CD8αβ Has Two Distinct Binding Modes of Interaction with Peptide-Major Histocompatibility Complex Class I*

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Interaction of CD8 (CD8α or CD8αβ) with the peptide-major histocompatibility complex (MHC) class I (pMHCI) is critical for the development and function of cytolytic T cells. Although the crystal structure of CD8α-α′pMHCI complex revealed that two symmetric CD8α subunits interact with pMHCI asymometrically, with one subunit engaged in more extensive interaction than the other, the details of the interaction between the CD8αβ heterodimer and pMHCI remained unknown. The Ig-like domains of mouse CD8αβ and CD8α are similar in the size, shape, and surface electrostatic potential of their pMHCI-binding regions, suggesting that their interactions with pMHCI could be very similar. Indeed, we found that the CD8α variants CD8αβ BRA and CD8αβ E27A, which were functionally inactive as homodimers, could form an active co-receptor with wild-type (WT) CD8β as a CD8αβ BRA or CD8αβ E27A β heterodimer. We also identified CD8β variants that could form active receptors with WT CD8αβ but not with CD8αβ BRA. This observation is consistent with the notion that the CD8β subunit may replace either CD8α subunit in CD8αα′pMHCI complex. In addition, we showed that both anti-CD8α and anti-CD8β antibodies were unable to completely block the co-receptor activity of WT CD8αβ. We propose that CD8αβ binds to pMHCI in at least two distinguishable orientations.

CD8 is a co-receptor that enhances the presentation of peptide antigen complexed with MHC class I molecule (pMHCI) to the T-cell receptor (1–3). Cell-surface CD8 is assembled as either CD8αα′ homodimers or CD8αβ heterodimers (4–6). Although CD8αα′ and CD8αβ are structurally similar (7), they differ in tissue distribution, ligand specificity, and efficiency of antigen presentation (8, 9). Although CD8αβ is expressed primarily on the surface of αβ TCR+ thymocytes and peripheral T cells, CD8α has a much broader expression pattern, including the αβ TCR+ and γδ TCR+ intestinal intraepithelial lymphocytes, natural killer cells, and dendritic cells (9–11). CD8αβ has been shown to be a more efficient co-receptor than CD8α for presentation of a given antigen (12–14). However, the underlying mechanism for the enhanced efficiency is not well understood.

Both the extracellular domain and the cytoplasmic tail of the CD8β subunit have been implicated in providing increased efficiency of CD8αβ (12–16). We reported previously that the extracellular domain of the CD8β subunit is critical for this enhanced efficiency (14) and that introduction of the CD8β stalk region is sufficient to confer a CD8αβ-like co-receptor efficiency to the CD8αα′ homodimer (17). In addition, the sialylation of the O-linked glycans in the CD8β stalk region is differentiation stage-dependent and may modulate the intrinsic activity of CD8αβ during the transition from double-positive (CD4+CD8+) to single-positive (CD8+) T cells (18, 19). It was also reported that palmitoylation of a membrane-proximal cysteine residue in the cytoplasmic tail of CD8β during T-cell activation facilitates partition of CD8αβ heterodimers into lipid rafts, where it associates with the CD3 component of TCR complexes (20). Lastly, the kinase activity of p56lck, which is associated only with the cytoplasmic domain of CD8α, can be enhanced by CD8β (21). It is conceivable that the extracellular domain and the cytoplasmic tail of CD8β may independently contribute to the enhanced co-receptor activity of CD8αβ.

The interaction of CD8αβ and pMHCI has been elucidated through x-ray analyses of the crystal structures of human CD8αα′HLA-A2 and mouse CD8αα′H2-Kb (22, 23). This structural information confirms the results of many mutational studies. The complementarity-determining region-like loops of CD8α and the MHC α3 domain CD loop (residues 220–228) are the most critical for CD8αα′pMHCI interaction (24–26). In both human and mouse CD8αα′pMHCI complexes, two symmetric CD8α subunits clamp asymmetrically onto the relatively rigid MHC α3 domain CD loop. One subunit, CD8α1, is involved in more than 70% of the total interaction surface between CD8αα′ and the MHC α3 domain and β2-microglobulin (β2M). The second subunit, CD8α2, is less engaged and interacts only with the MHC α3 domain (22, 23). To simplify reference to these interaction sites, we designated the site occupied by the CD8α1 subunit of CD8αα′pMHCI as site 1 and the site occupied by the CD8α2 subunit as site 2.

