418 at 37°C and 5% CO_2. Murine DAP.3 L cells were transfected with wild-type or mutant $A\alpha^d$ cDNA expression plasmid DNA (5 μg) together with wild-type $A\beta^d$ cDNA expression plasmid DNA (5 μg) and the selective marker pMo-neo (0.4

μg) using calcium phosphate coprecipitation (22, 25). Homogenous populations of cells expressing high levels of wild-type or mutant $A\alpha^dA\beta^d$ heterodimers were selected by antibody-mediated magnetic bead sorting (22). The structural integrity of mutant $A\alpha^dA\beta^d$ molecules was determined by antibody dilution assays (26) using the mAbs K24-199 (27) (specific for polymorphic residues in the α1 domain), MK-D6 (28) (specific for polymorphic residues in the β1 domain), and M5/114 (29) (specific for a β1 epitope of assembled α/β heterodimers). Transfected L cells were maintained in DMEM containing the same additives as described above for RPMI 1640 at 37°C and 10% CO_2.

Mutagenesis. Mutations were generated either by the gapped heteroduplex method (22, 30) for S125G, S127T, V128A, G131A, Y133S, and βE137A.V142A, or by PCR and splicing by overlap extension (22, 31). PCR and splicing by overlap extension conditions were as follows: 1 min at 94°C, 1 min at 40°C, 1 min at 50°C, 2 min at 72°C for 30 cycles followed by one cycle of 7 min at 72°C in a DNA thermal cycler (Ericomp, Inc., San Diego, CA). Mutant cDNAs were subcloned into the EcoRI site of the expression vector pcEXV-3 (32), and sequenced from double-stranded templates either by dideoxynucleotide sequencing using Sequenase version 2.0 T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, OH) and the Multiwell sequencing kit (Amersham Corp., Arlington Heights, IL) or by cycle sequencing using Taq DNA polymerase (Perkin-Elmer Corp., Norwalk, CT). Cycle sequencing reactions were analyzed on a DNA sequence analyzer (model 373A; Applied Biosystems, Inc., Foster City, CA).

Functional T Cell Assays. DO.11.10 hybridoma cells were stimulated with the synthetic ovalbumin peptide 323-339 and 171.3 cells with the bacterial superantigen staphylococcal enterotoxin A (Toxin Technology, Sarasota, FL). Duplicate cultures of T hybridoma cells (5×10^4 cells/well) with $A\alpha^dA\beta^d$-expressing L cells (5×10^4 cells/well) in flat-bottomed 96-well plates in a total vol of 200 μl of complete RPMI 1640 medium containing the indicated antigen concentrations were cultured for 24 h. Culture supernatants of duplicate wells were pooled and assayed for IL-2 and IL-3 content by their ability to support the proliferation of the indicator cell lines CTLL-2 and FD.C/1, respectively. For each culture supernatant, three threefold (1:6 to 1:54 final concentration for IL-2) or four fourfold (1:38 to 1:3,056 final concentration for IL-3) dilutions were tested in triplicate on 1×10^4 CTLL-2 or 5×10^3 FD.C/1 cells. Incubations were done in flat-bottomed 96-well plates in a total volume of 200 μl complete RPMI 1640 for 28 and 44 h, respectively. For the last 4 h of the incubation, 1 μCi of [^{3}H]thymidine was added. Cells were then harvested with a harvester (Filtermate 196; Packard Instrument Co., Inc., Meriden, CT) and [^{3}H]thymidine incorporation was determined using a direct beta counter (Matrix 9600; Packard). Lymphokine units were calculated as described (26), and are expressed in units per ml^{-1}. To test for experimental rather than biological variability, select assays were cultured in triplicate and supernatants were carried through separately to obtain triplicate measurements of the cytokine response per concentration point. At lymphokine concentrations below those giving the maximum proliferation of the indicator cells, the results were essentially identical to those obtained when replicate supernatants were pooled before determining lymphokine concentrations. In addition, there were no differences in the qualitative conclusions concerning the effects of individual mutations whether lymphokine concentrations were determined from dilution curves or the counts per minute incorporated at each supernatant dilution were plotted against antigen concentration. Lymphokine unit assays, however, more accurately reflected differences in responses at high antigen concentrations.

Molecular Modeling. The MHC class II images were generated using Quanta software (Polygen Corp., Waltham, MA) on a Silicon Graphics workstation (Mountain View, CA) from coordinates corresponding to HLA-DR1 as determined by Brown et al. (20).

