

Recognition of Multiple Peptide Cores by a Single T Cell Receptor

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Summary

We present evidence that a single T cell clone can recognize at least five different overlapping peptides, each with its distinct core structure, in the context of the same major histocompatibility complex (MHC) molecule. Distinct core residues are crucial for triggering the T cell receptor (TCR) in each case. These results suggest that the TCR (*a*) has multiple sets of contact residues for alternative peptide–MHC ligands, the binding to any one of which can trigger the cell; and/or (*b*) is able to attach to the peptide–MHC complex in more than one orientation. In this sense, the TCR is a multisubsite structure capable of being stimulated by a variety of peptide ligands associated with the same MHC molecules.

TCRs recognize an antigen only when the antigen's peptide fragments are lodged in a groove-like site on MHC molecules (1). How do the TCRs interact with the peptide–MHC complex? The answer is not clear because the crystal structure of the TCR–peptide–MHC complex still has not been solved. In the meantime, several models for the ternary molecular interaction have been proposed in which the CDR3 loops (encoded by the V-(D)-J junctional region) of both chains of the TCR molecule bind the nominal peptide, whereas the germline-encoded CDR1 and CDR2 loops bind the α helices of the MHC molecule, which flank its peptide-binding groove (1–4).

Using variants of each of the three participating molecules of the TCR–peptide–MHC complex, extensive attempts have been made to test these predictions (4–9). The results from these studies have neither entirely supported nor contradicted these models; nevertheless, several characteristics of the interacting trimolecular complex have emerged. It thus seems clear that a single peptide–MHC ligand can induce several distinct TCR molecules, with each TCR molecule recognizing a distinct aspect of the peptide in the MHC groove. Different TCR molecules displaying specificity for the same peptide–MHC ligand may interact with different and overlapping residues of the peptide (4–8). In addition, studies with MHC mutants at the residues pointing inward along the wall of the peptide-binding groove of the MHC molecule showed that different TCRs displaying identical specificity for the ligand were differentially affected by such mutant MHC molecules (8). These results emphasize that alterations of the particular conformation of the peptide–MHC complex, induced by the association of the peptide with the altered peptide-

binding groove, can result in loss of recognition. Taken together, these results indicate strict constraints in interactions between the TCR molecule and the peptide–MHC complex.

However, how precise are the requirements for peptide structure, distribution of charge and hydrophobicity for a peptide to be a stimulatory one for a given TCR? Can only a single sequence from the antigen associated with a given MHC molecule meet the constraints of TCR recognition? We have asked this question by using a previously reported panel of I-A^d-restricted sperm whale myoglobin (SWM)¹ peptide 110-121-specific T cell hybridomas (10, 11). The approach used was to truncate this determinant from one end so as to abrogate T cell recognition but to retain MHC binding, extend the determinant at the other end, and then examine mutations in the new peptide that could restore TCR recognition.

We now report T hybrid CJM4-16 (from the above mentioned panel of T hybrids) could be stimulated by five different peptides presented by I-A^d. Different core residues are crucial for triggering the TCR in each case. These results were obtained by (*a*) using a set of 180 unique peptides derived by substituting every residue, one by one, in the truncated and extended peptide 105-116 with 15 other amino acids; and (*b*) further analysis of the core structure of three of the six stimulatory mutants of peptide 105-116. Our findings have important implications for models of TCR interaction with peptide–MHC ligands, and they suggest that the TCR is a multisubsite structure capable of being stimulated by a variety of peptide ligands presented by the same MHC molecule.

¹ Abbreviation used in this paper: SWM, sperm whale myoglobin.

Materials and Methods

Mice

Mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Antigens and Peptides

SWM was obtained from Accurate Chemicals & Scientific Corp. (Westbury, NY). SWM peptides 110-121, 105-118, and 105-116 were synthesized and HPLC purified, as described previously (10-12). SWM peptide 105-116 and all series of mutant peptides were synthesized on polyethylene pins followed by cleavage into 96-well plates (10-12). Bulk SWM peptide 105-116 and variants D109, V112, I114, and L114 were synthesized on pins, as well as by conventional methods by Cambridge Research Biochemicals, Inc. (Wilmington, DE). The G107, G108, and P113 variant peptides used to define the cores of these mutants (see Fig. 3) were synthesized in bulk (5 mg) on the pins. Mass spectroscopic analysis done on 16 of the 24 peptides used in Fig. 3 showed the expected molecular mass and 15 of the 16 peptides consisted of a single product. OVA peptide 323-339 was synthesized as described earlier (13).

