Changes at Peptide Residues Buried in the Major Histocompatibility Complex (MHC) Class I Binding Cleft Influence T Cell Recognition: A Possible Role for Indirect Conformational Alterations in the MHC Class I or Bound Peptide in Determining T Cell Recognition

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Summary

Recent crystallographic studies on two peptide complexes with the mouse K\(^b\) molecule have shown that peptide binding appears to alter the conformation of the class I α-helical regions that flank the antigen binding cleft. Given that this study also showed that much of the foreign peptide is buried within the class I binding cleft with only a small portion accessible for direct interaction with the components of the T cell receptor, this finding suggests that at least some component of T cell specificity may arise as a consequence of peptide-induced conformational changes in the class I structure. To assess this possibility, we have made systematic substitutions at residues within the K\(^b\)-restricted determinant from ovalbumin (OVA\(^{257-264}\)) that are thought to be buried on binding to the class I molecule. We have found that changes in this determinant at the completely buried second residue (P2) can influence T cell recognition without affecting binding to K\(^b\), suggesting that the substitutions may indirectly determine T cell recognition by altering the conformation of the class I molecule or the bound peptide.

The products of the MHC class I loci bind small peptides derived from cytoplasmic degradation of foreign and self proteins (1-2). Such complexes are recognized by the antigen-specific receptors on the surface of CD8\(^+\) T cells, triggering their peripheral activation and their initial selection within the thymus (3, 4). Allelic variations at pockets within the antigen binding cleft are thought to result in selective binding of peptide fragments that bear the correct motif amino acid sidechains to anchor and stabilize peptide–MHC association (5–10). The more exposed sidechains of the nonanchor amino acids would then be free to directly contact the TCR and determine T cell recognition (7, 9). Whereas the recent crystallographic analysis of two complexes between viral peptides and the mouse K\(^b\) molecule by Fremont et al. (9) revealed that the central region of the bound peptide fragments were particularly exposed for potential TCR recognition, the majority of the peptide antigen remained buried within the class I binding cleft. These workers speculated that given the limited extent of peptide exposure and the homology of TCR polypeptides to the Fab fragment of antibodies (11–14), T cell recognition would necessitate interactions over a relatively large surface area probably incorporating sizable regions of the MHC class I molecule itself. Furthermore, since the two peptide complexes chosen for study revealed the existence of peptide-induced conformational changes within the class I structure, this raises the possibility that peptide-dependent alteration in class I may play a critical role in determining TCR recognition (9).

In an effort to assess what role such conformational changes might have on T cell recognition, we have made alterations at residues within the K\(^b\)-restricted determinant from OVA, SIINFEKL (OVA\(^{257-264}\)) (15, 16) that are expected to be completely buried within the class I binding cleft (10). We reasoned that modulation of T cell recognition by substitutions at buried residues would identify the influence of indirect conformational changes.
Materials and Methods

Cell Lines and Synthetic Peptides. The K\(^{b}\)-restricted, OVA-specific CD8\(^{+}\) T cell hybridomas B3.1 and GA4.2 have been described previously (17), and 149.13.13 and 149.42.12 CD8\(^{+}\) T cell hybridomas were derived from a TCR \(\beta\) chain transgenic mouse expressing the TCR \(\beta\) chain from B3.1 (17). 5A.K\(^{b}\) is an L cell transfectant expressing the K\(^{b}\) gene (18), whereas RMA-S is an H-2\(^{d}\) antigen presentation-defective cell line that expresses low surface levels of class I molecules devoid of endogenous peptides (19, 20). Synthetic analogs of the octomeric OVA257-264 determinant substituted at the peptide positions 2, 5, and 8 (P2, P5, and P8) with various natural amino acids were synthesized by the Multipeptide Synthesis System (Chiron Mimotopes, Clayton, Australia) with free NH\(_{2}\) and COOH termini (21).