Results and Discussion

Experimental Approach. Structural homologies (19, 20, 33–36) and similar functions (37) of MHC class I and class II molecules suggest analogies in the mechanism of CD8-MHC class I and CD4-MHC class II interactions. Indeed, the previous identification of the class II β2 domain's interaction with CD4 (amino acid residues 137–143) was based in part on the assumption that the binding site for CD4 might lie in a position analogous to the CD8- binding site on MHC class I molecules. An x-ray crystallographic structure had been determined for the human class I molecule, HLA-A2 (38), and a binding site for CD8 on the α3 domain of HLA-A2 had been identified (39) before these published CD4 studies. Although the class II β2 domain was the predicted homologue of the class I α3 domain, the lack of a crystallographic structure for class II at that time left open the possibility that the class II α2 domain might actually be the relevant domain for CD4 binding. Sequence alignment of the β2 domain of MHC class II with the class I α3 domain yielded a high degree of similarity, but alignment of the class II α2 domain with the MHC class I–associated β_2-microglobulin was not as good. In fact, the MHC class II α2 domain aligns better with the class I α3 domain than with β_2-microglobulin. We therefore introduced mutations in both the class II β2 and the α2 domains. Analysis of mutants carrying amino acid substitutions in a surface-exposed loop of the β2 domain rapidly revealed the presence of a critical CD4 contact site, but several clustered mutations in the α2 domain also diminished function with CD4. We have now carefully analyzed this highly conserved second region comprising Aα amino acids 125–133 (numbering of residues is according to Kabat et al.[40]).

Single or double mutations were made in an $A\alpha^d$ cDNA throughout this region. Two types of mutations were generated: human for mouse substitutions and alanine replacements. In addition, to sterically interfere with CD4-MHC class II interactions, we attempted to introduce potential N-linked glycosylation sites at positions 127 and 129 of the $A\alpha^d$ molecule. These mutants, however, were not expressed on the cell surface of transiently transfected COS or stably transfected mouse L cells. On the other hand, the corresponding control constructs carrying an asparagine residue at positions 127 or 129 and an alanine or glycine residue at positions 129 or 131, respectively, were surface expressed, and are included in this analysis.

The mutant $A\alpha^d$ cDNA constructs were cotransfected into L cells together with a cDNA construct encoding wild-type $A\beta^d$ and a plasmid carrying a neomycin-resistance gene. Stable transfectants were selected in G418-containing medium, and cells expressing high levels of the A^d α/β heterodimer were isolated by antibody-mediated magnetic bead sorting.

All mutant $A\alpha^d$ chains included in this report supported efficient surface MHC class II expression. These mutant heterodimers reacted appropriately with a panel of monoclonal anti-A^d antibodies, indicating that the mutations did not grossly affect the folding and conformation of the class II α chain or the assembled α/β heterodimer (data not shown).

The various transfected L cells were used as antigen-presenting cells for the stimulation of two sets of T cell hybridomas, derived from the parental lines DO.11.10 (23) and 171.3 (24). Members of each set express the same TCR, but differ in coreceptor expression, having on their surface either human CD4 (hCD4), mouse CD4 (mCD4), mouse CD8 (mCD8), or no coreceptor at all. The DO.11.10 hybridoma responds to stimulation by APC plus peptide antigen with lymphokine secretion even in the absence of CD4 expression. It is therefore possible to selectively assess the effect of sequence changes in $A\alpha^d$ on the capacity of the α/β heterodimer to elicit CD4 coreceptor function by comparing the responses to mutant vs. wild-type class II molecules of DO.11.10 cells with different coreceptor expression. Mutations with selective effects on CD4 coreceptor function but without effects on peptide binding or TCR recognition will not affect the responses of the CD4-negative T cell hybridomas, which therefore can serve as a control to render this assay independent of the precise level of class II expression on the individual transfectant L cell lines, or of differences in the absolute level of cytokine production by the individual T hybridoma cell lines. In contrast, the 171.3 hybridoma requires CD4 expression for responses to peptide or toxin. By using transfectants with comparable levels of cell-surface MHC class II expression, the hybridoma set based on 171.3 can be used to confirm data obtained using the DO.11.10-based set of hybridomas. This is the same assay used previously to identify the CD4 interaction site on the class II $\beta 2$ domain (22).

