Analysis of the Structure of Empty and Peptide-loaded Major Histocompatibility Complex Molecules at the Cell Surface

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Summary

We compared the conformation of empty and peptide-loaded class I major histocompatibility complex (MHC) molecules at the cell surface. Molecular conformations were analyzed by fluorescence resonance energy transfer (FRET) between fluorescent-labeled Fab fragments bound to the α2 domain of the MHC heavy chain and fluorescent-labeled Fab fragments bound to β2-microglobulin. No FRET was found between Fab fragments bound to empty H-2Kb, but FRET was detected when empty H-2Kb molecules were loaded with peptide. The magnitude of FRET depended on the sequence of the peptide used. The results imply that empty H-2Kb molecules are in a relatively extended conformation, and that this conformation becomes more compact when peptide is bound. These changes, which are reflected in peptide-dependent binding of monoclonal antibodies, affect the surfaces of MHC molecules available for contact with T cell receptors and hence may influence T cell–receptor recognition of MHC molecules.

The MHC antigens are polymorphic cell surface glycoproteins with critical roles in the development of immune responses (1). The class I MHC complex consists of a polymorphic heavy chain, which associates with an antigenic peptide and a nonpolymorphic light chain β2-microglobulin (β2m) (2–5). This trimolecular complex is required for stable cell surface expression of class I MHC molecules. Some mutant cell lines, including RMA-S, T2, and EE2H3, have been derived that fail to associate peptides with class I MHC molecules (6–11). These cell lines produce class I MHC molecules that are either devoid of endogenous antigenic peptides or may contain low affinity MHC-binding peptides. These MHC molecules are largely retained in the endoplasmic reticulum, where they are degraded. Functionally, these class I molecules are “empty” in that they are unstable at physiological temperature, and high affinity MHC-binding peptides can be loaded into these empty MHC molecules (8, 11).

The structural bases for the instability of empty MHC molecules and their stabilization by exogenous peptides in situ have not been characterized. In contrast, the three-dimensional structure of peptide-loaded MHC molecules has been well characterized (12–17). Three-dimensional x-ray crystallographic solutions of all of the solved structures indicate that the peptide-binding groove consists of two sides composed of α helices and a base formed by strands of β-pleated sheets (12–14). Antigenic peptide fragments bind to the class I peptide-binding groove in an extended conformation, and some peptides bulge out from the central portion of the peptide-binding groove (15, 17, 18).

An important question in understanding the molecular mechanisms T cells use to recognize antigenic peptides is whether only exposed variable parts of a peptide are recognized by TCRs or if buried residues of the antigenic peptide influence T cell recognition by altering the MHC conformation. Previously, we and others (19–23) have shown that serological epitopes expressed on MHC molecules loaded with specific peptides were peptide dependent. Whereas peptide-dependent serological epitopes could be secondary to peptide side chains interfering with access to certain mAb-binding sites on MHC molecules, substitution of buried anchor residues also affected the binding of mAbs (19, 20, 23). This supports the hypothesis that peptide-dependent serological epitopes reflect conformational changes in H-2Kb molecules. Experiments analyzing the three-dimensional structure of a soluble purified class I molecule cocristallized with two different peptides showed that the conformation of the MHC molecule around the groove depends on the particular peptide bound (15, 24, 25). Recent work analyzing a larger

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1 Abbreviations used in this paper: β2m, β2-microglobulin; FRET, fluorescence resonance energy transfer; TcR, Texas red; VSV NP, vesicular stomatitis virus nucleoprotein.
number of peptides bound to a HLA-A2 class I MHC also showed small differences in the MHC conformation (17). In these studies, the structure of the bound peptide was thought to be the essential determinant of the antigenic identity of the MHC–peptide complex. All of these studies indicate that peptides influence exposed MHC residues, thus supporting a role for the recognition of “altered” MHC by TCRs.

Class I MHC conformation in situ, the structure of empty class I MHC molecules, and the conformation of peptide-loaded class I MHC molecules may be probed by use of the biophysical technique of fluorescence resonance energy transfer (FRET) (26). This technique detects proximity between fluorescent donor and acceptor molecules in terms of quenching of donor molecules and enhanced fluorescence of acceptor molecules. If \( r \) is the distance between donor and acceptor, then the magnitude of FRET varies as \( r^{-6} \). For the commonly used donor and acceptor pairs of fluorescein and rhodamine, a 25% change in \( r \) results in an 85% change in FRET. Thus, small changes in molecular conformation can be detected by FRET. This method has been used to analyze MHC molecules on intact cells and detects features of class I MHC structure (27) and the proximity of class I MHC molecules to one another (28).

