Minimal peptide length requirements for CD4\textsuperscript{+} T cell clones—implications for molecular mimicry and T cell survival

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Abstract

CD4\textsuperscript{+} T lymphocytes usually recognize peptides of 12–16 amino acids in the context of HLA class II molecules. We have recently used synthetic peptide combinatorial libraries to dissect in detail antigen recognition by autoreactive CD4\textsuperscript{+} T cell clones (TCC). The results of these studies demonstrated that antigen recognition by T cells is highly degenerate and that many cross-reactive ligands can be defined, some of which much more potent than the selecting autoantigen. Based on these observations, we examined the response of a myelin basic protein-specific HLA class II-restricted CD4\textsuperscript{+} TCC to truncation variants of optimal ligands. Surprisingly, pentapeptides, tetrapeptides and even tripeptides derived from different segments of the optimal ligands were recognized by the TCC, and some were even more potent than the selecting autoantigen. In addition, these peptides enhanced the survival of the TCC at low concentration. The relevance of this finding was supported by the generation of pentapeptide-specific CD4\textsuperscript{+} TCC from peripheral blood lymphocytes. These observations not only change existing views on the length requirements for activation of CD4\textsuperscript{+} HLA class II-restricted T cells, but also extend our knowledge about the flexibility of TCR recognition and the potential for cross-reactivity in the immune system.

Introduction

T cell activation of CD8\textsuperscript{+} and CD4\textsuperscript{+} T cells results from the recognition of antigenic peptides in the context of self MHC class I or class II molecules respectively. In recent years, the interaction between the TCR and the MHC–peptide ligand has been functionally analyzed (1), and their crystal structures elucidated (2–4). It is now firmly established that peptides are able to bind to a given MHC molecule provided that the peptide contains the allele-specific anchor motifs (5,6). On the T cell side, productive engagement of the TCR with the MHC–peptide complex will result when critical amino acids are present in certain positions of the peptide (7). However, it was also demonstrated that residues that are not in TCR contact positions can influence recognition and modulate the functional outcome of the T cell response (8,9). Our recent data argue that, while there is a hierarchy in terms of the importance of individual amino acids contacting the TCR, none of them is strictly required (10). Similar to what has been demonstrated for the interaction of the peptide with the MHC molecule (6,11), each amino acid of the peptide contributes largely independently to the overall affinity between the TCR and the MHC–peptide complex (10). Whether T cell activation will occur depends on a number of factors: the affinity of the TCR for the MHC–peptide complex (12), the density of TCR and MHC–peptide complexes, as well as the expression of co-receptors, adhesion molecules and co-stimulatory molecules on the interacting cells (13,14).

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With respect to peptide length, it was assumed that class II-associated peptides fall into a certain range of lengths to fulfill the above requirements. This notion stems from sequencing MHC class II-associated self-peptides, from systematic binding studies (15,16), and from the elucidation of the X-ray crystal structure of MHC–peptide complexes (17,18). While the peptide length for MHC-restricted T cell responses is generally thought to be 8–9 amino acids (class I) and 12–15 amino acids (class II), it was shown that shorter peptides may be sufficient in certain instances, although at much higher concentrations (19–22).

We and others have recently utilized synthetic peptide combinatorial libraries in the positional scanning format (PS-SCL) to systematically assess the stimulatory value of each of the 20 naturally occurring L-amino acids at each position of the peptide and to define the spectrum of agonist ligands for individual T cell clones (TCC) (23–26). This approach has allowed the identification of agonist ligands for myelin basic protein (MBP)-specific TCC that were several orders of magnitude more potent than the autoantigenic peptide used to select the clone (24). Here, we employ such optimal ligands (effective in the lower picomolar range) to determine the minimal peptide length for HLA class II-restricted CD4+ T cell responses is generally thought to be 8–9 amino acids (class I) and 12–15 amino acids (class II), it was shown that shorter peptides may be sufficient in certain instances, although at much higher concentrations (19–22).

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**Methods**

**Peptides**

Peptides were initially synthesized by pin/F-moc techniques (Chiron Mimotopes, Clayton, Australia). To ensure the purity of the peptides and to exclude contamination by longer peptides, the same set of antigens including all peptides <6 amino acids were resynthesized by simultaneous multiple peptide synthesis (SMPS) methodology and purified by HPLC (28). The peptides were characterized using HPLC and mass spectrometry. Peptides were dissolved in H2O, except peptides VVIFFKNVVIK and VIIFFKNVVIK that required the addition of 0.1% acetic acid.