Unlike CD8αα′pMHCI, the structure of CD8αβpMHCI is not known. Two different binding models have been proposed for the interaction of pMHCI with mouse and human CD8αβ (22, 23). Considering that the stalk region of CD8α is longer than that of CD8β (44 versus 35 amino acids), which allows the
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The overall protein architecture of CD8αβ is similar to that of CD8αα (22, 23) and resembles the Ig-variable domain fragment of an antibody (Fig. 1A). Despite the low degree of protein sequence identity between CD8α and CD8β (Fig. 1C), superimposing their structures reveals only limited differences in the loop regions, including the CDR1- and CDR2-like loops (Fig. 1B). The CDR-like loops are involved in ligand binding and are expected to be more variable than other regions. However, the nine residues that are identical in human and mouse CD8α and CD8β are important for maintaining an Ig-fold structure and dimeric interface (7). This conservation ensures similarity in the overall structure of α and β subunits (Fig. 1B). The apparent structural similarity between CD8α and CD8β raises the possibility that CD8αα-pMHCI interactions may be very similar to those of CD8αβ-pMHCI, with the β subunit replacing either one of the α subunits in CD8αα-pMHCI complex.

RESULTS

Structural Basis for CD8β Occupancy of Both Site 1 and Site 2—The overall protein architecture of CD8αβ is similar to that of CD8αα (22, 23) and resembles the Ig-variable domain fragment of an antibody (Fig. 1A). Despite the low degree of protein sequence identity between CD8α and CD8β (Fig. 1C), superimposing their structures reveals only limited differences in the loop regions, including the CDR1- and CDR2-like loops (Fig. 1B). The CDR-like loops are involved in ligand binding and are expected to be more variable than other regions. However, the nine residues that are identical in human and mouse CD8α and CD8β are important for maintaining an Ig-fold structure and dimeric interface (7). This conservation ensures similarity in the overall structure of α and β subunits (Fig. 1B). The apparent structural similarity between CD8α and CD8β raises the possibility that CD8αα-pMHCI interactions may be very similar to those of CD8αβ-pMHCI, with the β subunit replacing either one of the α subunits in CD8αα-pMHCI complex.

CD8β Subunit of Heterodimeric CD8αα-E277β Occupies Site 1—We recently showed that CD8ααββ is functionally active (17–28). This suggests that Argβ residue of the CD8α subunit is dispensable for the co-receptor function of CD8ααββ. The Argβ is not only critical for the ability of CD8α to occupy site 1 but also is the antigenic epitope recognized by mAb H59 (17). Hence, it is possible that mAb H59 blocks the interaction of pMHCI and CD8α1 but not that of pMHCI and CD8α2. If so, mAb H59 would be expected to block the co-receptor activity of CD8αα but not that of CD8ααββ. However, as the binding epitope of mAb H59 is lost in CD8ααββ (17), this variant is not suitable for performing the antibody blocking experiment. Because both Argβ and Glu27 form hydrogen bonds with Lys58 of βM in the crystal complex of CD8αα-H-2Kb (Fig. 1, D and E), we engineered a substitute variant, CD8ααE277A and tested whether, like CD8ααββ, CD8ααE277Aβ is functionally active. Fig. 2A shows that despite comparable surface expression levels of CD8 and TCR among the CD8 transfectants, cells expressing

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CONSTRUCTS OF MOUSE CD8α AND CD8β VARIANTS AND N15 TRANSFECTANTS EXPRESSING CD8β VARIANTS—We generated alanine-substituted mouse CD8β variants, including CD8ααE277A, CD8ααK55A, CD8ααS101A, and CD8ααK103A, using the PCR-based mutagenesis (14). Cell lines expressing variant CD8ααE277A or CD8ααS101A were generated by transfecting the CD8αα-pMHCI complex, the CD8α subunit occupies site 1, and the CD8β subunit occupies site 2. Among a panel of CD8β variants that were unable to occupy site 1, we identified two CD8β variants that were capable of forming functional co-receptor with WT CD8α. Therefore, the mouse CD8β subunit can also occupy site 2 in the CD8αα-pMHCI complex. In addition, we showed that an anti-CD8α antibody only blocked activity of co-receptors in which CD8α was occupying site 1 but not site 2. These results support the notion that CD8αβ interacts with pMHCI in at least two distinguishable binding orientations.