Effects of Mutations. In the presence of wild-type $A\alpha^dA\beta^d$, DO.11.10 T cells expressing either hCD4 or mCD4 secreted more IL-3 than mCD8-bearing cells (Fig. 1, *A* and *F*, and Fig. 2 *A*), responding more vigorously than the mCD8-expressing line at any given dose of antigen. Substitution of glycine for serine at position 125 in the α chain strongly reduced the response of the mCD4-expressing DO.11.10 cells at all antigen concentrations, but did not affect the response of the hCD4-bearing cells (Fig. 1 *C*). This mutation is an example of an isotypic substitution at an amino acid highly conserved between alleles of a particular isotype, with serine occurring in all alleles of $A\alpha$, and glycine in $E\alpha$ (as well as all human class II MHC isotypes). This finding is consistent with functional data suggesting CD4 interacts less well with $E\alpha E\beta$ than with $A\alpha A\beta$ (41). Another mutation of this type, substitution of serine for tyrosine at position 133, had no effect on either the hCD4 or the mCD4-expressing hybridomas (Fig. 3). Alanine substitution at positions 129 (Fig. 2 *C*) and 131 (Fig. 1 *D*) in the α chain substantially decreased responses from both hCD4 and mCD4-bearing cells, as did substitution of asparagine for threonine at position 129 (Figs. 1 *G* and 2 *D*), and two different double mutations also involving residue 129 (Figs. 1 *H*, 2 *E*, and 3). In contrast, the capacity of these mutants to elicit responses from the mCD8-expressing DO.11.10 cells remained wild type–like. As a comparison, we have included the previously described double alanine mutation in the $A^d\beta 2$ domain at positions 137 and 142 (Fig. 1 *J*), which abrogates CD4 coreceptor function in this assay (22). Mutations decreasing CD4 function in these DO.11.10 experiments also reduced cytokine production by both hCD4- and mCD4-expressing 171.3 cells relative to stimulation with wild-type $A\alpha A\beta$ molecules (Fig. 1, *K–N*).

Asparagine substitution of threonine 129 drastically reduced function with both hCD4 and mCD4 (Figs. 1 *G* and 2 *D*). Function with hCD4 was also eliminated by combining the alanine substitution at position 125 with one at position 129 (Figs. 1 *H* and 2 *E*). Conversely, substituting glycine for serine at position 125 (a change that introduces the conserved human residue at this position) dramatically decreased function with mCD4, but did not affect function with hCD4. To determine the individual contributions of alanine substitutions at positions 125 and 129, we compared the former with the double alanine mutation and the asparagine for threonine substitution at 129 (Fig. 2). This experiment demonstrated the major contribution of the alanine for serine substitution at 125 to the decrease in function with hCD4, and the equivalency of alanine and asparagine substitutions at 129.

The effects of mutations in the $\alpha 2$ domain of $I\text{-}A^d$ appeared greatest at low to moderate antigen concentrations. Mutations judged to decrease function with CD4 reduced responses by $CD4^+$ T hybridomas to a level equal or only slightly higher than that seen with the $mCD8^+$ control hybridoma cells. As seen in some of the antigen dose-response curves depicted in Fig. 1, the effects of a partial loss of MHC class II–induced CD4 function could be overcome at very high antigen concentrations. To standardize data from multiple experiments in which absolute responses varied, we used a transformation formula that accounts for the range in the dose-response curve covered by low to moderate antigen concentrations. This formula gives an estimate of the residual CD4 coreceptor function induced by the respective mutant $A\alpha^dA\beta^d$ molecule with wild type–like function represented by a fractional value of 1 and a value of 0 given to mutant $A\alpha^dA\beta^d$ molecules that fail to induce an increased response by the $CD4^+$ T hybridoma cells as compared with the $CD8^+$ control cells. The fractional CD4 coreceptor function was defined as: $F = \log f$ [mutant $A\alpha^dA\beta^d$]/$\log f$ [$A\alpha^dA\beta^d$]; where f represents the ratio between the antigen concentration necessary to half-maximally stimulate the $CD8^+$ control T hybridoma and the antigen concentration required to cause equal cytokine secretion by T hybridoma cells bearing either mCD4 or hCD4.

Fig. 3 summarizes the results of several independent experiments of the type illustrated in Figs. 1 and 2 using the transformation formula just described. In each individual experiment, this formula attributes a value of $F = 1$ to wild-type $A\alpha^dA\beta^d$. The shaded area in the panels of Fig. 3 represents the range of F values seen in repeated experiments with mutations we judge to have wild-type function with CD4, because they led to only small effects of inconsistent