**Synthesis and analysis of soluble peptide combinatorial libraries**

The decapetide PS-SCL as well as subsequent peptide mixtures and individual peptides were synthesized using SMPS methodology as described (28,29).

**TCC TL5F6 and CSF-3**

TCC TL5F6 was established from peripheral blood mononuclear cells (PBMC) by a limiting dilution split-well technique with MBP and characterized as described (30). The TCC is restricted by DR2b (DRα + DRβ*1501). The TCR usage for the TCC is TCRBV6.2. TCC CSF-3 was established with a limiting dilution split-well technique with a lysate of Borrelia burgdorferi from the cerebrospinal fluid of a patient with chronic Lyme disease. The TCC recognizes several B. burgdorferi-derived as well as human peptides in the context of DR2b. The TCR usage is TCRBV14 (26).

**T cell proliferation**

TCC were rested for 8–12 days, washed and resuspended at 1 × 10^5 cells/ml in complete medium (CM = IMDM containing 5% human serum, 1% penicillin/streptomycin and 0.2% gentamicin). Then 100 µl of this cell suspension was added to each well of 96-well U-bottom plates containing 5 × 10^4 irradiated (3000 rad) PBMC and varying concentrations of peptides. Cells were cultured at 37°C for 72 h or as indicated. During the last 8 h of culture, 1 µCi [3H]thymidine was added to each well. Cells were then harvested and incorporated radioactivity was measured by scintillation counting.

**TCR signaling**

HLA-matched B cells (2 × 10^5) were pulsed with MBP peptide (83–99), or short peptides FFK, VVI or NVVI for 2 h at 37°C. Antigen-presenting cells (APC) were then washed 3 times with T cell medium to remove unbound peptide. T cells (1 × 10^6) were added to the B cells and, after centrifugation (10 s at 1000 r.p.m.), were incubated at 37°C for 5 min. Samples were then washed once with PBS and placed in lysis buffer [1% NP-40, 10 mM Tris–HCl, pH 7.2, 140 mM NaCl, 2 mM EDTA, 5 mM iodoacetamide, 1 mM Na 3VO 4 and complete protease inhibitor cocktail (Boehringer, Mannheim, Germany)] for 25 min on ice. After removing nuclear debris, lysate supernatants were subjected to immunoprecipitation by incubation with rabbit anti-ZAP-70 (provided by Dr L. Samelson, NCI) at 4°C for 12 h. Samples were analyzed by SDS–PAGE and immunoblotting with 4G10, a mouse mAb to phosphotyrosine (Upstate Biotechnology, Lake Placid, NY).

**T cell survival assays**

T cell survival assays were performed as described (31). Seven days after re-stimulation of the TCC TL5F6, cells were washed and resuspended at 2 × 10^5 cells/ml. Then 100 µl of the suspension was added to a 96-well U-bottom plate containing 5 × 10^4 irradiated (3000 rad) PBMC and various concentrations of peptides. After 6 days, T cells were rechallenged with irradiated PBMC and MBP (83–99). Cells were cultured for another 72 h at 37°C. During the last 8 h of culture, 1 µCi [3H]thymidine was added to each well. Cells were then harvested and incorporated radioactivity was measured by scintillation counting. Control cultures were set up under the same conditions to measure primary proliferative responses and cytokine secretion on day 3, 4, 5 and 6 after initial stimulation by the short peptides. In a flow cytometry-based survival assay, 1 × 10^5 rested T cells (TCC TL5F6) and 1 × 10^5 PBMC were seeded in triplicate wells of U-bottom 96-well plates in the presence or absence of the short peptides (VVI, FFL, LRE and KDS, 10 µg/ml). After 5 days, T cells were stained with CyChrome-conjugated anti-CD3 antibody.
Short peptides activate CD4+ T cell clones

Table 1. Summary of the proliferative response for TCC TL5F6 to decapeptide PS-SCL

<table>
<thead>
<tr>
<th>PS-SCL</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
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<tbody>
<tr>
<td>PS-SCL</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>K</td>
<td>I</td>
<td>I</td>
<td>K</td>
<td>I</td>
<td>I</td>
<td>K</td>
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<td>not [CDEGHK]</td>
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<td>M</td>
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<td>I</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MBP (87–96)</td>
<td>V</td>
<td>H</td>
<td>F</td>
<td>K</td>
<td>N</td>
<td>I</td>
<td>V</td>
<td>T</td>
<td>P</td>
<td></td>
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<td></td>
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<td>92</td>
<td>93</td>
<td>94</td>
<td>95</td>
<td>96</td>
</tr>
</tbody>
</table>

Amino acids corresponding to the most active mixtures deduced from the response of TCC TL5F6 to a decapeptide PS-SCL are displayed in the upper part of the table. Amino acids in parentheses represent mixtures due to frameshift of the PS-SCL (24). The corresponding MBP sequence is displayed at the bottom. MHC anchor positions in the MBP sequence are indicated by boxes.