Generation of T Cell Hybrids

The cloned T cell hybridoma CJM4-16 was derived from continuously growing SWM peptide 110-121-specific T cell lines, as described previously (10, 11). Briefly, T cell blasts from antigen-specific cell lines were fused with the α - β variant of BW5147 as a fusion partner and cloned by limiting dilution in the presence of SWM or its peptides, 110-121, and 105-118 (10, 11). The antigen-reactive hybrids were analyzed for function and TCR V β expression. The hybrid CJM4-16 was further subcloned by limiting dilution and analysis for antigen specificity.

Specificity Assays

T Cell Hybrids. T hybridoma cells (5×10^4) were cultured with various concentrations of a given peptide with 5×10^5 irradiated BALB/c (I-A^d, I-E^d) spleen cells as APCs in 0.2 ml supplemented DMEM (Flow Laboratories, Ayrshire, Scotland). All cultures were done in triplicate, except those with the 180 variants of peptide 105-116, which were done in single wells for a given dose of peptide in each experiment. The culture supernatants collected 24 h later were assayed for IL-2 activity with the IL-2/IL-4-dependent cell line, HT-2, as described earlier (10, 11). Briefly, 10^4 HT-2 cells were cultured with medium alone or with supernatants for 48 h, and proliferation was measured by adding 1 μ Ci [³H]thymidine during the last 18 h of culture, and incorporation was assayed by liquid scintillation counting. Peptides 110-121 and 105-118 (synthesized by conventional methods) were used as positive control peptides in all experiments. In our previous extensive use of peptides synthesized on pins, we (reference 11; Sercarz, E., unpublished observations) have not experienced cases of false negatives or positives in comparison to conventionally synthesized peptides. Negative results with certain peptides and particular hybrids have been matched by positive results with other T cells.

Binding of Peptides to I-A^d

I-A^d was affinity purified from cell lysates of A20 lymphoma cells using anti-I-A^d mAb MKD-6 (13). OVA peptide 323-339 was radiolabeled with ¹²⁵I using the chloramine T method. Peptides were tested for binding to I-A^d as measured by their capacity to inhibit the binding of ¹²⁵I-radiolabeled OVA peptide 323-339, as described previously (13).

Results

Panels of I-A^d-restricted SWM peptide 110-121-specific T cell hybridomas (10, 11) have been reported previously. The minimal core of the peptide determinant recognized by this panel of T hybrids includes amino acids 110-118. The "core" of a peptide determinant is defined as the minimal residues required for both (a) binding to MHC molecules and (b) interacting with the TCR molecule (11). By removing the two amino acids essential for interaction with the TCR from the minimal core, we generated a truncated-core peptide that could still bind the MHC molecule but not stimulate the T cell population. SWM peptide 105-116 is such a truncated-core peptide for T cell hybrids that recognize SWM peptide 110-121, where the original stimulating core is peptide 110-118.

Peptide 105-116 Binds to the I-A^d Molecule but Fails to Stimulate SWM Peptide 110-121-specific T Cells

Fig. 1 shows dose-response curves for activation of CJM4-16 by peptides 105-116 and 110-121 (the peptide antigen used to derive these T cell hybrids), and it shows that peptide 105-116 was nonstimulatory, even at a concentration of 70 μ M. As shown in Table 1, in a direct binding assay to purified I-A^d, peptide 105-116 could bind to I-A^d better (threefold, see the legend of Table 1) than peptide 110-121 (Table 1). That the failure of peptide 105-116 to stimulate CJM4-16 was a result of the absence of core TCR-interactive residues 117 and 118 and not of negative influences (14) exerted by the extra NH₂-terminal sequence 105-109 was demonstrated by the fact that peptide 105-118 was found to be highly stimulatory (Fig. 1).

Six Mutants of the Truncated-core Peptide 105-116 are Stimulatory to T Hybrid CJM4-16 and Subclone CJM4-16-4

In the next series of experiments, every amino acid within peptide 105-116 was substituted, one by one, with 15 alter-

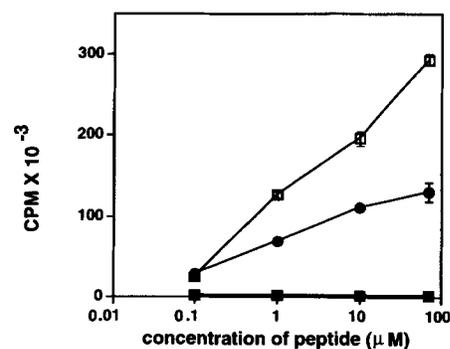


Figure 1. Peptide 105-116 does not stimulate T hybridoma CJM4-16 specific for peptide 110-121 of SWM. CJM4-16 T hybridoma cells (5×10^4) were cultured with various concentrations of the peptides 110-121 (●), 105-116 (■), and 105-118 (□) with 5×10^5 irradiated BALB/c spleen cells as APCs (10, 11). All cultures were performed in triplicate in 0.2 ml. Supernatants from these cultures were then used to assay proliferation of HT-2 cells as described before (10, 11) and in Materials and Methods, and the data are expressed as means \pm SE of each triplicate culture. The mean \pm SE value for medium controls for this experiment was $1.09 \pm 0.37 (\times 10^{-3})$.

