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The Importance of Pairwise Interactions Between Peptide Residues in the Delineation of TCR Specificity

Graham R. Leggatt,1,* Anne Hosmalin,2* C. David Pendleton,* Anita Kumar,† Stephen Hoffman,‡ and Jay A. Berzofsky3*‡

A minimal, nonamer epitope (TEMEKEGK1) from the reverse transcriptase protein of HIV-1, restricted by H-2Kb, was identified and the function of individual residues determined. Besides classical anchor residues at positions 2 and 9, methionine at position 3 was identified as an important MHC anchor and improved binding of a different (malarial) nonamer epitope to H-2Kb, albeit while also abolishing CTL recognition. Lysine at position 5 was replaceable by alanine for CTL raised against wild-type peptide but abolished recognition for CTL raised against the variant 5ALA peptide, indicating a unidirectional cross-reactivity. Interestingly, one CTL line raised against the 5ALA substituted peptide was permissive for a double substitution at positions 5 and 6, in which lysine was permissive at position 5 only if the adjacent glutamic acid was replaced by alanine. Extensive analysis revealed three distinct patterns of responses with peptides doubly substituted in this region: recognition of both single substitutions but not the double substitution, recognition of only one single substitution but also the double substitution, or recognition of both single substitutions and the double substitution. A second complementary substitution can therefore restore function lost through a first substitution. Thus, no residue acts independently of its neighbors, and pairs of substitutions may give results not predictable from the effects of each taken singly. This finding may have bearing on viral infections (such as HIV), in which the accumulation of two mutations in the epitope may lead to the reengagement of memory CTL previously silenced by the initial mutation. The Journal of Immunology, 1998, 161: 4728–4735.

Cytotoxic T lymphocyte recognition of short peptides in the context of a particular MHC class I molecule is important in determining the specificity and effector function of the immune system in its surveillance of the intracellular environment of cells. While much is being learned from the crystal structures of peptides interacting with MHC class I molecules (1–5) and MHC/peptide engaged with TCR (6, 7), ultimately we wish to better predict the functional outcome of TCR interaction with the peptide/MHC complex, particularly in situations in which viral pathogens are capable of mutating the specific peptide being recognized. For a given TCR, single substitutions within the peptide may result in enhancement of recognition by the TCR, a neutral effect, or loss of recognition (8–11). Recently, a further complication was observed when it was found that some mutations producing an apparent loss of recognition actually generated antagonist peptides (12–15) or partial agonists (16, 17). Consequently, understanding the TCR flexibility in recognition of the peptide/MHC complex is crucial to predicting effector functions and determining which cell populations will be activated in vivo.

Although MHC-bound peptide is viewed as a three-dimensional structure, conventional approaches to probing the TCR involve single mutations (usually alanine) along the length of the peptide, making the assumption that each amino acid side chain is independent in its effect on TCR recognition (18). One recent study, utilizing a class II MHC-restricted peptide epitope, has recently shown that double substitutions within a peptide can be recognized by T cells (19). Our study, conducted concurrently, extends this concept in a class I presentation system to show that nonconservative changes in amino acid side chains, which apparently do not interact directly with the TCR, can also influence TCR recognition of MHC class I peptide complexes, and that a second substitution can rescue recognition abrogated by a substitution at an adjacent position. In this study, we have mapped the effect of single substitutions in an HIV-1 reverse transcriptase (RT) peptide and utilized this information to examine the effect of double substitutions in the middle of the epitope. We show that single substitutions do not always allow the prediction of the outcome of double substitutions and that CTL silenced by a single mutation can be reengaged by a compensating second substitution. This may have importance in hypervariable viral epitopes, where multiple mutations may actually limit the spread of virus by reengagement of memory CTL.