The availability of cells expressing empty MHC molecules allows one to quantitatively load cell surface MHC molecules with antigenic peptides. These uniformly loaded MHC molecules help amplify signal differences detected by FRET. Using this approach, we find that H-2K\(^{b}\) molecules exist in a variety of conformational states as evidenced by differences in FRET and in the binding of mAbs. Empty H-2K\(^{b}\) molecules have a flexible and extended conformation. This “open” intermediate is accessible by peptides. Upon peptide binding, H-2K\(^{b}\) molecules adopt a more compact conformation. Since the amount of FRET depends on the sequence of the bound peptide, it appears that this compact MHC conformation is influenced by the peptide.

**Materials and Methods**

**Cell Transfections and Culture Conditions.** E-3K\(^{b}\) cells were established as previously described (19) by transfecting a cDNA encoding H-2K\(^{b}\) (29) into EE2H3 cells via calcium phosphate/DNA-mediated transfection. EE2H3 is an embryonic cell line that expresses mRNA for both the class I heavy chain and \( \beta_{M} \) but only expresses low levels of cell surface class I MHC molecules (30). These cells have defects in TAP gene expression, which accounts for the low level of cell surface MHC molecules. These MHC molecules are empty and, similar to other empty class I MHC molecules (11, 31), they are temperature sensitive and can be quantitatively loaded with MHC-binding peptides. Previously we characterized H-2K\(^{b}\) molecules expressed on the transfected E-3K\(^{b}\) cells and showed that they are also empty in that they can be quantitatively loaded with specific H-2K\(^{b}\)-binding peptides but not with other irrelevant peptides or peptides that bind to other MHC molecules (19).

E-3K\(^{b}\) cells were maintained by 1:20 passage three times weekly in RPMI-1640 supplemented with 2 mM glutamine, nonessential amino acids, 50 \( \mu \)g/ml gentamicin, 5 \( \times \) \( 10^{-5} \) M 2-ME, and 10% FCS (Hyclone Laboratories, Inc., Logan, UT).

**Peptide Loading of Cells.** E-3K\(^{b}\) cells were incubated at 25°C overnight in FCS-containing RPMI-1640 medium with or without human \( \beta_{M} \) (5 \( \times \) \( 10^{-7} \) M) (Sigma Chemical Co., St. Louis, MO) in a 5% CO\(_2\) incubator. In the morning, E-3K\(^{b}\) cells were incubated with antigentic peptides (100 \( \mu \)M) in FCS-containing RPMI-1640 medium for 1 h at 25°C and then for an additional 2 h at 37°C. An aliquot of the cells, which was not incubated with any peptide, was maintained at 22°C. At the end of the incubation period, cells were washed extensively to remove all of the excess peptides that did not bind to MHC molecules and prepared for flow microfluorometric analysis by staining with various mAb and for FRET analysis as described below.

**Peptides.** Peptides were made in the Johns Hopkins University Biopolymer Laboratory peptide synthesis facility by F-MOC chemical synthesis and then purified by preparative HPLC. The two H-2K\(^{b}\)-binding peptides used in these experiments were derived from either OVA amino acid residues 257–264 (32, 33) or vesicular stomatitis virus nucleoprotein (VSV NP) amino acid residues 52–59 (34) peptides. Sequences of the synthetic peptides were confirmed by amino acid analysis and peptide sequencing.

**mAbs.** The H-2K\(^{b}\)-reactive mAbs used in these experiments have previously been mapped to binding sites on either the \( \alpha_{1} \) domain (20.8.4) (35) or the \( \alpha_{2} \) domain (5F1, 28.8.6, and 34.4.20) (36, 37) of H-2K\(^{b}\). Fab fragments of mAb 5F1 and BBM.1 were prepared as previously described (38). For the Fab fragments used in these experiments, the fluorescein/protein molar (m/m) ratio was 1.5 for 5F1, and the Texas red (TxR)/protein ratio was 0.8 for BBM.1. In the reciprocal energy transfer experiments, the TxR/protein (m/m) ratio was 1.9 for 5F1 and the fluorescein/protein ratio was 2.8 for BBM.1.