Table 1A. Binding to MHC I-A^d Versus Stimulation of T Hybrid CJM4-16-4 by Peptide 105-116 and its Stimulatory Mutants

Peptide	Sequence	Relative binding to MHC*	Stimulation of CJM4-16*
105-116	EFISEAIIHVLH	1.5	–
-G107	—G—	0.34	+++
-G108	—G—	0.1	+++
-P113	—P—	1.8	+++
-L114	—L—	0.18	+++
-I114	—I—	0.01	+++
110-121	—SRHPG	0.59	+++

Table 1B. Binding of Peptides Constituting the First Peak of the 105-116-G108 Series of Peptides to MHC I-A^d: Strength of Stimulation is not Directly Related to Strength of MHC Binding

G108 analogues	Sequence	Relative binding to MHC*	Stimulation of CJM4-16*
101-112	IKYLEFIGEAIH	0.003	+ / –
102-113 [†]	KYLEFIGEAIH	0.011	+++
103-114	YLEFIGEAIH	0.040	+
104-115	LEFIGEAIHVL	0.013	++

* The binding capacity of each peptide is expressed relative to I-A^d binding of unlabeled OVA peptide 323-339 (13). The 50% inhibition value for unlabeled OVA peptide 323-339 was 131 ± 34 nM in the experiments considered. The values shown in the Table are means of at least two independent experiments. The quantitative values for stimulation are shown in Fig. 2 for Table 1 A and Fig. 3 for Table 1 B. The stimulation of CJM4-16 is depicted as +++ when proliferation of HT-2 cell is 15-fold or more than the background (medium control), ++ when it is between 5–10 times the background, + when it is 3–5 times the background, and + / – when proliferation is 2–3 times the background.

[†] The first peak of G-108 mutants.

native amino acids, thus creating 180 unique peptides. Response to the 180 peptides was examined for cloned T hybridoma CJM4-16 (core: 110-118) and its subclone, CJM4-16-4, to ensure that all responses were definitively caused by a single T cell. The results from a representative of three experiments for T hybrid CJM4-16-4, at the optimal peptide concentration (7 μ M) are shown in Fig. 2 A and were nearly identical to those obtained with CJM4-16 (data not shown). Surprisingly, it was found that several singly substituted analogues of truncated-core peptide 105-116 could trigger the SWM peptide 110-121-specific T hybridoma CJM4-16 or its subclone and this pattern of stimulation was repeated in each experiment studied at three peptide concentrations (see legend of Fig. 2 A).

Fig. 2 B shows the dose-response curves for five stimulatory mutants and one nonstimulatory analogue (Q110) of peptide 105-116 over a large range (0.1–70 μ M) of peptide concentrations. It is clear that the nonstimulatory mutant peptide does not stimulate even at a 70- μ M concentration of peptide.