T Cell Hybridoma Assays. T cell hybridoma stimulation by the K\(^{b}\)-presented peptides was assessed by quantitating the antigen-specific release of IL-2. 50 \(\mu\)l of complete DME media with 10% FCS containing 4 \(\times\) 10\(^5\) 5A.K\(^{b}\) cells were aliquoted into each well of a 96-well flat-bottomed plate (Costar, Cambridge, MA) and the cells allowed to adhere by overnight incubation. At the end of this time, synthetic peptides were added to the appropriate wells to give a final concentration of 10\(^{-8}\) M. After incubation for 1 h at 37°C, the plate was washed three times with 200 \(\mu\)l of DME media followed by the addition of 200 \(\mu\)l suspension of the T cell hybridomas (10\(^5\) cells) in DME media with 10% FCS. After a 24-h incubation at 37°C in 6.5% CO\(_2\), 50 \(\mu\)l of supernatant was collected and the IL-2 content measured using the indicator cell line CTLL by a colorimetric assay employing 3-(4,5-dimethykhiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (22). Activation was measured at OD\(_{570}\).

Assay for Peptide Binding to K\(^{b}\). Relative peptide binding was assessed by measuring the folding and stabilization of K\(^{b}\) on the surface of RMA-S cells after culture in media containing the appropriate peptides (19, 20). RMA-S cells (3 \(\times\) 10\(^5\)) were incubated for 12-14 h in 400 \(\mu\)l of complete DME media with 10% FCS and various dilutions of the peptide analogs in 24-well plates. The cells were then harvested and stained using the K\(^{b}\)-specific antibody Y3 (23) and a fluoresceinated anti-mouse antibody (Silenus Laboratories, Hawthorn, Vic., Australia) before flow cytometry using a FACScan\(^{\circ}\) (Becton Dickinson & Co., Sunnyville, CA). Peptide binding to K\(^{b}\) is proportional to the observed increase in mean fluorescence and demonstrated highly reproducible saturable binding characteristics.

Results and Discussion

Falk et al. (6) sequenced a mixture of natural peptides bound to K\(^{b}\) and found that they had a preferred length of eight amino acids with motif residues of Leu at peptide position 8 (P8) and Phe or Tyr at P5. Shibata et al. (24) have shown that these residues are critical for interaction with K\(^{b}\) since Ala substitutions at the P5 and P8 anchor residues in a vesicular stomatitis virus nucleoprotein (VSV NP) peptide prevented K\(^{b}\) binding. The crystal structure of the VSV NP peptide complexed with K\(^{b}\) confirms that the P5 and P8 sidechains are completely buried in deep pockets within the K\(^{b}\) antigen binding site (8). In addition, the structure of this peptide–class I complex and that involving the K\(^{b}\)-restricted Sendai virus nucleoprotein (SV NP) determinant predict that the nonmotif P2 sidechain for K\(^{b}\)-bound peptides should also be buried within the antigen binding site and consequently be inaccessible for direct T cell interaction (10).

We have extended the previous studies of Shibata et al. (24) to the OVA257-264, K\(^{b}\)-restricted determinant and substituted each of the buried residues at P2, P5, and P8 with 16 other natural amino acids. The ability of the analogs to stimulate four CD8\(^{+}\) OVA-specific T cell hybridomas is shown in Fig. 1. Substitutions of the P8 Leu from OVA257-264 with the conserved amino acids of Ile and Val to give the 8I and 8V analogs, respectively, do not appreciably affect T cell recognition. The less conserved substitutions of Ala or Gly at the P8 residue (8A or 8G) attenuate or abolish the potency of these peptide analogs. All four T cell hybrids have similar reactivity to these analogs, showing a reduced but reproducible response to stimulation by 8A and no response to 8G.