**Assay of Fab Affinity.** Cells were prepared for binding assay by incubation overnight with human \( \beta_{M} \) (5 \( \times \) \( 10^{-7} \) M final concentration). The next morning, cells were pulsed with the OVA or VSV peptides as described (100 \( \mu \)M final concentration). At the end of the incubation period, cells were harvested and stained with various concentrations of fluorescein-labeled 5F1 (Fl-5F1) or fluorescein-labeled BBM.1 (Fl-BBM.1) Fab fragments. Mean channel fluorescence data were normalized to equivalent soluble fluorescein molecules by use of standardized fluorophore-coupled beads (Flow Cytometry Standards Corp., Research Triangle Park, NC). Nonspecific binding plus autofluorescence for Fl-5F1-labeled cells were determined by the addition of a 50-fold molar excess of unlabeled mAb, 5F1. Nonspecific binding plus autofluorescence for Fl-BBM.1 Fab-labeled cells were determined by measuring the fluorescence of E-3K\(^{b}\) cells stained with Fl-BBM.1 Fab that were not pulsed with human \( \beta_{M} \).
fluorescein or TxR molecules by use of standardized fluorophore-coupled beads (Flow Cytometry Standards Corp.). On the logarithmic scale of our flow cytometer (Epics 752; Coulter Corp., Hialeah, FL), a 25-channel shift in fluorescence intensity is equivalent to a twofold change in the level of emission. A 32 channel shift in TxR intensity is equivalent to a twofold change in the level of emission. The standards for maximum donor fluorescence were cells labeled with Fl-5F1 Fab and unlabeled BBM.1 Fab. The standards for minimum acceptor fluorescence were cells labeled with TxR-BBM.1 Fab.

Enhancement of 5F1 Fab fragment binding secondary to stabilization by the anti-β2m mAb BBM.1 was determined by analyzing Fl-5F1 binding, either alone or in the presence of unlabeled BBM.1 Fab, for empty and peptide-stabilized H-2Kβ molecules.

With use of either Fl-5F1 Fab and excess unlabeled BBM.1 Fab or Fl-BBM.1 Fab and excess unlabeled 5F1 Fab, there was no evidence of cross-blocking by either of the Fab fragments.

**Labeling for Flow Microfluorimetry.** Approximately 10⁶ cells were incubated for 60 min at 4°C with 50 µl of indicated purified mAb diluted to 10 µg/ml final concentration or for the FRET experiments with labeled Fab fragments as described. Cells were washed twice in PBS and incubated for an additional 40 min at 4°C in 50 µl/40 dilution of fluorescent Fab(ab')₂; goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA) when directly conjugated Fab's were not used.

**Results**

E-3Kβ cells were established from EE2H3 cells as previously described (19) and express empty H-2Kβ molecules that are temperature sensitive and can be quantitatively loaded with H-2Kβ-binding peptides (see Materials and Methods). Serological analysis of E-3Kβ cells cultured overnight with human β2m showed that human β2m alone stabilized all the conformational epitopes recognized by the anti-H-2Kβ-specific mAbs (compare black columns in Fig. 1, A and B). This is similar to previously reported effects of human β2m in stabilizing H-2Dα with H-2Kβ (31). Analysis of cells expressing empty H-2Kβ molecules cultured overnight with human β2m and then loaded with different H-2Kβ-binding peptides showed that peptides stabilized mouse MHC molecules containing human β2m in serologically discrete conformations (Fig. 1, A). This is similar to our own and others' (19, 20) previous findings for peptide-dependent serological epitopes associated with H-2Kβ not stabilized by human β2m. The epitopes recognized by mAb 5F1 and 20.8.4 were stabilized by the addition of β2m alone, and only slight increases in mAb binding were seen with the addition of either of the two H-2Kβ-binding peptides OVA (257-264) or VSV NP (52-59) (Fig. 1 A). The epitope recognized by mAb 28.8.6 was stabilized by the addition of β2m alone and by OVA (257-264) peptide and β2m; however, mAb 28.8.6 binding was markedly reduced when H-2Kβ was stabilized by VSV NP (52-59) peptide and β2m. The epitope recognized by 34.4.20 was significantly decreased when stabilized by OVA (257-264) peptide and β2m, but was not changed by addition of the VSV NP (52-59) peptide. Control MHC-binding peptides, which bind to other MHC molecules but not H-2Kβ, did not affect any of the mAb reactivity analyzed and gave results similar to those obtained when no peptides were added (data not shown).