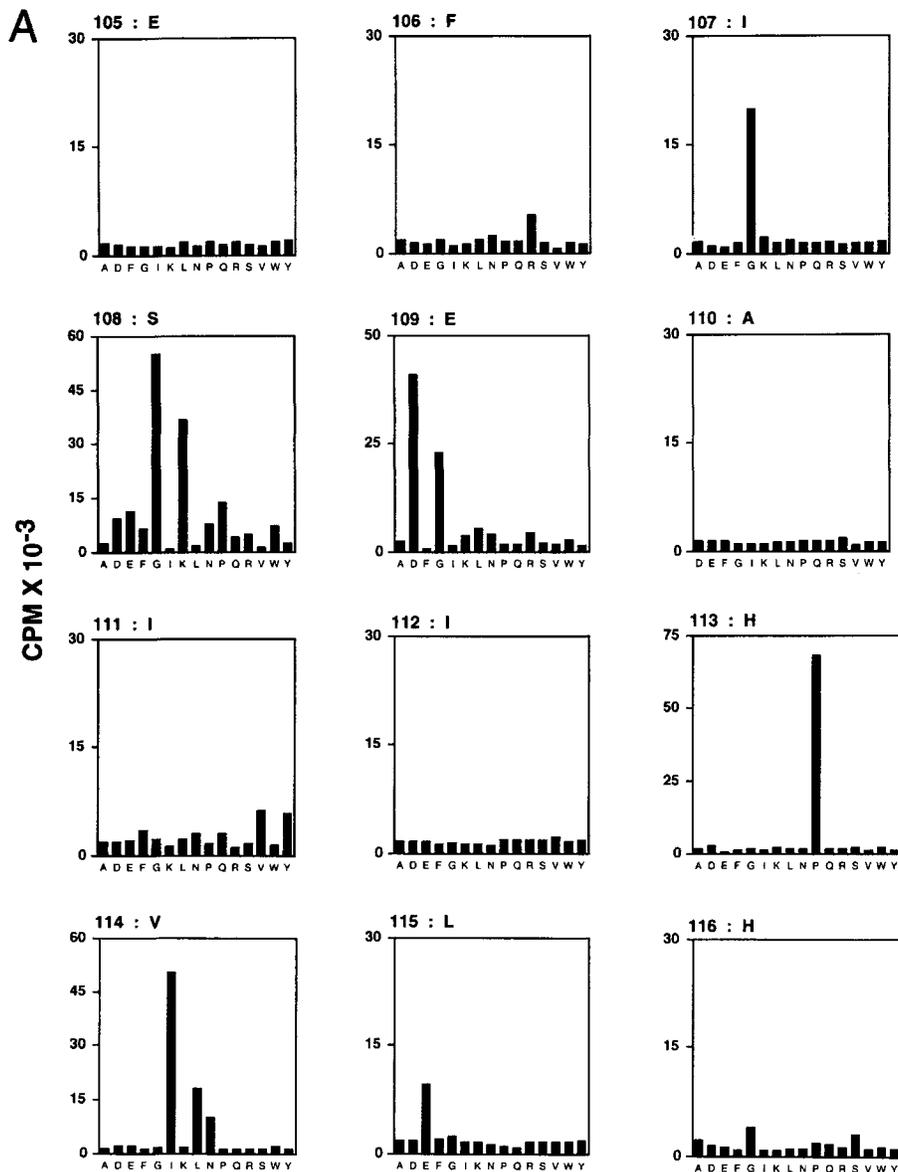
The six singly substituted mutants of peptide 105-116 that were strongly stimulatory in all the experiments could be divided into two categories:

Replacements Outside the Minimal Determinant Core (110-118) that Convert the Truncated-core Peptide 105-116 into a Stimulatory Fragment. (a) I-107 to G-107, (b) S-108 to G-108, and (c) E-109 to D-109 or G-109 can convert the inactive peptide 105-116 into a highly stimulatory peptide.

Replacements within the Original Core Residues That Also Convert the Truncated-core Peptide 105-116 into a Stimulatory Peptide. (d) H-113 to P-113, (e) V-114 to I-114, or (f) to L-114. Note that several other clones within our panel of SWM 110-121-specific CD4 T hybrids recognized a different pattern of mutant peptides, while certain other T hybrids were not stimulated by any of the variants of peptide 105-116 (data not shown).

Stimulatory Variants of Peptide 105-116 Bind to I-A^d Within the Same Range as the Nonstimulatory 105-116

Table 1 A shows that the stimulatory mutants of peptide 105-116, as well as SWM 110-121, display a relative binding to purified I-A^d in a comparable range (range of relative binding = 0.1–1.8, see legend of Table 1) with the exception of peptide 105-116-[I114], which shows low binding to I-A^d but can nevertheless stimulate T hybrid CJM4-16 very well



CJM4-16-4 : 105-116 VARIANTS

between the ability of a peptide to bind the MHC molecule and to activate a T cell. The nonstimulatory peptide 105-116 is one of the best binders to I-A^d, binding about threefold better than the original immunogen, SWM 110-121 (see Table 1).

Analysis of the Core Structure of the Stimulatory Mutants of p105-116

Three of six stimulatory mutants of the nonstimulatory peptide 105-116 were selected to analyze the core structure recognized within the mutant peptides. The core regions within the G-107, G-108, and P-113 mutant peptides were studied by using three series of 12-mer peptides, sequentially

traversing the sequence 100-118 with G always at position 107 or 108, respectively, or spanning region 102-120, with P at positions 113 (see Fig. 4).