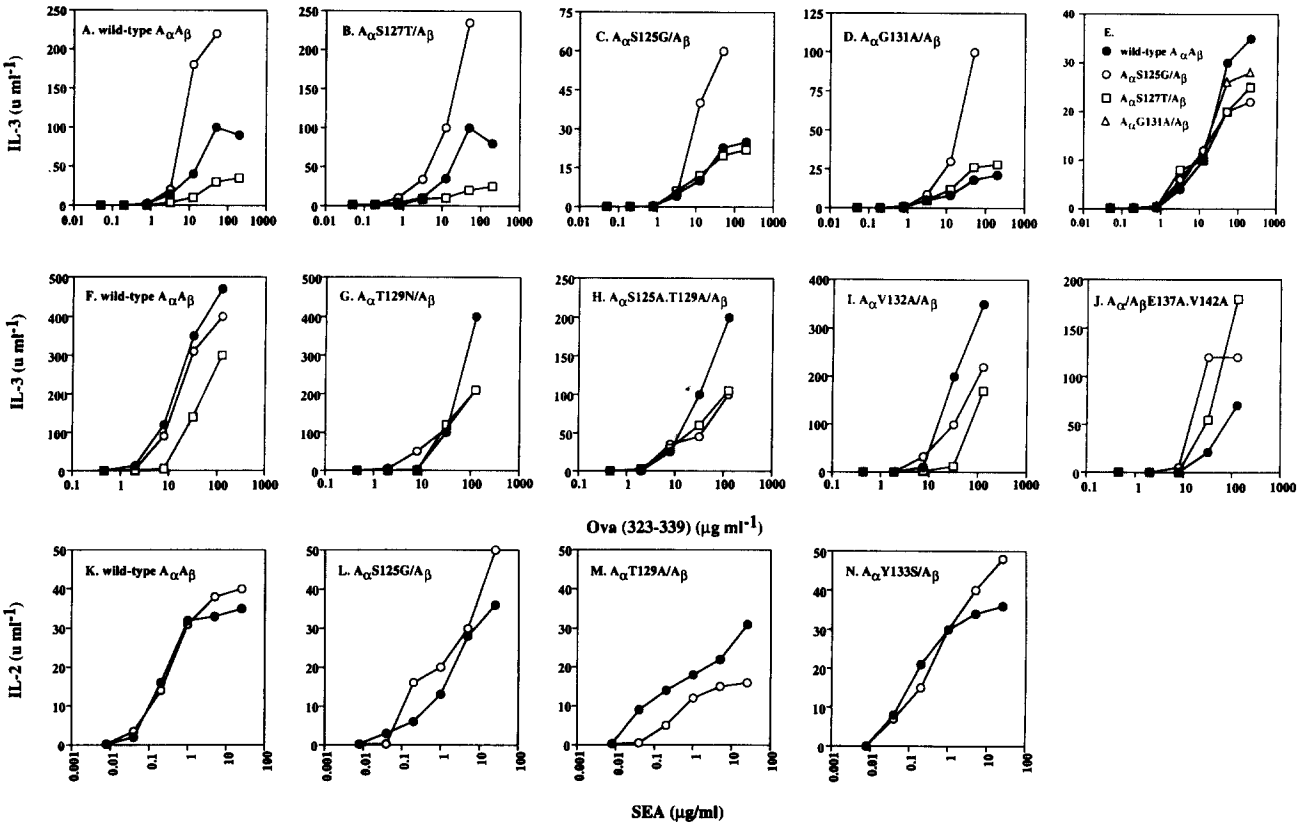


Figure 1. Selective mutations in the region of Aα formed by amino acids 125–133 decrease CD4 coreceptor function in T cell assays. Antigen dose-response curves for murine T hybridoma DO.11.10 cells expressing either mouse (*closed circles*) or human CD4 (*open circles*), or mouse CD8 (*open squares*) after stimulation with antigen (ovalbumin peptide 323.339) presented by wild-type or mutant $A\alpha^dA\beta^d$ transfected L cells (*A–J*), and for hybridoma 171.3 cells expressing either mCD4 (*closed circles*) or hCD4 (*open circles*) after stimulation with staphylococcal enterotoxin A and similar transfectants (*K–N*). Coreceptor-negative 171.3 cells did not secrete lymphokine at any concentration of antigen tested. Numbering of residues is according to Kabat et al. (40). Mutants are identified by the wild-type residue at the indicated position followed by the new residue present in the mutant protein. Data in panels presented in one row are from the same experiment. Results for the following transfectants are shown: (*A*) wild-type $A\alpha^dA\beta^d$; (*B*) AαS127T; (*C*) AαS125G; (*D*) AαG131A. In *E*, dose-response curves for the mCD8+ DO.11.10 stimulated with antigen plus wild-type or mutant $A\alpha^dA\beta^d$-expressing L cells are depicted to show the comparable capacity of the different $A\alpha^dA\beta^d$ molecules to provide CD4-independent T cell stimulation (*closed circles*, wild-type $A\alpha^dA\beta^d$; *open circles*, AαS125G; *squares*, AαS127T; *triangles*, AαG131A); (*F*) wild-type $A\alpha^dA\beta^d$; (*G*) AαT129N; (*H*) AαS125A.T129A; (*I*) AαV132A; (*J*) the previously described mutant AβE137A.V142A is shown for comparison; (*K*) wild-type $A\alpha^dA\beta^d$; (*L*) AαS125G; (*M*) AαT129A; (*N*) AαY133S. In each row of panels, one mutation that did not affect function with either mCD4 or hCD4 is shown (*B*, *I*, and *N*). For experiments with the 171.3 hybridomas, wild-type and mutant transfectants were selected for closely matched levels of surface class II expression.