To see if the peptide-stabilized forms of H-2Kβ reflect conformational changes in the molecular structure, we analyzed cell surface MHC molecules by FRET measured in a flow cytometer (26). FRET was used to analyze the conformational relationship of two regions in the MHC-β2m-peptide complex, the α2 region of H-2Kβ and β2m. The donor fluorophore, fluorescein, was conjugated to Fab fragments of mAb 5F1 (Fl-5F1), which binds to an epitope on the α2 region of H-2Kβ. This epitope was chosen since it is on the α2 helix, which has been shown to undergo slight peptide-dependent conformational changes with peptide binding (15, 17). Also the 5F1 epitope is stabilized by the addition of both peptides used (Fig. 1). The acceptor fluorophore, TxR, was conjugated to Fab fragments of BBM.1(TxR-BBM.1), which binds human β2m even when associated with mouse MHC heavy chain.

The efficiency of FRET depends on the spectral properties of a particular donor/acceptor pair, their spatial orientation, and the inverse sixth power of the distance between the two.

**Figure 1.** H-2Kβ-binding peptides stabilize specific epitopes on cell surface H-2Kβ molecules treated overnight with human β2m. E-3Kβ cells were incubated overnight with (A) or without (B) human β2m. Cells were then pulsed with either OVA (257-264) or VSV (52-59) peptides or without any added peptides and cultured at 37°C or left at 22°C and analyzed by flow cytometry. Cells pulsed with peptides were cultured as described.
For our donor and acceptor pair, the distance for half-maximal energy transfer Ro is 39Å (Hochman, J., and M. Edidin, unpublished data). Hence, assuming an average orientation of donor and acceptor molecules ($\kappa^2 = 2/3$), FRET should be detectable only when the two are separated by $\approx 80$ Å or less.

If peptide binding significantly changed the affinity of Fab fragments for H-2Kβ molecules, then Fab fragments might bind to H-2Kβ in slightly different, peptide-dependent, configurations. Were this the case, FRET measurements could simply reflect a difference in the relative orientation of the Fab molecules and not reflect changes in the MHC conformation. To control for this, we measured the affinity of the 5F1 and BBM.1 Fab fragments for empty and peptide-loaded H-2Kβ molecules. Scatchard analysis of specific binding of F1-5F1 Fab fragments showed that there were $\sim 45,000$ sites with an average $K_A$ of $8.2 \times 10^6$ (M$^{-1}$) in cells expressing empty H-2Kβ molecules (Fig. 2A). As expected, peptide loading increased the number of accessible H-2Kβ sites by 4–10 fold. However, the affinity of the 5F1 Fab for OVA (257–264)-loaded H-2Kβ molecules was not significantly altered, $K_A \approx 8.0 \times 10^6$ (M$^{-1}$) (Fig. 2B). The affinity of 5F1 for VSV-stabilized H-2Kβ molecules consistently showed a two- to fourfold increase over its affinity for empty MHC molecules, $K_A \approx 3.1 \times 10^6$ (M$^{-1}$) (data not shown). Peptide loading did not significantly influence either the number of sites or the affinity of the anti-β2m BBM.1 Fab fragment for β2m (Fig. 2, C and D). In both empty and peptide-treated cells, there were $\sim 300,000$ sites with an average $K_A$ of $2.0–2.4 \times 10^6$ (M$^{-1}$). Thus, peptide loading did not significantly alter the affinity of the Fab fragments for the MHC complex. In our system, differences in FRET would be expected to directly measure changes in the MHC conformational structure rather than a change in the orientation of Fab fragments relative to the MHC molecule.

When FRET was measured, cells that expressed empty class I MHC molecules showed no quenching of F1-5F1 fluorescence in the presence of TxR-BBM.1 (Fig. 3AII) and a low level of apparent sensitized emission of TxR-BBM.1 fluorescence (Fig. 3 BI). In contrast, when empty H-2Kβ was loaded with OVA (257–264) peptide, both marked quenching of donor fluorescence and sensitized emission of acceptor fluorescence were observed (Fig. 3, AII and BII). The histograms are displayed on a logarithmic scale on which quenching is seen as a leftward shift of the distribution of 5F1 fluorescence (Fig. 3 AII) and sensitized emission is seen as a rightward shift of the distribution of TxR-BBM.1 fluorescence without a significant change in the shape of the distribution (Fig. 3 BII). Control peptides had no effect on energy transfer (data not shown). The magnitude of energy transfer was quantitated by use of standardized fluorophore-coated beads, by converting mean channel fluorescence to average number of fluorophore molecules per cell. In the experiment shown, the fluorescence signal was quenched by an average of 22% in OVA-treated cells.