EXPERIMENTAL PROCEDURES

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CD8α, CD8β, and CD8αβ exhibit co-receptor activity about 1000-fold less efficiently than cells expressing WT CD8αβ (Fig. 2B). Thus, like CD8α R8A, CD8α E27A, CD8α E27, and CD8α E27A, CD8α E27A α E27A is incapable of interacting productively with pMHC1. In addition, the co-receptor activity of CD8α E27A β is indistinguishable from that of WT CD8αβ (Fig. 2C). Therefore, similar to CD8α R8A β, CD8α E27A β can...
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productively engage with pMHCI. mAb H59 binds to cells expressing WT CD8αβ and CD8αE27Aβ variants (Fig. 2A) indicating that the binding epitope of mAb H59 is retained. Fig. 2E shows that mAb H59 completely inhibits the co-receptor activity of CD8αα, yet it does not affect that of CD8αE27Aβ (Fig. 2D). We also tested the co-receptor activity of CD8αE27Aβ in the presence of an anti-CD8β mAb YTS156. In contrast to mAb H59, mAb YTS156 completely blocks the activity of CD8αE27Aβ (Fig. 2D).

The fact that mAb H59 has no effect on the co-receptor activity of CD8αE27Aβ is consistent with the prediction that the interaction between the MHC class I α3 domain and site 2-occupying CD8αE27A is minimal, and the binding of mAb H59 does not compromise the productive interaction between CD8αE27Aβ and pMHCI. Conversely, the complete inhibition of the co-receptor activity of CD8αE27Aβ by mAb YTS156 indicates that heterodimeric CD8αE27Aβ is responsible for all observed co-receptor activity. As CD8αE27A is no longer capable of occupying site 1, these results indicate that in the CD8αE27Aβ·pMHCI complex, the CD8β subunit occupies site 1. Significantly, the “all-or-nothing” inhibitory effect of mAb YTS156 and mAb H59 on the co-receptor activity of CD8αE27Aβ suggests that the occupation of site 1 by CD8β is the only binding mode in the interaction between CD8αE27Aβ and pMHCI.

CD8β Subunit of Heterodimeric CD8αβα·pMHCI Occupies Site 2—Because CD8αRRAβ and CD8αE27Aβ interacted with pMHCI in only one orientation, the CD8αRRA co-transfection system also provided a measure to identify residues of CD8β that were critical for CD8β being able to occupy site 1. We recently reported that the Lys55 in the CD2R-like loop and also Ser101 and Lys103 in the CD8β CD3R-like loop is critical for the co-receptor activity of CD8αRRAβ, as co-expression of each of these CD8αβ variants with CD8αRRA does not lead to detectable co-receptor activity (28). In light of the observation that heterodimeric CD8αRRAβ is functionally active (17), we questioned whether each of these CD8β variants could form a functional co-receptor with WT CD8α. The co-receptor activity of the CD8αRRAβ would indicate that each of these CD8β variants can form a functional co-receptor with WT CD8α. We found that cell lines expressing similar levels of CD8α and CD8β, CD8βK55A, CD8βS101A, or CD8βK103A proteins on the cell surface (data not shown) exhibited co-receptor activity (Fig. 3A). These results suggest that the heterodimeric CD8αRRAβ can productively interact with pMHCI.

However, WT CD8α can form functional CD8αα homodimers in these transfectants (17) and contribute to the observed co-receptor activity (Fig. 3A). We reported previously that the co-receptor activity of WT CD8αβ and CD8αα can be triggered by peptide antigen at concentrations of 10^{-9} M and 10^{-7} M, respectively (14). Thus, the CD8αRRAβ heterodimers are likely to be functionally active if cells expressing CD8αα and CD8αRRA are responsive to peptide antigen at a concentration lower than 10^{-7} M. This result suggests that heterodimeric CD8αβK55A and CD8αβS101A are functionally active. Because cells expressing CD8αα and CD8βS101A exhibited a CD8αα-like antigen sensitivity of 10^{-7} M, it appears that CD8αβS101A was not functionally active.