direction (e.g., 0.78–1.1 for S127T with mCD4; 0.96–1.18 for V132A with mCD4). Bars that do not reach these shaded areas or extend beyond them therefore identify mutations that consistently affect function with CD4 in a single direction (decrease or increase, respectively) and to an extent greater than the experimental variability in this system. The data indicate that amino acids 125–131 of Aα are part of a site necessary for optimal class II–dependent CD4 coreceptor function. Threonine 129 appeared to be particularly important for mediating this interaction, because both alanine substitution and the relatively conservative asparagine replacement drastically reduced function with both hCD4 and mCD4. Glycine for serine substitution at position 125 drastically reduced function with mCD4, but did not affect function with hCD4. On the other hand, alanine for serine substitution at 125 affected function with both. The differential effect of glycine vs. alanine at position 125 on human CD4 vs. mouse CD4 function could reflect the coevolution of the human coreceptor and class II for proper interaction involving this site directly. Alternatively, structural data indicate that residues 125–133 are part of a surface-exposed loop between the C and D strands of the α2 domain (20) (Fig. 4 *A*). Both ends of this loop are defined by glycines in most class II molecules, and mutating residues 125 and 131 may affect the overall conformation of the loop.

Several of the substitutions augmented function with CD4. For example, substitution of serine for tyrosine 133 strongly enhanced function with both mouse and human CD4. Serine is the amino acid occurring at this position in all alleles of HLA-DR and DQ, whereas tyrosine at this position is conserved among alleles of I-A. The increase in function resulting from the serine for tyrosine substitution may be a reflection