The results depicted for T hybrid CJM4-16-4 (Fig. 3) clearly show a surprising "dual-core" pattern of response within each of the three series of overlapping mutant peptides that were tested (Fig. 3). Thus hybridoma CJM4-16-4 optimally recognizes two core structures in both mutant peptides G-107 and G-108: (a) 102-113 and (b) 105-116. It is not clear whether or not this dual-core pattern within this sequence is a result of hindrance or facilitation in presentation of the peptide by the MHC molecule as one moves along the frame of the peptide. Table 1 B shows the binding to I-A^d of peptides defining the NH₂-terminal core 102-114-[G108], and it is clear that the dual-core pattern of response is not a direct

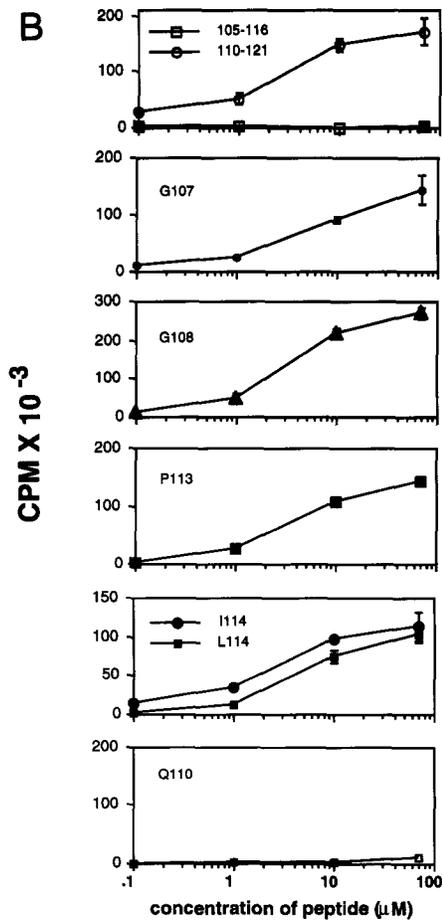
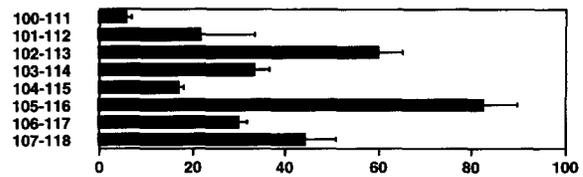


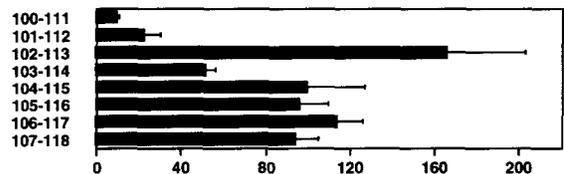
Figure 2. (A) Several of the 180 mutants of the nonstimulatory, truncated core peptide 105-116 regain antigenicity for T hybridoma CJM4-16-4. Every amino acid within peptide 105-116 (EFISEAIIHVLH) was substituted, one by one, with 15 alternative amino acids, thus creating 180 unique peptides. One representative of three experiments is depicted at the optimal dose of 7 μM peptide. The vertical axis refers to proliferation with the peptide minus that obtained with the medium alone control. Proliferation was assayed as in Fig. 1. The horizontal axis refers to the substituted amino acid in the single letter code. At the top of each panel is shown the native amino acid and its specific position in peptide 105-116. The 14- and 3.5- μM doses of peptides gave results similar (data not shown) to the 7- μM dose of peptide. The mean \pm SE values of triplicates for medium, peptides 105-116, and SWM peptide 110-121 in this experiment were $0.33 \pm 0.005 \times (10^{-3})$, $0.69 \pm 0.005 \times (10^{-3})$, and $29.7 \pm 3.5 \times (10^{-3})$. The stimulatory potential of four mutant peptides (105-116-[D109], 105-116-[I112], 105-116-[I114], and 105-116-[L114]), synthesized by conventional methods (10, 11), was not different from that shown by peptides synthesized by the pin methodology (data not shown; references 11, 31). All the stimulatory analogues, when tested using B10.GD (I-E⁻) spleen cells as APCs, showed the same results (data not shown) as with BALB/c (I-E^{d+}) APCs. Thus as with the original immunogen, peptide 110-121, responses with the stimulatory analogues were restricted to I-A^d. (B) The stimulatory and nonstimulatory mutant peptides show a consistent pattern over a large concentration range. Shown is the stimulation of T hybrid CJM4-16-4 with five stimulatory analogues of peptide 105-116 (G107, G108, P113, I114, and L114) and one nonstimulatory analogue (Q110) of peptide 105-116 at a concentration range of 0.1–70 μM . The nonstimulatory analogue Q110 shows no stimulation even at the 70- μM peptide concentration. The original peptides, 110-121 and 105-116, are shown as positive and negative controls.