To further verify that CD8αβK55A and CD8αβK103A are indeed functionally active, we also examined their co-receptor activity in the presence of anti-CD8β mAb YTS156 or anti-CD8α mAb H59. Because mAb YTS156 is β chain-specific, it is expected to block the activity of the CD8αRRAβ heterodimeric co-receptor. Conversely, mAb H59, which is specific for the α chain, would block the co-receptor activity associated with CD8αα homodimers and CD8αRRAβ heterodimers in which CD8αα subunits occupy site 1. Fig. 3B shows that mAb YTS156 was able to block some co-receptor activity associated with cells transfected with WT CD8α and CD8βK55A, indicating that CD8αβK55A is indeed a contributor to that activity. Similar results were obtained from the cell line expressing WT CD8αα·pMHCI.
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and CD8αβK103A (Fig. 3C). Thus, although CD8αβK55A and CD8αβK103A are unable to occupy site 1 (7), they are capable of forming functional co-receptors with WT CD8α by occupying site 2. Surprisingly, very little, if any, co-receptor activity associated with cells expressing CD8α and CD8αβS101A is inhibited by mAb YTS156 (Fig. 3D), indicating that CD8αβS101A does not contribute to the co-receptor activity shown in A. This is consistent with the observation that these cells exhibit CD8αα-like antigen sensitivity (10⁻⁷ M). In contrast to mAb YTS156, mAb H59 completely blocks the co-receptor activity in all three cell lines (Fig. 3, B–D) suggesting that all functional co-receptors expressed must interact with pMHCI with a CD8α subunit occupying site 1 and that the portion of co-receptor activity that cannot be blocked by mAb YTS156 is contributed by CD8αα. These results show that the β subunit of heterodimeric CD8αβK55A or CD8αβK103A occupies site 2.

Wild-type CD8αβ Heterodimer Has Two Distinct Binding Modes—Using variants of CD8α or CD8β, we are able to restrict the interaction between each of these heterodimeric variants and pMHCI in only one orientation. It would be important to determine whether the WT CD8αβ heterodimer indeed interacts with pMHCI in both orientations. We analyzed the inhibitory effect of mAb H59 and mAb YTS156 on the co-receptor activity of WT CD8αβ. Three possible CD8β:pMHCI complexes were expected: CD8αα:pMHCI, CD8αβ:pMHCI with the α subunit occupying site 1, and CD8αβ:pMHCI with the β subunit occupying site 1 (Fig. 4A). mAb H59 was expected to block the first two complexes but not the third. mAb H59 did not block all of the co-receptor activities (Fig. 4B), indicating that CD8αβ:pMHCI interaction with CD8αα occupying site 2 contributed at least some co-receptor activity. Because mAb YTS156 inhibited the activity of CD8αβ complexed with pMHCI in either orientation (Figs. 2 and 3), the CD8αα homodimers were responsible for the co-receptor activity that was not affected by this antibody. These results are in agreement with our proposal that WT CD8αβ can bind to the pMHCI complex in at least two distinct modes, one sensitive and the other insensitive to inhibition by mAb H59.

DISCUSSION

The structure of the CD8αα:pMHCI complex reveals that the two α subunits of the CD8αα homodimer bind to the peptide-MHCI complex asymmetrically, with one subunit interacting extensively with pMHCI (site 1) and the other subunit interacting only with the α3 domain of pMHCI (site 2). Based on this information, we identified residues Argβ and Gluβ³⁷ as being critical to the ability of CD8α to occupy site 1. Using CD8αRRA and CD8αS27A, we found that CD8β could form a functional co-receptor with either variant. Therefore, in the context of CD8αβ:pMHCI interaction, CD8β can occupy site 1. We also used the CD8αRRA variant to identify three CD8β variants incapable of occupying site 1. Two of these three CD8β variants could form a functionally active co-receptor with WT CD8α indicating that although, CD8βSRA could not occupy site 1, it could still occupy site 2. The concept that CD8αβ and pMHCI can form a complex in different orientations is also supported by the observations that anti-CD8α mAb H59 completely blocks the co-receptor activity of CD8αβSRA, yet it does not affect that of CD8αRRAβ and only partially blocks that of WT CD8αβ. Our results support a two-binding-mode model in which heterodimeric CD8αβ can interact with pMHCI with two distinct binding orientations, one sensitive to mAb H59 and the other not.