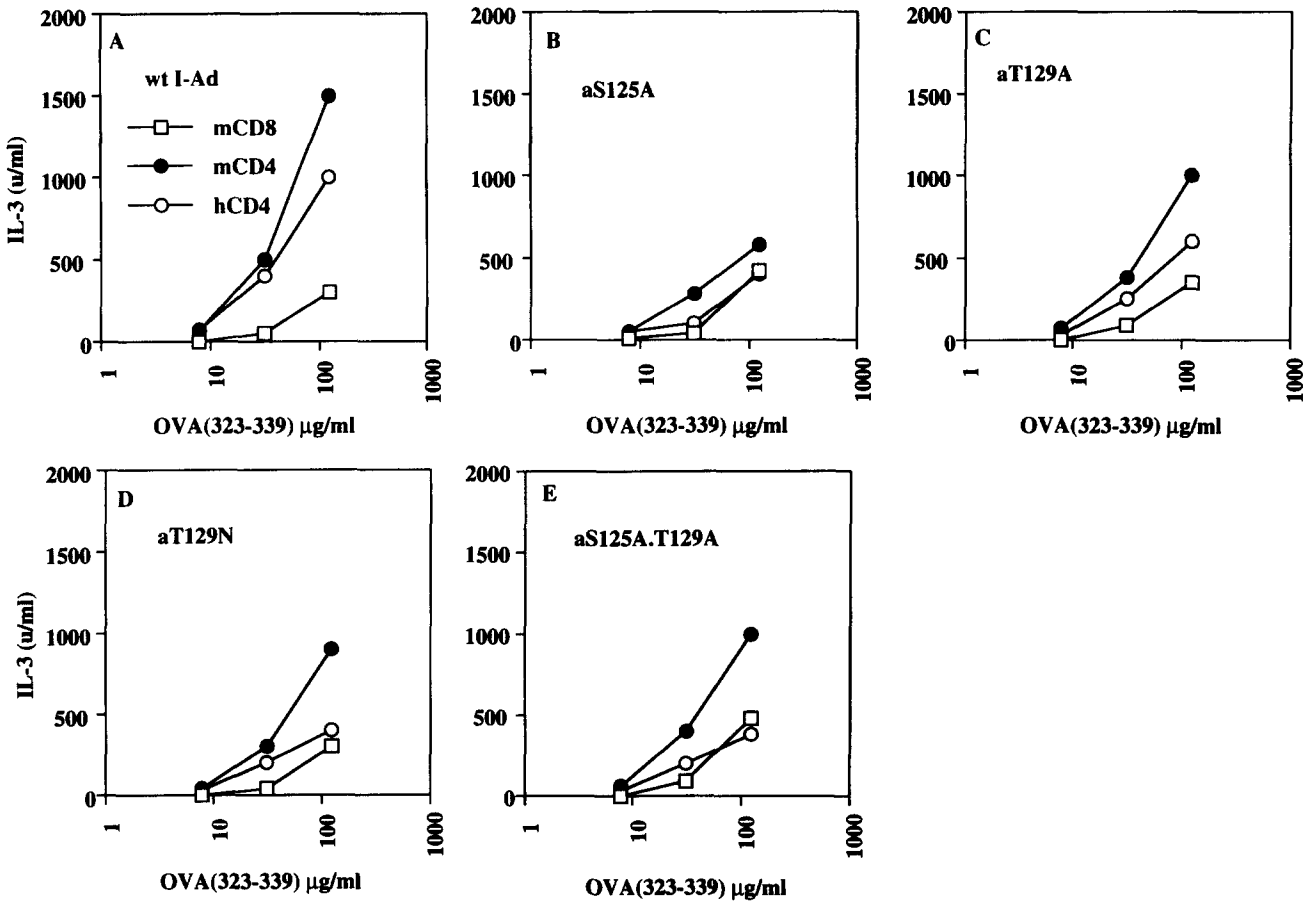


Figure 2. Mutating amino acids 125 and 129 of Aα causes drastic decreases in CD4 coreceptor function. Antigen dose-response curves for T hybridoma DO.11.10 cells after stimulation with ovalbumin peptide 323–339 and wild-type or mutant $A\alpha^dA\beta^d$ transfected L cells are shown. For an explanation of symbols refer to Fig. 1.

of the higher affinity of human CD4 for human class II compared to mouse CD4 and mouse class II (21, 42), and the contribution of serine at this position to stronger binding between these proteins. A simple structural explanation is not as readily apparent for the ability of conservative substitutions S127T and V128A to selectively enhance function with human but not mouse CD4.

Structural and Functional Implications. Computer docking studies show that the elongated, rigid structure of the CD4 D1 and D2 domains involved in interaction with class II (43, 44) and the positioning of the β2 and α2 sites on opposite faces of the class II heterodimer (Fig. 4 *A*) prevent these critical binding regions of single CD4 molecules from simultaneously interacting with both sites on an individual class II molecule (Fleury, S., and R. N. Germain, unpublished observations). Yet the ability of either α or β chain mutations to strongly decrease the capacity of class II molecules to support CD4 function indicates that both sites are required for inducing full CD4 coreceptor activity. Together, these observations provide the first evidence that during T cell activation by physiological ligands, CD4 and class II molecules must undergo a structurally precise dimerization or oligomerization for the effective contribution of CD4 to receptor-dependent signal transduction.

Two different general models for the organization of the CD4–class II complex can be envisioned that satisfy the structural constraints revealed by this study and the available data on CD4 regions affecting interaction with class II (44). In the first model, two CD4 molecules would associate with a single TCR–MHC class II complex, one CD4 molecule binding to the α2 site and the other to the β2 site (Fig. 5 *A*). The two CD4 molecules thus would form a clamp tethering the class II ligand to the TCR. This would also provide a mechanism for activating the protein tyrosine kinase, $p56^{lck}$, upon monovalent TCR–MHC class II interaction, based on CD4 dimerization in this postulated complex. Furthermore, higher order oligomerization of the coreceptor-TCR–MHC class II units might occur, if the free face of each CD4 molecule could engage the alternative site of an adjacent, TCR-engaged class II molecule (Fig. 5 *B*). This model is consistent with data suggesting that two opposite surfaces of the D1-D2 segment of CD4 contribute to class II recognition (44), and with the possible role of such higher order oligomers ("immunons") in lymphocyte signal transduction (45).