Association of K\(^{b}\) with peptide results in the increased expression of K\(^{b}\) on the surface of the processing-defective mu

![Amino Acid Substitution](https://example.com/fig1.png)

**Figure 1.** T cell hybridoma reactivity with P2, P5, and P8 peptide substitutions of the OVA determinant. 5A.K\(^{b}\) APCs were pulsed with 10\(^{-4}\) M of OVA257-264 (SIINFEKL) (*) or synthetic peptide analogs substituted at P2 (a), P5 (b), and P8 (c) before the addition of the T cell hybridomas B3.1, GA4.2, 149.13.13, and 149.42.12. IL-2 secretion by the activated T cells was measured using the IL-2-dependent cell line CTLL in a colorimetric assay, and is expressed at OD\(_{570}\).
tart RMA-S (19, 20). Thus, the relative increase in K\textsuperscript{b} expression induced by the various peptide analogs can be used to quantify this binding. The results in Fig. 2 demonstrate that substituting the P8 Leu in OVA\textsuperscript{257-264} with a Val or Ile has little or no effect on peptide binding to K\textsuperscript{b}. In contrast, the Ala, and in particular the Gly P8 substitutions, attenuate this binding considerably. Thus, the combined T cell stimulation and K\textsuperscript{b} binding data demonstrate that changes at P8 appear to affect T cell stimulation by reducing the ability of the peptide determinant to bind to K\textsuperscript{b}.

The data for the P5 substitutions essentially mirrors that found for the P8 substitutions. The 5Y analog stimulates the four OVA\textsuperscript{257-264}-specific T cell hybrids, whereas substitutions containing the nonconserved Ala or Gly at P5 fail to do so. It is surprising that 5Y is only marginally better at binding K\textsuperscript{b} than 5A (Fig. 2). More significantly, 5V associates more efficiently with K\textsuperscript{b} than the more potent T cell stimulator 5Y. Whereas the four T cell hybrids did not recognize 5V at the $10^{-8}$ M peptide concentration used in Fig. 1, they could all be activated by $10^{-6}$ M concentrations of this peptide (data not shown). The results suggest that whereas substitutions at P5 in the OVA\textsuperscript{257-264} peptide primarily influence peptide binding to K\textsuperscript{b}, they also have an indirect effect on T cell recognition.

This indirect effect on T cell stimulation is more pronounced for the P2 substitutions of the OVA\textsuperscript{257-264} peptide. Whereas 2A, 2G, and the parental OVA\textsuperscript{257-264} show similar binding to K\textsuperscript{b} (Fig. 2), they have different potencies for T cell stimulation (Fig. 1). Crystallographic studies have shown that P2 is completely buried in the K\textsuperscript{b} complexes with the VSV NP peptide (Gly at P2), and the 5V NP peptide (Ala at P2) (9). The four T cell hybrids used in our studies show a different fine specificity for the analogs with Ala and Gly substitutions at P2. For example, the B3.1, GA4.2, and 149.13.13 hybrids recognize 2G, whereas 149.42.12 fails to do so. In contrast, GA4.2, 149.13.13, and 149.42.12 respond to 2A, whereas B3.1 does not. Although Fig. 2 shows that the Ala and Gly substitutions at P2 do not disrupt peptide binding to K\textsuperscript{b}, certain substitutions at P2 can affect peptide-K\textsuperscript{b} affinity as evidenced by the poor binding of the 2F and 2Y analogs. This confirms that P2 in the K\textsuperscript{b}-bound determinant is buried within the class I binding cleft, as proposed by Matsumura et al. (10). Whereas this also implies that P2 is important in peptide-MHC interactions, our data showing K\textsuperscript{b} presentation (Fig. 1) and binding (Fig. 2) by analogs with divergent P2 substitutions would suggest that it is not a major anchor residue such as P5 or P8. This is consistent with the finding that P2 was not identified as a motif position for K\textsuperscript{b} bound peptides (6). The ability of analogs with diverse substitutions such as 2N, 2S, and 2T to stimulate OVA-specific T cells in a clone-specific manner (Fig. 1) would further suggest that P2 sidechains are unlikely to directly contact TCR. Nonetheless, changes at this position appear to influence T cell recognition. We would argue that these results from conformational alterations at other more exposed parts of the peptide or at regions of the MHC that are in direct contact with the TCR.