function of binding of various peptides to the MHC I-A^d. Thus, loss of K-102 in 103-114-[G108] results in a sizable reduction of TCR activation by 103-114-[G108] (Fig. 3, middle panel), even though it shows good binding to I-A^d (Table 1 B). Two stimulatory peaks can also be defined within the P-113 mutant at frames 103-114 and 108-117 (Fig. 3, bottom panel). Importantly, the dose response of all peptides shown in Fig. 4 over a large range of concentrations (0.1–70 μM) showed the same bimodal response at all the stimulatory doses (data not shown). To summarize, there are four distinct peptide structures that are stimulatory to this single T cell clone

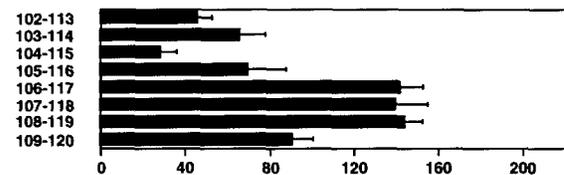
G107 peptides



G108 peptides



P113 peptides



CPM X 10⁻³

Figure 3. Two peak stimulatory sites occur within the three stimulatory mutant peptides of 105-116. Response of CJM4-16-4 is shown to a series of 12-mer peptides, sequentially traversing the peptide from its NH₂ to its COOH terminus and spanning residues 100-118 (native sequence: PIKYLEFISEAIIHVLHSR) for the mutant peptides 105-116-[G107] (top panel) and 105-116-[G108] (middle panel) and 102-120 (native sequence: KYLEFISEAIIHVLHSRHP) for the mutant peptide 105-116-[P113] (bottom panel). One representative of three experiments is shown at the optimal dose of 7 μM for each peptide. Activation of CJM4-16-4 is represented as the mean \pm SE of triplicate cultures. The mean \pm SE values from triplicate cultures for the medium control, peptide 105-116 and SWM peptide 110-121 in this experiment were $8.9 \pm 0.69 \times (10^{-3})$, $3.5 \pm 0.63 \times (10^{-3})$ and $58.2 \pm 0.86 \times (10^{-3})$, respectively. The mean value of each peak was compared with the nearest lowest mean value in the preceding trough by the Student's *t* test (one tail). For example, the means at the first and the second peaks obtained with peptides 102-113-[G107] and 105-116-[G107], respectively, were compared with the means obtained with peptides 100-111-[G107] and 104-115-[G107], respectively. *P* values for each of the peaks were: (a) for the G-107 mutants, <0.005; (b) for the G-108 mutants, <0.005 and <0.025; and (c) for the P-113 mutants, <0.05 and <0.005.

CORE SEQUENCES OF THE PEPTIDES THAT CAN STIMULATE T HYBRID CJM4-16-4

NATIVE CORE	
110-121	
<u>AIHVLHSRHPG</u> *	
NOVEL CORES	
102-113-G108	103-114-P113
<u>KYL</u> EFIGEAIH	<u>YL</u> EFIGEAIIPV
105-116-G108	105-117-P113
<u>E</u> FIGEAIHVLH	<u>E</u> FIGEAIIPVLHS

Figure 4. The “minimal core” of a peptide determinant is defined as the minimal residues required for both binding to MHC molecules and interacting with the TCR molecule (11). As an example, 108-117-[P113] is depicted above as one of the minimal cores in the stimulatory peptide 105-117-[P113] because loss of residue 108 at the NH₂ terminus (109-120-[P113]) and the loss of residue 117 at the COOH terminus (105-116-[P113]) (see Fig. 3) results in a considerable loss of activation of the T hybrid CJM4-16. * The residues underlined represent the minimal core residues in each of the minimal stimulatory peptides.

(see Fig. 4): NH₂-terminal cores (a) 102-113 with G at 107 or 108, (b) 103-114 with P at 113; and COOH-terminal cores (c) 105-116-[G107/G108] and (d) 108-117-[P113]. It should be pointed out that each of these structures is different from the core structure in the native core sequence 110-118 (Fig. 4).

We predicted residue K-102 of the core 102-113-[G108] (Fig. 4) to be a TCR contact residue of the core 102-113-[G108] since the COOH-terminal positively charged residue R-118 was the TCR-interacting for the core 110-118. It is clear from Table 1 B that K-102 is not required for MHC binding. Substitution of K-102 with A-102 results in complete loss of the ability of the variant peptide 102-113-[G108] to activate the T hybrid CJM4-16 (Fig. 5). Thus, we propose that the NH₂-terminal positive charge at K102 is important for stimulation of T hybrid CJM4-16 by core 102-113-[G108].