It is interesting to note that we found that binding of unlabeled anti-β2m Fab enhanced binding of 5F1 to empty H-2Kβ (Fig. 3C). Detection of the 5F1 epitope was only enhanced by BBM.1 when the anti-β2m Fab was added before or simultaneous with the addition of FI-5F1 (data not shown).

![Figure 2](image-url)

**Figure 2.** Affinity of 5F1-Fab fragments for empty and peptide-loaded H-2Kβ. E-3Kβ cells were incubated overnight with β2m. The next morning, the cells were either not treated with any additional peptides (A and C) or pulsed with the OVA (257–264) peptide (B and D) as described. Cells were then stained with either varying amounts of FI-5F1 Fab fragments (A and C) or FI-BBM.1 Fab fragments (C and D). Nonspecific binding plus autofluorescence of FI-5F1 Fab-stained cells was always <20% for empty H-2Kβ molecules and <5% for peptide-loaded H-2Kβ molecules. Nonspecific binding plus autofluorescence of FI-BBM.1 Fab-stained cells was always <10%. Mean channel fluorescence data were normalized to equivalent soluble fluorescein molecules by use of standardized fluorophore-coupled beads. By use of a linear fit, Scatchard analysis of the 5F1 binding data showed that $B/F = (3.81 \times 10^4) - (8.65 \times 10^4)B$ [R$^2 = 0.87$] for empty H-2Kβ molecules and $B/F = (1.16 \times 10^4) - (8.03 \times 10^4)B$ [R$^2 = 0.85$] for OVA (257–264)-loaded H-2Kβ molecules. Scatchard analysis of the BBM.1 binding data showed that $B/F = (6.69 \times 10^4) - (2.16 \times 10^5)B$ [R$^2 = 0.86$] for empty H-2Kβ molecules and $B/F = (7.18 \times 10^4) - (2.49 \times 10^5)B$ [R$^2 = 0.81$] for OVA (257–264)-loaded H-2Kβ molecules.
shown). Anti-β2m had no effect on the amount of 5F1 epitope in peptide-loaded H-2Kb molecules (Fig. 3 CII). These results imply that Fab binding to β2m can stabilize the conformation of a physically distant part of the empty MHC complex, the α2 domain of the molecule. Thus, compared to peptide-loaded H-2Kb, empty H-2Kb molecules appear to be extended and flexible.

To verify that the effects seen are due to changes in the MHC conformation and not local membrane artifacts, we compared FRET between Fl-5F1 Fab and TxR-BBM.1 Fab with FRET between Fl-BBM.1 Fab and TxR-5F1 Fab. FRET was analyzed for both empty and peptide-loaded H-2Kb molecules (Fig. 4). Empty H-2Kb molecules did not show any significant quenching of either the Fl-5F1 signal or the Fl-BBM.1 signal (Fig. 4, A and B). When H-2Kb was stabilized with OVA (257–264), quenching of Fl-BBM.1 in the presence of TxR-5F1 was 24% (Fig. 4 D), comparable to quenching seen with use of Fl-5F1 and TxR-BBM.1 (Fig. 4 C). In the experiment shown, fivefold less Fl-5F1 Fab was used to stain OVA (257–264) peptide-loaded cells (Fig. 4 C) than for cells expressing empty H-2Kb molecules (Fig. 4 A). This was done to show that FRET was seen in the peptide-treated cells even at fluorescence levels comparable to the level of fluorescence seen when cells expressing empty H-2Kb molecules were analyzed. As expected, there was no peptide-associated increase in the level of expression of BBM.1 epitope (compare Fig. 4, B and D), as was seen for the expression of the 5F1 epitope.

The magnitude of FRET depended on the peptide used to stabilize H-2Kb. The greatest quenching (22%) and most sensitized emission (50%) was observed when H-2Kb was stabilized by OVA (257–264) (Fig. 5). When H-2Kb was sta-
Discussion

Epitopes expressed by empty class I MHC molecules may be lost or enhanced when these molecules bind peptides (19, 20, 22). We found these same changes in epitopes for H-2K\(^b\) molecules associated with human \(\beta_2m\), rather than with the mixture of murine and bovine \(\beta_2m\) that is usually bound to the class I MHC heavy chain of murine cells in culture. Exchange of human for endogenous \(\beta_2m\) allowed us to probe the conformation of empty and peptide-loaded MHC molecules by FRET, by use of pairs of fluorescent donor and acceptor Fab. One member of each pair, 5F1, bound to the \(\alpha2\) domain of the class I MHC heavy chain, whereas the other, BBM.1, bound to the \(\beta2m\).