The upper surfaces of the class I \(\alpha\)-helices are thought to directly contact the TCR (25) and are likely to play an important role during positive selection, possibly determining the requirement for self restriction. Our results show that changes at peptide residues buried within deep pockets could indirectly alter TCR interaction with class I. It is possible that corresponding allele-specific changes at these same class I pockets could also result in a similar effect and explain how such variation at sites buried in the class I cleft can influence self restriction (3, 4). For example, the P2 sidechains for K\textsuperscript{b}-restricted peptides bind at a completely buried pocket within the class I antigen-binding cleft lined by amino acids 9, 24, 45, and 67 (pocket B in reference 10). A variant of the K\textsuperscript{b} molecule with changes at this site, K\textsuperscript{b67} is known to abolish the thymic selection of Kb-restricted T cells specific for the OVA\textsuperscript{257-264} determinant without affecting selection for T cells specific for the VSV NP determinant (3). This differen-

\begin{figure}[h]
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\includegraphics[width=\textwidth]{peptide-induced-stabilization.png}
\caption{Peptide-induced stabilization of K\textsuperscript{b}-expression on the surface of the presentation defective cell line RMA-S. The presentation-defective cell line RMA-S was incubated with increasing concentrations of the OVA\textsuperscript{257-264} (SIINFEKL) (○) or synthetic analogs substituted at P2 (a), P5 (b), and P8 (c) with residues Ala (■), Gly (□), Ile (△), Phe (△), Tyr (○), or Val (▲). The increase in K\textsuperscript{b} expression was measured by flow cytometry after staining using the K\textsuperscript{b}-specific antibody Y3, and is expressed as a mean channel fluorescence.}
\end{figure}
tial selection by the K<sup>bm8</sup> molecule might simply reflect differences in binding affinity for particular selecting self peptides. Alternatively, differences in positive selection between K<sup>b</sup> and K<sup>bm8</sup> might arise as a result of constraints imposed by P2 interaction with pocket B resulting in class I conformation changes. Peptide-specific alteration in the class I conformation could therefore play a dominant role in T cell recognition during T cell activation and, more importantly, in the selection for self restriction during the thymic maturation process.