Discussion

The current report shows that a single TCR molecule can be stimulated by at least five essentially “different” peptides, each with a distinct core structure, in the context of the same MHC molecule. It demonstrates that single amino acid alterations of a nonstimulatory I-A^d-binding peptide can generate stimulatory peptides for the I-A^d-restricted T hybrids such as CJM4-16-4. Furthermore, we demonstrate that the region 100-121 of SWM binds the MHC I-A^d in several different registers; one register, 110-121, which was used as the immunogen, is stimulatory, but the other registers, 105-116 or 102-113, are nonstimulatory. However, several mutants of 105-116 and 102-113 are able to stimulate the CJM4-16-4 T cell; these stimulatory mutant peptides, despite sharing

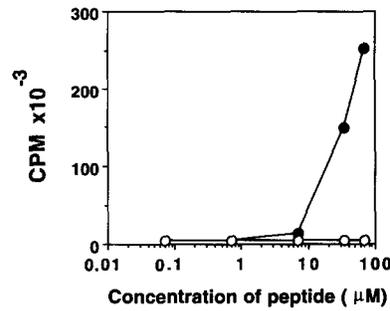


Figure 5. K at position 102 is required for the core 102-113-[G108] to stimulate T hybrid CJM4-16. Shown is the stimulation of T hybrid CJM4-16-4 with the stimulatory mutant peptide 102-113-[G108] (●) and its variant in which K-102 has been replaced with A-102 (○). The response of the T hybrid was assayed as described in Fig. 1. K-102 is not required for binding to MHC I-A^d (see Table 1).

three residues with peptide 110-121, seem essentially very different in charge and hydrophobicity distribution.

Are the shared Ala-Ile-Ile residues (110A-111I-112I, [A-I-I]) in the five peptide cores depicted in Fig. 5 responsible for stimulation of CJM4-16-4? These residues are placed at the (a) NH₂ terminus in the peptide core 110-118, (b) at the COOH terminus in cores 102-113-[G108] and 103-114-[P113], and (c) in the middle of the 12-mer 105-116-[G108] and 105-117-[P113]. Thus, if the residues A-I-I are responsible for triggering the TCR of CJM4-16-4, models of TCR-peptide-MHC interactions will have to account for their relative positioning in different peptides (discussed below).

The crystal structures of the class II molecule and several peptide-MHC class I molecules clearly show that (a) the peptide is lodged in a groovelike site on the MHC molecules, where the walls of the groove are formed by two α helices and the floor by the β-pleated sheets encoded by the MHC molecule; and (b) the orientation of the peptide in this groove is fixed and thus the NH₂ terminus of all peptides interacts with one end and the COOH terminus with the other end of the groove (14-16). However, what can be said about the orientation of the TCR molecule interacting with its ligand, the peptide-MHC complex? It was recently reported that the TCR is oriented over the peptide-MHC complex in such a way that the TCR α chain contacts the α helix formed by the β chain of the class II molecule and the TCR β chain contacts the class II α chain (4). In a different system, using mutants of MHC and TCR molecules, a reverse orientation of the TCR was reported earlier by Janeway and colleagues (17) with the TCR α chain contacting the class II α chain. Each of these experiments implies that a given TCR interacts with its peptide-MHC ligand in only a single orientation, albeit opposite.

It was recently shown that alteration of a single-charged residue elicited a TCR with a complementary alteration in charge in a specific residue within the CDR3 region (4). These elegant experiments made two important points: (a) there is relevant and direct contact between the nominal peptide and the CDR3 region of the TCR molecule (4, 18); and (b)

formation of an essential charge interaction between specific residues of the TCR and the peptide is important and of much greater significance than TCR-MHC interaction, which is less demanding (4, 19).

Since the interaction of the TCR with its ligand(s) involves hydrophobic and/or H bond interactions with the side chains of the peptide residues and/or salt bridges between residues with opposite charges on the peptide and the TCR, respectively (1, 4), a consideration of the sequences of the different core peptides shown in Fig. 4 suggests the following non-mutually exclusive possibilities:

Recognition by a Single TCR Through Multiple Sets of Contact Residues

(a) The strongly charged residues within the stimulatory sequences are not only qualitatively and quantitatively different, but also occupy different positions relative to the COOH or the NH₂ terminus (Fig. 4). Thus, the peptides 103-114-[P113], 105-116-[G107/G108], and 108-117-[P113] have no strongly positive charged residue, but rather one to two negatively charged residues, whereas the original peptide 110-121 (and its 110-118 core) had the strongly basic arginine at 118 and no negatively charged residue. If formation of a salt bridge involving a strongly basic residue on the peptide were essential for TCR interaction with its ligand, it is clear that each of the four peptide sequences could form a salt bridge only at unique positions along the TCR molecule.