Apparent FRET may occur because of factors unrelated to changes in MHC conformation. However, the results of control experiments rule out important alternative explanations of our results and show that the changes in FRET between labeled Fab are likely to reflect molecular changes in the MHC conformation. First, Fab fragments bind to empty and peptide-loaded MHC with about the same affinity, \(K_A \approx 8.0 \times 10^6 \text{ M}^{-1}\) for 5F1 Fab and \(\sim 2 \times 10^5 \text{ M}^{-1}\) for BBM.1 Fab. From crystallographic solutions of the interaction of Fab molecules with protein ligands (39), it is clear that the complex of Fab fragments with ligands involves upwards of 30 amino acids on the surfaces of the Fab and the ligand. The specificity of interaction is believed to result from the remarkable shape complementarity, which leads to the almost total exclusion of water molecules from the interface. If peptide loading significantly altered the binding of Fab by blocking some critical sites on the MHC molecule, then one would expect to see a large change in Fab affinity. The absence of such large changes in the affinity of the Fab for peptide-loaded MHC molecules strongly suggests that average orientation of the bound Fab fragments is the same in all forms of class I MHC molecules considered here. Second, the same results for donor fluorescence quenching were seen whether the 5F1 Fab or the BBM.1 Fab fragments were labeled with the fluorescent donor fluorophore, fluorescein. Hence, the results are not artifacts of the local environments of donor or acceptor fluorophores.

The measured FRET is likely to reflect intra- rather than intermolecular energy transfer. Intermolecular energy transfer might occur under two different conditions: at very high surface concentrations of class I MHC molecules or when MHC molecules are clustered. The surface concentration of class I MHC molecules is of the order of \(10^2/\mu\text{m}^2\). At this concentration, the average distance between monomeric mole-

Figure 5. Decrease in donor fluorescence (quenching) or increase in acceptor fluorescence depends on the peptide added. Percentage changes were calculated from the values of equivalent soluble fluorophores standardized with fluorescent beads as described in Fig. 2.
induced conformational changes would propagate from the presence of peptide, B2m may bind deeper into the class I binding site causes the observed changes in FRET. In the physiological temperatures unless stabilized by addition of peptides or mAb to the MHC molecule (8, 10, 11, 31, 41, 42). Our second observation, consistent with the conformational changes in the B2m can propagate to the heavy chain. Our finding that peptide-loaded MHC molecules consistently showed differences in amount of FRET is consistent with those findings. Comparison of FRET for peptide-loaded H-2Kb shows higher FRET for OVA (257–265)-loaded H-2Kb than for VSV (52–59)-loaded H-2Kb. These differences could reflect greater structural differences between the molecules in solution than are evident in crystal structures, which may have constraints imposed on them by the crystallization process.

In an analysis of the influence of peptides on serological epitopes expressed on MHC molecules, not only exposed peptide residues but also buried anchor residues but also buried anchor residues influenced expression of serological epitopes (19, 20, 23). This was seen when the buried anchor tyrosine residues at positions 3 and 5 in the VSV (52–59) peptide were substituted. Our preliminary data suggest that these differences can also be detected using FRET (Catipović, B., unpublished data). This could be secondary to a buried amino acid residue in the antigenic peptide altering the surface of the MHC molecule and may explain why most anti-VSV (52–59)-specific H-2Kb-restricted CTLs do not recognize target cells pulsed with VSV A5 (46). Use of FRET to analyze the MHC conformation may provide a system to help determine the influence of engagement of peptide anchor residues on the MHC conformation.

The epitope on H-2Kb recognized by mAb 5F1 is exposed at the surface of the molecule and available for contact by the TCR. Since a relatively small part of an antigenic peptide bound to H-2Kb is available for interaction with the TCR (15, 25), these changes in the MHC structure could significantly contribute to the transmission of information between the peptide–MHC complex and the TCR. These findings support the hypothesis that TCR recognize both foreign antigenic peptide bound to an MHC molecule as well as a portion of an “altered” self-MHC molecule.

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