Peripheral blood lymphocytes obtained by leukapheresis and countercurrent centrifugal elutriation were separately stained with a mixture of FITC-conjugated anti-CD3 and -CD19 antibodies (Exalpha, Boston, MA), and added to the T cells at 3×10^5/condition. The survival rates were calculated as the number of CD3–CyChrome-positive cells divided by the number of CD3/CD19–FITC-positive cells×100.

T cell cloning with pentapeptides

TCC were established as described (30). PBMC (2×10^5) from normal donors or multiple sclerosis patients were seeded in 96-well U-bottom plates with either 25 µM peptides KNVVI (derived from an optimal ligand for TCC TL5F6) or KYVKQ (derived from influenza hemagglutinin). After 7 days, 10 U/ml rIL-2 (NCI, NIH) was added. After another 8 days, the cultures were split to determine wells that contained antigen-specific T cells by proliferation. Positive wells were re-stimulated with PBMC, peptide and IL-2, and re-tested at least twice to confirm specificity. In parallel to the third test, a FACS pheno-type analysis for the expression of CD4 and CD8 was performed on a FACSCalibur (Becton Dickinson, Mountain View, CA) using mAb (both from Becton Dickinson) as described (32).

Database search

Database searches of SWISS-PROT for peptide sequences or peptide motifs were performed with ScanProsite (http://www.expasy.ch/sprot/scnpsit2.html).

Results

Determination of optimal peptide ligands for TCC TL5F6

In order to define the minimal length requirements for an antigenic peptide recognized by a class II-restricted MBP-specific CD4+ TCC, we selected a human TCC that showed a strong response to a decapeptide PS-SCL, thus allowing determination of optimal ligands as previously described (24,25). A summary of the results is shown in Table 1. While many amino acids were tolerated in positions 1–3, mainly uncharged amino acids were optimal in position 1, aliphatic and aromatic amino acids in position 2, whereas F, M and Q were stimulatory in position 3. In contrast, the requirements in positions 4–10 were much more discriminatory in that only a few mixtures induced strong stimulation of the TCC, whereas all other mixtures in those positions elicited no response. The amino acids corresponding to the most active mixtures were F, Y and A in position 4, the positively charged amino acids K and R in position 5, N in position 6, K, V and T in position 7, I and V in position 8, aliphatic amino acids and A in position 9, and K in position 10 (Table 1). Based on these results, we synthesized a number of optimal 10 amino acid (as well as some 11 amino acid) peptides and tested them for their relative potency in proliferation assays. The results with one of these, decapeptide LIMFKNVVIK, is shown in Fig. 1. Similar to our previous data (24), peptides based on the TCR motif deduced from the response to PS-SCL were several orders of magnitude more potent than the corresponding 11 amino acid MBP peptide VVHFFKNVIK. If the first 3 amino acids LIM were replaced by the three corresponding amino acids of MBP (87–96) and an additional V added at the N-terminus, resulting in peptide VVHFFKNVIIK, no significant change was observed, corresponding to the small differences observed with the PS-SCL in positions 1–3. However, substitution of P for K in position 11 decreased the stimulatory potency of the peptide. The exchange of T for I in position 10 reduced
Short peptides activate CD4+ T cell clones

**Fig. 2.** Tripeptide variants are recognized by TCC TL5F6. The proliferative response of TCC TL5F6 to N-terminal truncations of superoptimal peptide LIMFKNVVIK (A) as well as N- and C-terminal truncations of superoptimal peptides VIIFKNVVIK and VIIFKNVVIK is shown (B and C). Peptides as short as 3 amino acids (FFK) can activate the TCC. The background was 333 c.p.m., averages ± SD of duplicates for each data point are displayed.

The response slightly, whereas I for V in position 8 (note, I is present in the native sequence) decreased it by several orders of magnitude (Fig. 1).