Materials and Methods

Peptides

The amino acid sequences of all HIV pol and Plasmodium falciparum circumsporozoite protein peptides used in this study are shown in Tables I and II. Peptides were synthesized on a Model 430A peptide synthesizer (Applied Biosystems, Foster City, CA) using conventional t-BOC chemistry (20) and cleaved from the resin by liquid HF. The purity and molar concentration were analyzed by reverse phase HPLC on a C18 column using a gradient of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, and were further purified by gel filtration on
Biogel P4 column (Bio-Rad, Richmond, CA) in 9% formic acid and/or, where necessary, by preparative reverse phase HPLC using a similar gradient.

**CTL assay**

The $^{51}$Cr release assay was conducted as previously described (21). Briefly, L cell fibroblast target cells (5 x 10$^5$) were labeled with 300 mCi Na$^{51}$CrO$_4$ in 200 to 250 µl T cell medium with 10% FCS for 2 h at 37°C before washing and dispersion of 30,000 or 60,000 cells/tube with peptide at the indicated concentrations for 2 h. The cells were then washed once and plated into a 96-well round-bottom plate at 3000 cells/well. Effector CTL were then added as indicated for a period of 4 to 5 h, after which supernatants were harvested and counted in an Isomedic gamma counter.

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**Table I. Peptide sequence and pol a lytic activity on L28 cells pulsed with AH2 (HIV-1 RT residues 203–219) alanine-substituted peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Lysis (%)</th>
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<tbody>
<tr>
<td>AH-2-1</td>
<td>EICT</td>
<td>35</td>
</tr>
<tr>
<td>AH-2-7</td>
<td>AICT</td>
<td></td>
</tr>
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<td>EAIT</td>
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</tr>
</tbody>
</table>

Table I. Peptide sequence and pol a lytic activity on L28 cells pulsed with AH2 (HIV-1 RT residues 203–219) alanine-substituted peptides

**Results**

*Boldface indicates a substituted amino acid residue.*
as the minimal epitope, because seven of the nine amino acid replacements had a substantial effect on T cell recognition. This region also contained the classical H-2Kk binding motif consisting of a glutamic acid at position 2 and isoleucine at position 8 or 9 (34–37), although it is clear from MHC class I elution studies that octamer peptides predominate in the binding groove (35–37).

On the basis of these results, the nonamer peptide, AH2-I9, was synthesized, along with two octamer peptides contained within this sequence (Table II). The minimal epitope was successfully identified as residues 206–214, because the smaller peptides did not produce a significant response (Fig. 1). Using transfected fibroblast lines expressing H-2Kk or H-2Dk, the MHC restriction was confirmed as H-2Kk (data not shown), which is in agreement with the earlier reported restriction for the longer epitope (23).

Mapping

To define the role of each residue in AH2-I9, peptides with single alanine substitutions covering the nine amino acid sequence of this peptide (Table II) were synthesized and examined for sensitization of targets at a pulsed peptide concentration of either 1 μM or 10 μM (Fig. 2A). Three alanine substitutions were seen to have no effect on recognition by the pol a CTL line, including those at positions 1, 5, and 7. This pattern was largely in agreement with the results for AH2 (Table I), except that the position 1 threonine was not replaceable in the longer epitope but was fully replaceable in the smaller nonamer. This difference suggests that the substitution in the longer peptide may affect processing to the minimal epitope. Both substitutions at position 1 (alanine for threonine) and position 7 (alanine for glycine) were relatively conservative, while the substitution at position 5 (alanine for lysine) was nonconservative. The titration of these peptides indicated that all had activity equal to or better than that of AH2-I9 using pol a CTL (Fig. 2B).

The binding of these peptides to H-2Kk was next analyzed using both a competition assay and up-regulation of H-2Kk expression on a T2-Kk cell line (deficient in TAP transport of endogenous peptide). Competition experiments (Fig. 3A) clearly defined positions 2, 3, and 9 as MHC binding residues, because substitution at
any of these positions produced peptides incapable of competing with the malaria Pf peptide that also binds to H-2K\(^\text{d}\) (22). The T2 MHC stabilization assay system confirmed that peptides substituted at positions 2, 3, and 9 were unable to effectively stabilize class I at the surface, while alanine substitution at peptide positions 4 and 6 consistently produced peptides of intermediate efficiency in stabilizing the surface class I (Fig. 3B). The discrepancy between the T2 binding and competition assay probably reflects the greater sensitivity of competition assays that are performed under CTL assay conditions and with physiologic levels of stable class I molecule (38).