The idea that CD8αβ can interact with pMHCI in two orientations prompted questions regarding whether the antigen presentations mediated by these two distinct CD8αβ:pMHCI complexes are qualitatively and/or quantitatively different. Note that the p56Lck kinase is only associated with the cytoplasmic domain of CD8α but not with that of CD8β (21). Thus, the distance between p56Lck and the ζ chains in a TCR complex that has CD8αβ engaged in one orientation is expected to be different from that in another TCR complex that has CD8αβ engaged in the opposite orientation. In addition, a specific pMHCIIa or pMHCIIb, such as H-2K, H-2D, H-2L, or Qa-1, Qa-2 plus H2-M3 (31), might favor the interaction with CD8αβ in one orientation over the other. It is also possible that one
specific binding mode might be better accommodated because of the particular position at which a specific TCR docks with pMHCI (29–31). Furthermore, the differentiation stage-related sialylation of the O-glycans associated with the stalk regions of CD8 proteins (18, 19) may be compatible with one, but not another, binding orientation. Therefore, a given TCR-pMHCI complex may preferentially or exclusively interact with CD8αβ in one binding mode but not the other. Such an asymmetric binding mode might facilitate a better engagement between a given TCR complex and the heterodimeric co-receptor that is not possible for its homodimeric counterpart.

Both CD8 and Ly49A NK cell receptors are shown to interact with pMHCI on the same cell (cis interaction) (32, 33). Specifically, the inhibitory Ly49A NK cell receptor can bind to its pMHCI ligand, H-2D^d, expressed on either the target cells (trans interaction) or the same NK cell (cis interaction) (33). Cis interaction of Ly49A with pMHCI reduces the number of Ly49A available for binding of pMHCI ligand on the target cells. By attenuating the inhibition on NK cells, Ly49A can fine-tune an optimal sensitivity for NK cells to discriminate normal versus abnormal host cells. Similarly, interaction of CD8αβ to pMHCI in cis may be compatible with one, but not another, binding mode. Future studies are needed to better understand the potential regulatory role of heterodimeric CD8αβ on antigen presentation.

The structural comparison of human and mouse CD8α shows that the corresponding residue of mouse Arg^8 is human Arg^4. Similar to the mouse CD8α^{RRA,RRA}, human CD8α^{RRA,RRA} homodimer cannot productively interact with pMHCI (34). However, unlike its mouse counterpart, human CD8α^{RRA} cannot form a functional co-receptor with WT human CD8β (34). Judging from the overall structural similarities between human and mouse CD8 as well as pMHCI proteins, it is difficult to understand the molecular basis for such a discrepancy. However, close examination reveals some differences that may explain this discrepancy. For example, human CD8β can be expressed on the cell surface as functionally inactive homodimers CD8ββ, whereas mouse CD8β cannot (27). This may drastically reduce the surface abundance of heterodimeric CD8αβ and may therefore explain why no apparent co-receptor activity was detected in the human experimental system. In addition, transient expression cultures, rather than established cell lines, were used for experimentation in the human system. The background noise inherently associated with transient expression systems may compromise the detection of any functional activity of heterodimers in a cell adhesion assay. Thus, it is conceivable that the observed discrepancy was because of differences in experimentation systems and that human CD8αβ, like mouse CD8αβ, also interacts with pMHCI with two distinct binding orientations.

Our results may also explain the apparent difficulty in obtaining protein crystals for structure determination of the CD8αβ-pMHCI complex, as WT protein mixtures may contain two types of complexes preventing formation of ordered crystals. In this regard, our CD8 heterodimeric variants capable of only one binding mode may provide an alternative to obtain the protein crystal of CD8αβ-pMHCI complexes and facilitate future efforts to elucidate their structures by crystallographic or other biophysical approaches. By generating transgenic mice expressing one heterodimeric variant, it will be possible to investigate the physiological significance of one binding mode without the complications associated with the second binding mode.

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