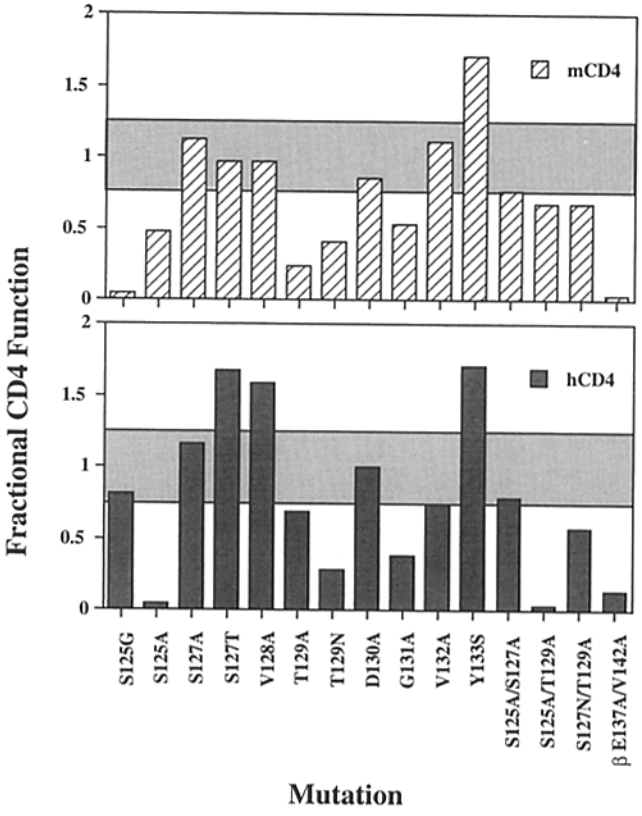
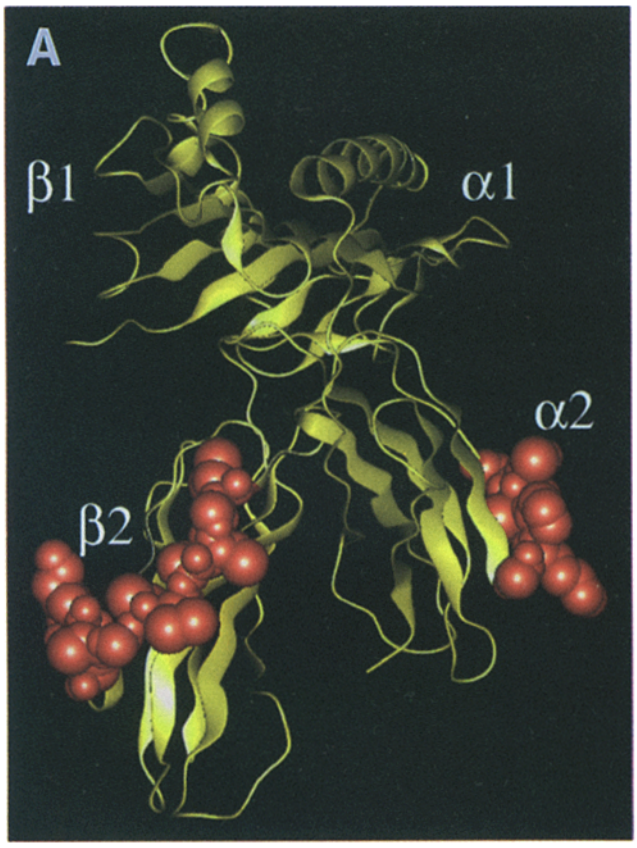
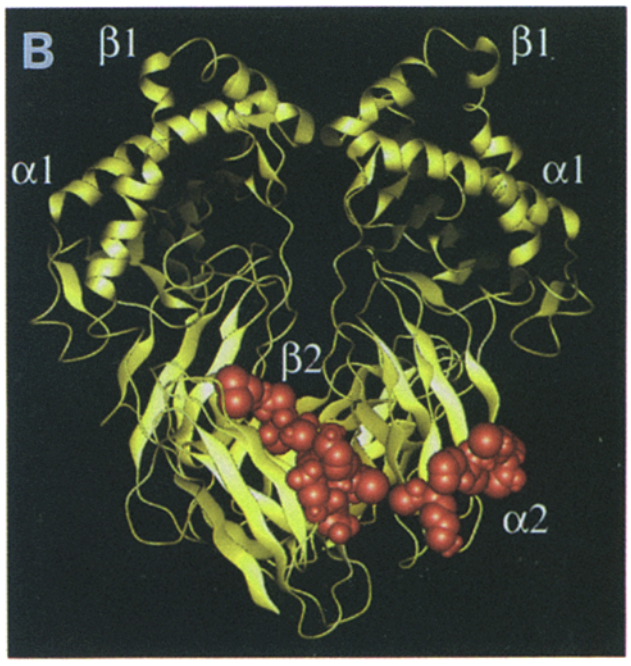


Figure 3. Residual coreceptor function of mouse and human CD4 in response to mutant $A\alpha^dA\beta^d$ molecules. A quantitative evaluation of coreceptor function with each mutant I-A molecule was derived from dose-response curves as in Figs. 1 and 2 by normalizing responses of CD4 coreceptor positive and negative T cell hybridomas to the responses of the same T cells to wild-type $A\alpha^dA\beta^d$. The bars represent mean values of two to six independent experiments (only one experiment was performed for A_αS125A.S127A with hCD4+ DO.11.10 cells). The shaded area represents the range of F values seen in independent experiments using mutant class II molecules considered wild-type in their function with CD4.