Consequently, from the binding data and CTL activity, we propose that position 8 is the major side chain interacting with the TCR, while positions 2, 3, and 9 form the major MHC anchor residues or agretopic residues (Fig. 3C). Positions 4 and 6 are probably secondary TCR contact residues because a portion of their weak response can be accounted for by their reduced binding efficiency, highlighted in the nonphysiologic T2 binding assay. Positions 1, 5, and 7 seem to have no effect.

Enhanced MHC binding with position 3 methionine

Glutamic acid at position 2 and isoleucine at position 9 have been well described as MHC anchor residues for H-2K\(^\text{d}\), but a role for methionine at position 3 has not (34). Given the sequence of a naturally occurring K\(^\text{d}\)-binding Pf peptide (Table II) with the two primary anchor residues, but an asparagine at position 3, we asked whether substitution of methionine for asparagine in this peptide might improve its binding to H-2K\(^\text{d}\). The methionine modified Pf peptide did show increased binding (~10-fold) in a competition assay (Fig. 4), suggesting that methionine is a favored anchor residue at position 3. Unfortunately, the modified peptide was not recognized by CTL specific for the wild-type Pf sequence, suggesting that the modification may also have affected the conformation of the peptide in the groove or the conformation of the MHC and thus resulted in a loss of TCR recognition.

**FIGURE 3.** Binding assays with alanine-substituted peptides based on the AH2-I9 sequence. A, Competition experiment. The alanine-substituted peptides were incubated with \(^{51}\)Cr-labeled L28 targets at the indicated concentrations for a period of 1.5 h at 37°C. Following this incubation, Pf peptide (which also binds to H-2K\(^\text{d}\)) was added to all tubes at a suboptimal concentration (1 \(\mu\)M) for a further 1.5 h before washing the cells once and plating at 3000 cells/well. The PFCTL line was added at an E:T ratio of 40:1, and the plate was incubated at 37°C for 5 h. Percent inhibition was calculated as follows: 1 – \((\text{percent lysis with competitor} – \text{percent background lysis})/\text{percent lysis without competitor} – \text{percent background lysis})\). Lysis in the absence of competitor was 49%. B, T2-K\(^\text{d}\) binding assay. T2 cells (TAP-deficient) transfected with the H-2K\(^\text{d}\) MHC molecule (1–2 \(\times\) 10\(^5\)/well) were incubated overnight at 37°C with the alanine-substituted peptides at the concentrations indicated. Following this incubation, the plate was placed on ice, and the FITC-conjugated anti-H-2K\(^\text{d}\) Ab was added to every well at 2.5 \(\mu\)g/ml. After a 1-h incubation, cells were washed and analyzed on a FACScan. The background fluorescence without peptide was 2.89, and the fluorescence index was calculated as: (sample fluorescence – background fluorescence)/background fluorescence. An arbitrary threshold of 1 (which represents a doubling in fluorescence) was chosen to indicate positive binding. C, Summary diagram indicating residues that may interact with TCR (upward arrows) and/or MHC (downward arrows) along with those that are apparently neutral positions (no arrows).
show a cross-reaction with AH2-I9 (data not shown), despite the fact that five out of nine amino acids were now identical in the two peptides. The silent lysine residue at position 5 Lysine, at position 5, located at the center of the epitope and neighbored on each side by a neutral/secondary epitopic residue for pol α CTL, was completely replaceable by alanine despite the fact that lysine has a large, charged side chain that might be expected to interact with the TCR. In other studies, the crystal structure of TCR interacting with MHC/peptide also indicates that the CDR3 region of the Vα- and Vβ-chain contacts the middle of the peptide bound in the MHC groove (6, 7). To investigate the apparent neutral effect of the lysine, we immunized mice with the peptide containing the sequence with alanine substituted at position 5 (5ALA) and generated CTL using different concentrations of 5ALA peptide. As seen in Figure 5B, specific CTL were generated that had different dose-response curves based on the Ag dose used in their generation, an observation our lab had made in an earlier publication with an HIV envelope peptide (39). These CTL were then screened against the wild-type peptide along with many of the monosubstituted alanine peptides used to determine epitopic and agretopic residues for wild-type-specific CTL (Fig. 5A). Interestingly, both CTL lines generated in this direction did not lyse targets pulsed with wild-type peptide, indicating a unidirectional cross-reactivity with the 5ALA replacement. Furthermore and surprisingly, the lower avidity line (10 μM 5ALA CTL) recognized the double substitution relative to 5ALA represented by the 6ALA peptide (i.e., 5Lys, 6Ala), while the higher avidity CTL line did not. In fact, titration of the 6ALA peptide indicated that the dose-response curve was within 10-fold of that for 5ALA (the peptide used to generate the CTL) (Fig. 5C). Thus, although the wild-type peptide with 5Lys was not recognized by the 5ALA-specific CTL, replacement of an adjacent residue with alanine allowed a peptide with 5Lys to be recognized.