**Definition of the minimal peptide length requirements for TCC TL5F6**

Next, we assessed how truncation analogues of an optimal ligand would be recognized by the TCC. The stepwise removal of single amino acids from the N-terminus of peptide LIMFKNVVIK resulted in a decline of the stimulatory potency by one or more orders of magnitude at a time (Fig. 2A). However, the truncated pentapeptide KNVI was ~1 log more potent than the hexapeptide KNVVIK, indicating that the removal of the terminal lysine in the short peptide enhances recognition.

Based on the surprising result that a pentapeptide stimulated the CD4+ TCC, we tested further truncations on both sides. Since the 11 amino acid long peptides VIIFKNVVIK and VIIFKNVVIK were equally well tolerated as the LIMFKNVVIK variant, we synthesized a new set of short peptides based on all three peptides. The removal of the N-terminal K from peptide KNVVI improved recognition by one order of magnitude (Fig. 2B). The resulting peptide NVVI was slightly more potent than the 11 amino acid MBP peptide (86–96) (data not shown). The N-terminal tetrapeptide of KNVVI, KNVV, was about two orders of magnitude less potent than NVVI and truncation of the N-terminal further reduced recognition. However, even the tripeptide VVI was still recognized to a low extent at the highest concentration. It is important to note that this sequence occurs twice, at the N- and C-terminal of the 11 amino acid superagonist peptide VVIFFKNVVIK.

To validate the above observations with peptides from the N-terminus, we truncated off the C-terminal 6 amino acids KNVVIK from one of the optimal 11 amino acid peptides (Fig. 2C). The N-terminal pentapeptide VIIFF is shown (B and C). Peptides as short as 3 amino acids (FFK) can activate the TCC. The background was 333 c.p.m., averages ± SD of duplicates for each data point are displayed.

**Fig. 3.** Response of TCC TL5F6 to MBP (86–96), a superagonist peptide and MOG (44–53). The background was 720 c.p.m.; triplicates for each data point are displayed. Amino acids that do not match the MBP sequence are underlined. The response of the TCC to the short peptides was strictly HLA-DR2b-dependent. No response to the short peptides designed for TCC TL5F6 was obtained from various TCC of different specificity. To confirm the importance of this finding, we tested the response of the TCC to a decapptide derived from myelin oligodendrocyte glycoprotein (MOG) that overlaps with the MBP peptide in 7 amino acids, matching only 4 amino acids in the sequence (Fig. 3). The peptide was recognized by the TCC although its potency was lower.
Short peptides activate CD4⁺ T cell clones

Fig. 4. Proliferative response of a DR2b-restricted, B. burgdorferi-specific CD4⁺ TCC, to a potent synthetic 10 amino acid long agonist and three overlapping hexapeptides spanning its sequence. The three panels represent proliferation in response to the indicated concentrations of the peptides as stimulation indices (mean c.p.m. in the presence of peptide/mean c.p.m. in the absence of peptide). Background proliferation was 10⁷ ± 25 c.p.m.

However, the result demonstrates that even truncated peptides matching only a few amino acids of the optimal TCR motif can be recognized. Furthermore, we extended our observation to a DR2b-restricted CD4⁺ TCC that recognizes several peptides derived from B. burgdorferi (26). A potent decapeptide agonist was synthesized based on the response to PS-SCL (data not shown) and tested for proliferation together with truncated hexamer peptides spanning its sequence (Fig. 4). High stimulation indices were observed at concentrations of the short peptides >1 µg/ml (Fig. 4), confirming that CD4⁺ TCC can be activated by short antigenic peptides.

Short antigenic peptides activate early TCR signaling events

Recently, correlations between the stimulatory capacity of a ligand and specific patterns of phosphorylation of components of the TCR signaling apparatus as seen by Western blot have been described. We investigated the capacity of short peptides to induce modifications of the pattern of phosphorylation of the TCR ζ chain. In order to guarantee that only peptides able to bind and remain bound to MHC would present to the TCC, the APC to be used were pulsed with MBP peptide (83–99), or short peptides FFK, VVI or NVVI and subsequently washed before incubation with the T cells. While T cells which received no stimulation showed no signal, incubation with unpulsed B cells showed an increase in p32. Stimulation with MBP (83–99) induced a full-agonist pattern, with p38 > p32. Stimulation with short peptides (FFK, VVI and NVVI) induced an increase in p32 and appearance of a p38 band (Fig. 5), compatible with a partial agonist signaling pattern.