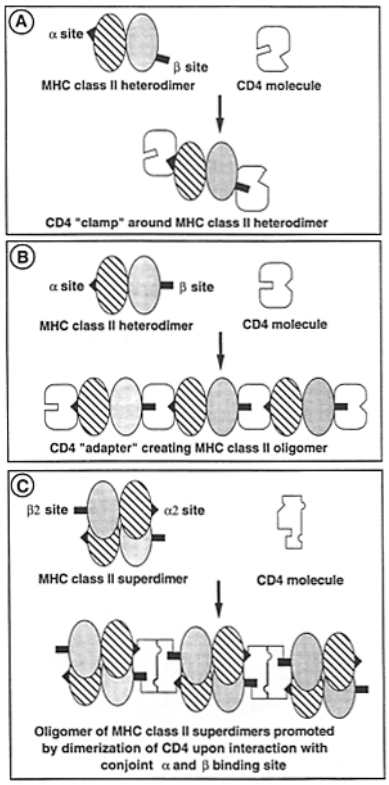


Figure 5. (*A*) Clamp model of CD4 function. One CD4 molecule binds to each of the functionally significant sites on a single MHC class II heterodimer. These two CD4 molecules and the single class II–peptide complex are postulated to interact with a single T cell receptor complex (not shown). (*B*) Adapter model of CD4 function. As in *A*, two CD4 molecules bind to each T cell receptor:MHC class II/peptide complex, but the CD4 also can link individual class II MHC heterodimers to one another by bridging between the α and β chain sites on different class II molecules. (*C*) MHC class II superdimer oligomerization by CD4 molecules with a single binding site for a conjoint $\alpha 2/\beta 2$ target region and a separate dimerization interface.

An intriguing alternative model comes from recognizing that the $\alpha 2$ and $\beta 2$ sites lie in close proximity on a single face of the dimer of dimers observed in crystals of the human class II HLA-DR1 molecule (20) (Fig. 4 *B*). In this configuration, the two discontinuous sites on a single class II dimer would now contribute to a single surface for interaction with CD4, possibly supporting a higher binding affinity than either site alone. CD4 would thus stabilize the interaction of the two heterodimer units of the class II "superdimer" and that of the associated pair of TCR complexes (Fig. 5 *C*). The mapping of regions contributing to CD4–class II interaction to discrete faces of CD4 might then reflect the presence of the actual binding surface on one face, and a possible dimerization interface on the other, that could link TCR–superdimer complexes into large oligomers.

A large body of literature derived primarily from antibody cross-linking experiments supports the importance of coclustering or coaggregation of CD4 and TCR for effective T cell activation, but these prior studies provided no information on the specific molecular associations necessary for proper signaling during physiological cell–cell interaction (6, 7, 15). The identification here of a second discrete binding site for CD4 on class II molecules strongly suggests that structurally specific, ordered oligomerization as opposed to generalized colocalization is a key feature in the assembly of useful signal transduction complexes on T cells. This result extends to the T cell receptor–coreceptor complex the general principle of ligand-induced receptor dimerization/oligomerization as a key event in the initiation of transmembrane signaling (46). The critical role of these stable, properly assembled dimers or oligomers in T cell activation also suggests that the partial agonist or antagonist properties of variant TCR–MHC:peptide complexes (47–49) may result from improper formation or altered stability of these critical molecular assemblies (50–53).

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Figure 4. (*A*) The two sites affecting class II–CD4 interaction lie on opposite sides of a single MHC class II heterodimer. The image is a side view of an MHC class II molecule (*yellow ribbon drawing*), with the residues of both the 137-147 loop in the $\beta 2$ domain and the 125–131 region in the $\alpha 2$ domain that affect interaction with CD4 shown in red. The domains of the class II molecule are labeled. (*B*) The two sites affecting class II-CD4 interaction lie next to one another in the dimer of dimers crystal structure of MHC class II. The image is a side view of the dimer of dimers (*yellow ribbon drawing*), with the residues of both the 137–147 loop in the $\beta 2$ domain and the 125–131 region in the $\alpha 2$ domain that affect interaction with CD4 shown in red. The domains of each class II molecule are labeled.

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