Multiple substitutions within the central residues of AH2-I9 To understand this restoration of recognition by a second substitution, we decided to investigate the central region residues (EAE for 5ALA peptide, EKE for AH2-I9) in greater detail to determine a chemical basis for the cross-reactivity. If position 5 and 6 were both alanine, recognition by 10 μM 5ALA CTL was maintained, suggesting that this CTL line was negatively influenced by two bulky residues at positions 5 and 6. This issue was then further investigated with several other amino acid combinations at this position.

The interpretation that the 10 μM 5ALA CTL was negatively affected by two bulky, charged residues at positions 5 and 6 was investigated by using other double substitutions in comparison...
with the single substitution at position 5 (Fig. 6). It was observed that isoleucine or glutamic acid at position 5, either as a single substitution (EIE, EEE) or as a double substitution with alanine at position 6 (EIA, EEA) did not result in lytic activity on peptide-pulsed targets. In contrast, peptides containing serine (ESA) or glutamine (EQA) as double substitutions or as a single substitution in the case of serine (ESE) were effective in sensitizing targets for lysis. This indicated that the cross-reactivity was not limited to the lysine-alanine double substitution (EKA) seen earlier, but also could not be explained simply by the presence of two bulky residues interfering with recognition of the 10 μM 5ALA CTL. Also, in contrast to 0.1 μM 5ALA CTL, it was observed that the 10 μM 5ALA CTL responded to targets bearing the serine single substitution (ESE) and double substitution (ESA). Finally, targets pulsed with alanine substitutions at position 4, 5 (AAE) were not recognized by the 10 μM 5ALA CTL line, suggesting that position 4 is epitopic for this CTL line given that the peptide had good binding to H-2Kk (data not shown). Substitution at position 5 and 6 (EAA) did not affect TCR recognition of the 10 μM 5ALA CTL line, indicating that position 6 is neutral/agreptopic, while a subset of changes were tolerated at position 5.