(b) Likewise, since proline at residue 113, replacing histidine, is a residue that causes a change in direction of a peptide sequence (20), the COOH-terminal VLH sequence in G-107/108 mutants and in the P-113 mutant (of peptide 105-116, Fig. 5) might occupy a different position and thus possibly contact different residue(s) in the TCR molecule.

(c) A third possibility has been postulated earlier (21), that each of the peptides is able to stimulate the TCR by virtue of its ability to somehow adopt a configuration within the MHC binding site that is equivalent in its constellations of charge, hydrophobicity, and shape (the "equivalence" or mimicry hypothesis). Although it cannot be ruled out, the physical and chemical topography of amino acid residues in these varied peptides do not seem to favor this possibility. The stimulatory peptides have qualitatively and quantitatively different charges, as well as completely different positions of hydrophobic centers (as discussed above).

It has recently been shown that individual T cell clones are able to express two functional TCR α chains (22). We have cloned and sequenced the α and β chains of T hybrid CJM4-16 by the PCR. Our results show the expression of a single α chain in clone CJM4-16 (Nanda, N. K., R. Fairhurst, and E. Sercarz, unpublished data), thus confirming that it is a single TCR that shows stimulation with a diverse set of peptides in the context of the same MHC molecule.

A Single TCR May be Able to Use Two Opposite Orientations to Recognize its Ligands

(a) The positively charged, carboxy-terminal R-118 in peptide 110-121 has been shown earlier by us to participate in

interacting with the TCR of CJM4-16 (11). In this report, we show that the NH₂-terminal K-102 in 102-113-[G108] is not required for binding to MHC (Table 1 B), but is important for triggering the TCR molecule (Fig. 5). If, as suggested by Brown et al. (15) and Davis and Chien (23), the peptide does not switch orientation in the groove of the class II molecule, R-118 (COOH-terminus in core 110-118) and K-102 (NH₂ terminus in core 102-113-[G108]) could play a role in TCR-ligand interaction with the same specific negatively charged residue on the TCR only if the TCR switches its orientation by 180°. This interpretation is supported by the fact that only a single negatively charged residue appears in the α and β sequences of the CDR3 regions of the CJM4-16-4 TCR (Nanda, N. K., R. Fairhurst, and E. Sercarz, unpublished data). However, it is relevant to note that there are other stimulatory peptides (e.g., 103-114-[P113]), which have no positive charge, indicating that this is not a sine qua non for reactivity (see below).

(b) Likewise, peptide 110-121 possesses a highly hydrophobic sequence A-I-I at its NH₂ terminus and two of these hydrophobic residues, 110-A and 111-I in peptide 110-121, have been shown previously by us to be important for interaction with the TCR of CJM4-16 (11). The identical hydrophobic sequence in the stimulatory peptide, 102-113-[G107/G108] (and 103-114-[P113]), is situated at or near the COOH terminus. Thus, important hydrophobic interactions among the TCR and A-110 and I-111 in peptides 110-121 and 102-113-[G107/G108], 103-114-[P113] are possible only if the TCR docks onto the peptide-MHC ligand in one orientation in the first case and in the opposite orientation (180° shift) in the second case (of 102-113-[G107/G108] and 103-114-[P113]). In this regard, it is noteworthy that when the sequences of α and β chains of the I-A^d molecule are compared, a few up-pointing residues (24) were found to be not only comparable in the antiparallel α and β chains of I-A^d, but also seemed to be symmetrically opposed, per the class II structure described recently (15). These residues are 79-G, 76-H, 72-A, and 69-Q in the α chain and 85-G, 81-H, 73-A, and 82-N in the β chain. Although the residue numbers are different, the relative position along the helices are equivalent. That a single TCR can interact with the same MHC in a variety of ways has also been suggested in a recent report (19).

In conclusion, our experiments reveal that, notwithstanding the general rule of exquisite antigen specificity of a TCR, the CJM4-16 TCR can be triggered by at least five different peptides in the context of the same MHC molecule. That TCR recognition as a rule may exhibit multispecificity is not only supported by the multispecificity exhibited by CJM4-16-4, but also by a previous report showing recognition of two different peptides by a T cell clone (25). It is of interest to note that in the latter case, at least one positively charged residue was critical for the ability of each peptide to stimulate the same TCR. Recognition of allogeneic MHC molecules by a single T cell clone specific for a nominal antigen is another example showing the potential multispecificity of a TCR molecule (26-29).

To summarize, our results, reminiscent of polyfunctional Igs, where a single immunoglobulin could bind several struc-

turally different haptens at different subsites within the antigen-combining site of the antibody molecule (30), suggest that the TCR may have a similar multisite structure capable of

being stimulated by a variety of peptides associated with a given MHC.

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