Tetrapeptides support T cell survival

The above data document that short peptide sequences are stimulatory for CD4⁺ MHC class II-restricted TCC. To address whether they can also support the survival of autoreactive TCC as reported previously for altered peptide ligands, we employed two different kinds of survival assays, described in detail in Methods. The first assay (31) uses the proliferative capacity of surviving T cells after exposure to substimulatory concentrations of short peptides as a readout for cell number. The proliferative capacity and therefore the total number of surviving cells (Fig. 6A) was greater in cultures which had been treated with short peptides than those exposed to autologous MHC with or without control peptides [LRE and KDS; Fig. 6A; influenza hemagglutinin (306–318); data not shown].

This effect occurred at concentrations that did not result in proliferation of the TCC measured 3–6 days after stimulation (Fig. 6B). Moreover, the short peptides that supported T cell survival did not induce significant secretion of cytokines (IL-4, IFN-γ and IL-2) compared to control cultures without peptides (data not shown).

The flow cytometry-based assay allows calculation of the survival rate as the percentage of T cells with respect to a standard reference (see Methods). T cells cultured for 5 days in the presence of APC and the short peptides VVI and FFK, but not the control peptides LDS and KRE, showed an
Short peptides activate CD4+ T cell clones

Fig. 6. Short peptides derived from optimal ligands support T cell survival. (A) The proliferative response of TCC TL5F6 to MBP (63–99) after prolonged culture with either no peptide or four different short peptides is shown. Short peptides VVI and FFK (10 µM) promoted T cell survival, while control peptides LRE and KDS did not. (B) At 10 µM concentration, none of the four peptides induced proliferation in a standard [3H]thymidine incorporation assay conducted at days 3–6. Averages ± SD of duplicates for each data point are displayed.

Fig. 7. Generation of pentapeptide-specific T cell lines from PBMC. Short-term T cell lines (TCL) specific for peptide KNVVI were generated from PBMC of a normal donor. The TCL were tested 3 times for specificity, and then stained for the expression of CD4 and CD8 markers. Shown are the c.p.m. of triplicates in response to the pentapeptide. The FACS analysis is shown as an inlet in the upper left corner of each graph. Proliferative responses and CD4 expression were confirmed twice.

Table 2. Increased survival of TL5F6 T cells in the presence of the short peptides VVI and FFK

<table>
<thead>
<tr>
<th>Peptides</th>
<th>No peptide</th>
<th>VVI</th>
<th>FFK</th>
<th>LDS</th>
<th>KRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells9</td>
<td>12.2</td>
<td>18.8</td>
<td>16.4</td>
<td>12.8</td>
<td>11.5</td>
</tr>
<tr>
<td>Peripheral blood lymphocytes</td>
<td>78.3</td>
<td>70.2</td>
<td>72.1</td>
<td>79.4</td>
<td>74.2</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>16</td>
<td>27</td>
<td>23</td>
<td>16</td>
<td>15</td>
</tr>
</tbody>
</table>

*per cent of total cells

increased survival rate (Table 2). These data demonstrate that tripeptides and tetrapeptides can support the survival of CD4+ class II-restricted TCC.

Generation of CD4+ MHC class II-restricted TCC with pentapeptides from peripheral blood lymphocytes

Following the observation that a CD4+ MHC class II-restricted TCC can be stimulated by short peptide fragments derived from an optimal ligand, we reasoned that one should also be able to select for TCC with receptors that can be activated by short peptides from the peripheral blood. We therefore cultured PBMC in the presence of either peptide KNVVI (derived from the TCR motif of TCC TL5F6) or KYVKQ (derived from influenza hemagglutinin (306–318); data not shown for the latter). Positive cultures, established by split-well cloning, were stained for surface marker expression. CD4 expression of two clones specific for KNVVI is shown in Fig. 7. Furthermore, these T cell cultures specifically proliferated upon stimulation with pentapeptides underscoring that CD4+ MHC class II-restricted T cell cultures can be generated with pentapeptides from bulk PBMC, although their long-term propagation is difficult due to a lower stimulatory capacity than longer peptides.

Determination of the frequency of peptide mimics

To determine the importance of the finding that 3–4 amino acid-long peptides are recognized by TCC TL5F6, we wanted to know how frequently short peptide mimics can be found among all naturally occurring proteins reported so far. A SWISS-PROT database sequence search was performed using the search engine ScanProsite with a set of truncated...
peptide ligands derived from an optimal ligand. In addition, we searched the SWISS-PROT database with truncations of the degenerate TCR recognition motif of TCC TL5F6 as defined by the PS-SCL approach. The results demonstrate that both peptide length and flexibility of the peptide sequence strongly influence the number of matches. Increasing numbers of sequence matches were found both with decreasing length of the peptide and increasing number of tolerated amino acids in each position (Table 3). The search demonstrates the high frequency of cross-reactive ligands when a CD4+ TCC can recognize tripeptides or tetrapeptides.