In contrast, it was noted that position 4, previously shown to be neutral/epitopic for pol a and 10 μM 5ALA CTL, and position 6, shown to be epitopic/neural for pol a, were not critical for TCR recognition by 0.1 μM 5ALA CTL (high avidity) because targets pulsed with the peptide sequence containing either AAE or EAA were effectively lysed (data not shown). At the same time, position 5 (alanine) was more limited, because an amino acid change to lysine or isoleucine resulted in peptide-pulsed targets that were not susceptible to lysis. Additionally, substitution of serine for alanine (ESE) in the 5ALA peptide was permissive for lytic activity of 0.1 μM 5ALA CTL. This suggests that alanine at position 5 may play some role as an epitopic residue for the 0.1 μM 5ALA CTL line. Both 5ALA CTL lines did not lyse targets pulsed with peptides containing alanine at both positions 5 and 8, indicating that the major epitopic residue at position 8 identified for pol a is retained as epitopic for these CTL. An interesting feature of the 0.1 μM 5ALA CTL was the ability to recognize wild-type 5ALA (EAE), single serine substitution (ESE), and single alanine substitution (EAA), but not the combination of these substitutions (ESA). The pol a CTL line did not recognize any of the doubly substituted peptides but did show extreme flexibility with position 5 substitutions as previously mentioned. The T-2 binding assay was used to determine the hierarchy of binding for the double substitutions. Consistent with the lower sensitivity of the T2 class I stabilization assay than CTL recognition (38), several peptides that induced CTL activity showed poor or unmeasurable binding to the T-2Kk cells. It was also observed that binding of KE, AE, AA at positions 5 and 6 was comparable but did not predict the binding of KA, which was significantly lower.

Discussion

Understanding the rules governing the specificity of TCRs is central to immunologic processes as diverse as the positive selection of immature T cells in the thymus (40), the maintenance of memory in the periphery (41), and possibly the initiation of autoimmune diseases (42). Given our current understanding that each T cell expresses a unique receptor, the repertoire of TCRs in the periphery is extremely large. Therefore, it is difficult to establish general rules for specificity, because several receptors may recognize the same MHC/peptide complex with different microspecificities (43). Despite this complication, the advent of peptide technology has allowed us to probe the TCR through the use of monosubstituted peptides. In this study, we have examined the recognition of a conserved RT epitope in the context of the H-2Kk MHC class I molecules as a first step to manipulating the T cell specificity with altered peptides. Results show that monosubstituted peptides do not tell the whole story.

In previous studies, the RT epitope was mapped using recombinant vaccinia viruses and peptides to a 17 amino acid region near the N terminus of the RT protein (23). In the current study, alanine replacements along the 17-mer peptide revealed a core region of nine amino acids containing a H-2Kk binding motif (34–37, 44) as the putative minimal epitope, and this was confirmed by testing the predicted nonamer peptide along with the two octamer peptides contained within this sequence. For H-2Kk, this length was surprising as nonamer peptides have been rarely identified in MHC class I elution studies from H-2Kk (37), and there is evidence that peptides shorter than 8 amino acids may have increased binding affinity for this class I molecule (36).

To further characterize the epitope, alanine-substituted peptides spanning the nonamer were then generated (Table II and Fig. 2) and gave some unexpected results of broader interest. First, substitution at position 1 was permissive for CTL activity in the nonamer, but not in the longer peptide. We speculate that this difference reflects a requirement for threonine in serum protease digestion of the longer peptide to its minimal size for MHC binding and recognition and an interference of alanine at this position with processing.

Second, two different binding assays, when applied to the alanine substituted nonamer peptides, suggested that position 3 as well as the known anchors at positions 2 and 9 were critical to MHC binding (Fig. 3). To test this hypothesis, we asked whether replacement of the asparagine for methionine at position 3 of a second, non-cross-reactive CTL epitope from the circumsporozoite protein of P. falciparum (22), also restricted by H-2Kk, might improve the binding of this Pf peptide to H-2Kk, given its essential role in the AH2-I9 peptide. As observed in Figure 4, substitution with methionine did improve the binding of the Pf peptide by at least a logarithm in several, independent competition assays, suggesting that methionine is indeed a preferred secondary anchor at position 3. Interestingly, however, the resulting peptide could not be recognized by a wild-type Pf peptide-specific CTL line, suggesting that the substitution had also led to a conformational change in the peptide resulting in the loss of recognition at the peptide/TCR interface (data not shown). Alternatively, the methionine substitution in the peptide may have forced a change in the MHC conformation. To confirm that the substituted peptide resulted in an altered conformation, we immunized mice with the methionine-substituted peptide and generated two specific CTL lines. As predicted, neither CTL line could recognize the wild-type peptide, despite displaying good lytic activity in response to the methionine-substituted peptide (data not shown). This result adds to the growing literature that indicates that a portion of peptides designed to have increased binding activity may fail to elicit responses to the native peptide. Further, anchor residues that bind primarily to the MHC molecule can also affect TCR recognition, presumably by affecting peptide conformation in the MHC groove or MHC conformation itself (45, 46).