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References

1. Townsend, A., and H.A. Bodmer. 1989. Antigen recognition by class I-restricted T lymphocytes. *Annu. Rev. Immunol.* 7:601.
2. Bjorkman, P.J., and P. Parham. 1990. Structure, function, and diversity of class I major histocompatibility complex molecules. *Annu. Rev. Biochem.* 59:253.
3. Nikolic-Zugic, J., and M.J. Bevan. 1990. Role of self-peptides in positively selecting the T-cell repertoire. *Nature (Lond.)* 344:65.
4. Sha, W.C., C.A. Nelson, L.D. Newberry, J.K. Pullen, L.L. Pease, J.H. Russell, and D.Y. Loh. 1990. Positive selection of transgenic receptor-bearing thymocytes by K<sup>b</sup> antigen is altered by mutations that involve peptide binding. *Proc. Natl. Acad. Sci. USA.* 87:6186.
5. Garrett, T.P.J., M.A. Saper, P.J. Bjorkman, J.L. Strominger, and D.C. Wiley. 1989. Specificity pockets for the side chains of peptide antigens in HLA-AW68. *Nature (Lond.)* 342:692.
6. Falk, K., O. Rötzschke, S. Stevanovic, G. Jung, and H.G. Ramnensee. 1991. Allele-specific motifs revealed by sequencing of MHC transgenic receptor-bearing thymocytes by K<sup>b</sup> antigen. *Nature (Lond.)* 351:290.
7. Madden, D.R., J.C. Goga, J.L. Strominger, and D.C. Wiley. 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature (Lond.)* 353:321.
8. Jardetzky, T.S., W.S. Lane, R.A. Robinson, D.R. Madden, and D.C. Wiley. 1991. Identification of self peptides bound to purified HLA-B27. *Nature (Lond.)* 353:326.
9. Fremont, D.H., M. Matsumura, E.A. Stura, P.A. Peterson, and I.A. Wilson. 1992. Crystal structures of 2 viral peptides in complex with murine MHC class-I H-2K<sup>d</sup>. *Science (Wash. DC)* 257:919.
10. Matsumura, M., D.H. Fremont, P.A. Peterson, and I.A. Wilson. 1992. Emerging principles for the recognition of peptide antigens by MHC class-I molecules. *Science (Wash. DC)* 257:927.
11. Novotny, J., S. Tonegawa, H. Saito, D.N. Kranz, and H.N. Eisen. 1986. Secondary, tertiary and quaternary structure of T-cell specific immunoglobulin-like polypeptide chains. *Proc. Natl. Acad. Sci. USA.* 83:742.
12. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of T-cell αβ receptor. *EMBO (Eur. Mol. Biol. Organ.)* J. 7:3745.
13. Davis, M.M., and P.J. Bjorkman. 1988. T-cell antigen receptor gene and T-cell recognition. *Nature (Lond.)* 334:395.
14. Claverie, J.M., A. Procknicha-Chalufour, and L. Bougneleret. 1989. Immunological implications of a Fab-like structure of the T-cell receptor. *Immunol. Today.* 10:10.
15. Moore, M.W., F.R. Carbone, and M.J. Bevan. 1988. Introduction of soluble protein into the class I pathway of antigen processing and presentation. *Cell.* 54:777.
16. Rötzschke, O., K. Falk, S. Stevanovic, G. Jung, P. Walden, and H.G. Ramnensee. 1991. Exact prediction of a natural T cell epitope. *Eur. J. Immunol.* 21:2891.
17. Carbone, F.R., S.J. Sterry, J. Butler, S. Rodda, and M.W. Moore. 1992. TCR α-chain pairing determines the specificity of residue 262 within the K<sup>b</sup>-restricted, ovalbumin<sup>257-264</sup> determinant. *Int. Immunol.* 4:861.
18. Pullen, J.K., H.D. Hunt, R.M. Horton, and L.R. Pease. 1989. The functional significance of two amino acid polymorphisms in the antigen-presenting domain of class I MHC molecules: molecular dissection of K<sup>bm3</sup>. *J. Immunol.* 143:1674.
19. Townsend, A., T. Elliott, V. Cerundolo, L. Foster, B. Barber, and A. Tse. 1990. Assembly of MHC class I molecules analyzed in vitro. *Cell.* 62:285.
20. Townsend, A., C. Ohlsen, J. Bastin, H.G. Ljunggren, L. Foster, and K. Kärre. 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. *Nature (Lond.)* 340:443.
21. Valerio, R.M., M. Benstead, A.M. Bray, R.A. Campbell, and N.J. Maeji. 1991. Synthesis of peptide analogues using the multipin peptide synthesis method. *Anal. Biochem.* 197:168.
22. Mossmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* 65:55.
23. Hammerling, G.J., E. Rusch, N. Tada, S. Kimura, and U. Hamerling. 1982. Localization of allodeterminants on H-2K<sup>b</sup> antigen by monoclonal antibodies and H-2 mutant mice. *Proc. Natl. Acad. Sci. USA.* 79:4737.
24. Shibata, K., M. Imai, G.M. van Bleek, S. Joyce, and S.G.
Nathenson. 1992. Vesicular stomatitis virus antigenic octapeptide N52-59 is anchored into the groove of the H-2K<sup>b</sup> molecule by the side chains of three amino acids and the main-chain atoms of the amino terminus. Proc. Natl. Acad. Sci. USA. 89:3135.

25. Ajitkumar, P., S.S. Geier, K.V. Kesari, F. Borriello, M. Nakagawa, J.A. Bluestone, M.A. Saper, D.C. Wiley, and S.G. Nathenson. 1988. Evidence that multiple residues on both α-helices of the class I MHC molecule are simultaneously recognized by the T cell receptor. Cell. 54:47.
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