**Discussion**

In recent years, the understanding of how T cells recognize peptide antigens has advanced significantly. It is now widely accepted that in some T cells recognition is highly flexible in that many peptide ligands may lead to productive TCR engagement and functionally elicit a graded response ranging from full agonism to partial/weak agonism to TCR antagonism (1). Although extensive studies have been performed with individual substitution analogues, the question of length requirements for peptide recognition has received little attention (11,19,34,35). This is not surprising, since studies involving elution and sequencing of peptides from MHC molecules (5,15,16), peptide binding measurements (33), T cell cloning experiments and, in particular, the resolution of structures of the MHC–peptide complexes (17,18,36,37) have all indicated that 8–9 amino acids are necessary for MHC class I recognition and 12–16 amino acids for class II recognition. Following studies on the degeneracy of TCR recognition of antigen (24,25), here we investigated whether length requirements for CD4+ T cell activation may be less strict than previously believed, thereby representing a possible mechanism by which this surprising flexibility is achieved. This appeared reasonable since short peptide fragments are likely to be widely available throughout the body, especially in inflammatory sites where proteases and peptidases are activated in local environments (38). In central nervous system inflammation, extracellular degradation of MBP by matrix metalloproteases may promote the generation and presentation of short antigenic peptides (39). In the current study, we demonstrate that truncation variants down to 3 amino acids can induce proliferation of a CD4+ class II-restricted TCC. The variants were generated from optimal peptides, as determined for a particular TCR by the PS-SCL approach. These optimal sequences did not precisely match the peptide used to select the TCC in vitro, but were several orders of magnitude more potent than the autoantigen (24). Different truncated, but non-overlapping segments of the peptide stimulated the TCC. These short peptides also promoted T cell survival in vitro at concentrations even lower than those required for proliferation. To confirm that recognition of short peptides is not a unique feature of TCC TL5F6 examined in this report, we extended the observation to another CD4+ TCC for which truncated peptides defined by the PS-SCL approach maintained agonist properties (Fig. 4) (26). We also established TCC with slightly longer peptides (5 amino acids) derived from two different sources (MBP and influenza hemagglutinin) from the peripheral blood. These results confirm that T cell recognition at least in some T cells is extremely flexible not only in terms of MHC–peptide structure but also with respect to peptide length.

These data raise questions about which mechanisms underlie the interaction between the TCR and its MHC–peptide ligand that allow recognition of tri- and tetrapeptides. A possible answer comes from recently resolved crystal structures of TCR–MHC–peptide complexes (3,4,40), showing that a large part of the flat contact surface between TCR and MHC–peptide complex is formed by direct TCR–MHC rather than TCR–peptide interactions. This suggests that the contribution of the peptide to overall affinity in productive TCR engagement by peptide–MHC complexes may vary considerably (4). In certain instances of allore cognition, no peptide contribution may be required (41). More commonly, antigenic peptides may lead to productive TCR engagement by modulating the pre-existing affinity between TCR and MHC. In this case, the presence of short peptides is necessary and sufficient to overcome the threshold of TCR activation leading to effective signaling as evident from the appearance of the
high molecular weight TCR ζ chain phosphoisoform (p38; Fig. 5). We propose that positively selected TCRs with affinity for self-MHC that is relatively high [although not enough for negative selection (42,43)] may require little additional peptide contribution for productive engagement. Such TCR may be more degenerate with respect to both number and length of peptides they recognize (25,27).

Since the number of potential mimics which are found in proteins of various sources dramatically increases with decreasing peptide length (Table 2), such cross-stimulatory peptide sequences could potentially drive autoreactive TCC and prolong their local survival in the target tissue. The observation that short peptides can induce experimental allergic encephalomyelitis further support their potential role in autoimmune diseases (35).

While many of the possible roles for short peptides are speculative at present, the observation that very short sequences can stimulate CD4+ HLA class II-restricted TCC at levels comparable to their nominal antigen may have implications for future therapies aiming at modifying cellular immune responses. Short peptides can be administered at high molar concentration, they may be able to cross biological barriers such as the gut epithelium or blood–brain barrier due to their low mol. wt and are more resistant to proteolysis than longer peptides. This may allow the use of such short peptides as therapeutics either to modify or to enhance immune responses in autoimmune disorders, inflammatory diseases and cancer.

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Short peptides activate CD4⁺ T cell clones