Third, residues, particularly ones with large, hydrophilic side chains, located at the center of CTL-peptide epitopes have been shown to play a key role in CTL recognition for lysis. Indeed, it is of interest that H-2Kk is the only murine class I molecule known with positions 152 and 156 both being acidic residues and pointing into the groove, and these are interspersed with two positively charged Arg residues at positions 155 and 157. This combination
might be expected to interact ionically with the Glu-Lys-Glu sequence at positions 4, 5, and 6 in the peptide, but clearly not all the results can be explained by such charge interactions. In this context, it was surprising that lysine at position 5 appeared fully replaceable by alanine, isoleucine, serine, and glutamic acid (Fig. 6C). This suggested that this residue may lie across the peptide groove and was unlikely to be interacting either with the negatively charged TCR-interacting glutamic acids on either side or with the TCR itself. To investigate the hypothesis that this residue was uninvolved in TCR contact sites, we generated two different CTL lines, varying in avidity (39), using the modified peptide (with alanine at position 5) as an immunogen, speculating that a truly neutral position would generate CTL capable of recognizing wild-type peptide. Two CTL lines responding to 5ALA were shown to respond weakly (or not at all) to the wild-type sequence (AH2-I9) (Fig. 5A). Thus, the cross-reactivity was unidirectional and under some circumstances residue 5 could play a role in CTL recognition. Given that position 8 and, to a lesser extent, positions 4 and 6 were epistatic for pol α, we mapped the recognition of the 5ALA lines using alanine substitutions combined with the alanine at position 5. Both 5ALA CTL lines retained position 8 as epistatic but differed at positions 4 and 6 (data not shown). Position 4 was epistatic for 5ALA CTL grown on 10 μM peptide (10 μM 5ALA CTL) and neutral for 5ALA CTL grown on 0.1 μM peptide (0.1 μM 5ALA CTL). Position 6 was neutral for both 5ALA CTL lines, while some alterations to position 5 now affected both lines, suggesting a secondary epitopic residue (Fig. 6). This suggests that secondary TCR contact residues, although presumably positioned toward the TCR, need not play any role in TCR recognition. Conversely, previously replaceable residues can acquire a role in TCR recognition by immunization with a peptide containing the altered sequence. The primary TCR contact remains associated with the TCR, presumably because it is the most surface exposed.

Surprisingly, a second substituted peptide (6ALA), with two substitutions relative to the immunizing 5ALA peptide, was able to stimulate the lower avidity CTL line (10 μM 5ALA CTL) (Fig. 5, A and C). Consequently, the combination of K and E did not efficiently stimulate 10 μM 5ALA CTL, but peptides in which either amino acid was paired with an alanine at the neighboring position produced similar dose-response curves to 5ALA itself for these CTL.

This study was not extended to clones, so we cannot necessarily attribute all the data to a single cross-reactive receptor, although the populations have been in continuous culture for >6 mo and show a single Vβ usage. Furthermore, it is highly unlikely that a subpopulation specific solely for the double mutation could possibly have been carried in long-term cultures that contain only the original immunizing Ag, 5ALA. More likely is the presence of a T cell population that cross-reacts between 5ALA and 6ALA. Because titration curves for these two peptides are almost identical, it suggests that the cross-reactive T cells form a major part, if not all, of the CTL line (Fig. 5, B and C).

The data to this point suggested that perhaps two bulky residues were detrimental to CTL recognition, and indeed when a peptide with alanine substitutions at both positions 5 and 6 was made, the peptide was effectively recognized by the CTL. Based on this hypothesis, we synthesized several other peptides with changes at position 5, position 6, or both and found that only a subset of these peptides was recognized by the low affinity 10 μM 5ALA CTL line (Fig. 6A). Peptides such as SS6A and SQ6A had reduced activity compared with 5ALA but were sufficient to indicate that the recognition of the double substitution KA (6ALA) by the 10 μM 5ALA CTL was not unique to that amino acid combination. However, the lack of recognition of SE6A and SI6A suggested that simple pairing of a bulky residue with a small side chain was not sufficient to produce a cross-reaction in all situations. Consequently, the situation is more complex than simply a negative effect from two bulky side chains and will depend on the nature of the side chain paired with alanine. Further delineation of the exact biochemical basis for this pairwise interaction would require the production of a much larger number of substituted peptides and is beyond the scope of the present study.

The 0.1 μM 5ALA CTL had a more restricted cross-reactivity but was unusual in its recognition of 5S6E, 5A6E, 5A6A, and yet not 5S6A, which represents the double mutation and the combination of two singly substituted peptides that were recognized (Fig. 6B). Consequently, this result would be difficult to predict from each substitution in isolation, especially given that all the peptides involved are within a logarithm of each other in the T2 binding assay (Fig. 6D). This contrasts with the same group of peptides used with the 10 μM 5ALA CTL, where each single substitution is recognized (5S6E and 5A6A), and the double substitution is also recognized (5S6A). Consequently, the data provide examples of three patterns of recognition: 1) one of the single substitutions is not recognized (5K6E), but the double substitution is recognized (5K6A); (i.e., restores function); 2) both of the single substitutions are recognized (5S6E, 5A6A), but not the double substitution (5S6A); or 3) both the single and double substitutions are recognized. The first pattern demonstrates that loss of recognition by substitution at one position can be offset by a complementary substitution at a second position that restores recognition, and thus implies pairwise interactions between residues that contribute to binding or recognition that cannot be predicted from current schemes, which make the simplifying assumption that each position in the sequence is independent.

It is also interesting that recognition pattern 2 for T cell recognition also occurs in the T2 binding assay for the KA pairwise where KE, AE, and AA all give comparable T2-K binding, whereas the double substitution (KA) is quite reduced (Fig. 6D). This result suggests that both TCR recognition and MHC binding can be unpredictable when making a double substitution based on the positive interaction of each single substitution. The fact that peptides with the KA double substitution and the EE substitution do not bind significantly in the T2-K binding assay and yet efficiently stimulate CTL suggests that the T2 binding assay is not sufficiently sensitive to predict all epitopes capable of stimulating CD8+ T cells, which is consistent with the findings of others (38).

T cell recognition of complementary double substitutions has been recently described in a concurrent study for a human class II-restricted myelin basic protein epitope, where a double change in both TCR contact residues (not directly adjacent) restored proliferation of the CD4 T cell clone to a level comparable with wild-type peptide (19). Our data confirm that study’s concept of complementary mutations and extend its observations. Using murine class I MHC-restricted CD8+ T cells, we show that immediately adjacent residues can interact in a complementary way to elicit activation or in a negative manner to negate an interaction predicted by the single substitutions. This was possible because we studied interactions involving residues not solely involved with TCR contact. We further suggest that MHC binding of single substitutions may not predict the effect of double substitutions. Both studies then complement each other and together contribute to establishing the principles that the TCR shows great flexibility in the recognition of peptide/MHC complexes and that compensatory changes can restore TCR recognition.

Importantly, our study demonstrates that a single escape mutation that eliminates CTL reactivity can be negated by the appearance of a second mutation that restores CTL to full capacity. This
may have wide implications with regard to the reactivation of memory T cell populations and the selection pressure exerted on highly mutable viruses such as HIV.

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References