Th1 but not Th0 cell help is efficient to induce cytotoxic T lymphocytes by immunization with short synthetic peptides

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Abstract

Immunization of BALB/c mice with peptide HVSGHRMAWDMMMNWA, encompassing residues 121–135 from hepatitis C virus E1 protein, induced CD4⁺ Th1 cells as well as a long-lasting CD8⁺ cytotoxic T lymphocyte (CTL) response in vivo when the peptide was administered s.c. with or without incomplete Freund’s adjuvant. Using truncated peptides from this sequence it was shown that the determinant recognized by cytotoxic T cells was encompassed by residues SGHRMAWD. Deletion of residues from the N-terminus or the C-terminus of the wild-type peptide abrogated its helper character. When Val122 of the wild peptide was replaced by Ala, the ability to induce a cytotoxic response was lost concomitantly with the loss of the Th1 pattern of cytokine production. Interestingly, the Ala-modified peptide, when co-immunized with a peptide encompassing residues 323–329 from ovalbumin (OVA), which is able to induce a Th1 response in BALB/c mice, restored the capacity of the modified peptide to induce CTL. However, co-immunization of the Ala-modified peptide with a peptide encompassing residues 106–118 from sperm whale myoglobin, which induces a Th0 cytokine profile in BALB/c mice, was much less efficient than the OVA peptide to restore CTL induction. These results demonstrate that CTL induction with a short synthetic peptide requires that this peptide contains domains recognized by Th cells as well as by Th1 cells. For those peptides that do not contain this type of Th domain, competent T cell help can be provided by co-immunization with a distinct peptide that is able to stimulate a Th1 response.

Introduction

Induction of cytotoxic T lymphocytes (CTL) is an important defensive mechanism of the immune system in response to infection with a virus or other pathogens (1–6). This induction plays a key role in the elimination of infected cells. Similarly, it is also known that tumor growth can be controlled by CTL (7–9). Because of the great potential of CTL in cancer therapy and in the treatment of viral infections, a number of groups have attempted to develop immunization strategies to induce CTL. Thus, the successful use of recombinant viruses (10–12), dendritic cells (DC) pulsed with antigens (13,14), particulate antigens (15,16), lipopeptides (17) and synthetic peptides has been reported. In this last case, several alternative approaches have been tested: short synthetic peptides (18–22), peptide constructs containing a determinant recognized by Th cells (TDh) and a determinant recognized by CTL (TDc) (23–26) or mixtures of TDh and TDc peptides not covalently linked (27–29).

Because synthetic peptides do not have any of the potential dangers associated with the induction of an infection, due to recombinant viruses, the use of peptides might be more acceptable for human therapy. Thus, understanding the molecular mechanisms of how synthetic peptides induce CTL is of paramount importance. Experiments carried out by others as well as by our group (19,23–25) demonstrated that CTL induction with synthetic peptides requires T cell help. Also, it has been reported that during infection a Th1 cytokine profile is required to induce CTL (30,31). All these considerations suggest that short synthetic peptides, that are able to induce a CTL response in vivo, should encompass a TDh (probably of the Th1 subtype) and a TDc in their sequence.
Peptides were synthesized by the solid-phase method of Merrifield (33) using the F-moc alternative (34) and a manual multiple solid-phase peptide synthesizer (35). The ninhydrin test of Kaiser was used to monitor every step (36). At the end of the synthesis, peptides were cleaved and deprotected, and washed 6 times with diethyl ether. They were lyophilized and analyzed by HPLC. The purity of peptides was >80% as judged by HPLC.

Methods

Peptide synthesis

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Mice

Six-week-old female BALB/c mice were purchased from IFFA Credo (Barcelona, Spain). They were housed in appropriate animal care facilities and handled following international guidelines required for experimentation with animals.

Immunization and measurement of CTL response

Groups of three mice were immunized by s.c. injection at the base of the tail and footpads with 60 μg of peptide emulsified in IFA at days 0 and 15. For immunizations using a TD₈ and a TD₆ peptide mixture, 60 μg of each peptide was injected. In some experiments, immunizations were carried out in the absence of adjuvant. This was done by i.p. injection of 60 μg of peptide in 0.5 ml of saline at days 0, 7, and 14. In both protocols the animals were killed 10 days after the last immunization and their lymph nodes (LN) and/or spleen removed. Cells (8×10⁵/well) were re-stimulated in vitro in 24-well plates in 2 ml of culture medium (RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, penicillin 100 U/ml, streptomycin 100 μg/ml and 5×10⁻⁵ M 2-mercaptopetanol) with 5 μg/ml of peptide. Cytotoxic activity was measured 5 days after initial culture using the ⁵¹Cr-release assay (37). P815 target cells (10⁶ cells) were radiolabeled with 50 μCi of Na₂⁵¹CrO⁴ at 37°C for 1 h, washed 3 times, and incubated with or without 5 μg/ml of peptide and different numbers of effector cells in triplicate. Spontaneous release was measured from wells without effector cells and maximum release from target cells incubated with 5% Triton X-100. The spontaneous release was in all cases <25% of total release. In selected experiments, 0.1 mg/ml of different antibodies was added during the incubation period. After 4 h of incubation, supernatants were harvested and radioactivity measured in a scintillation counter (Topcount; Packard, Meridian, CT).

In CTL precursor frequency measurement experiments, four different concentrations of LN cells (8×10⁵, 4×10⁵, 2×10⁵ and 1×10⁵) were placed in 24 replica cultures (for each dilution) in culture medium in the presence of 10 μg/ml of peptide E1(121–135), complemented with graded numbers of irradiated syngeneic spleen cells (3000 rad) to give a total number of 5×10⁵ cells/well (96-wells U-bottomed plates) in a final volume of 0.25 ml of medium. After 6 days of culture at 37°C and 5% CO₂, CTL activity of each individual well was measured using the ⁵¹Cr-release assay by transferring 100 μl of each well to a plate containing radiolabeled P815 cells and peptide E1(121–135), and another 100 μl to a plate with P815 radiolabeled cells without peptide. After 5 h of culture, 50 μl of supernatant were removed from each well and the percentage of lysis estimated in a scintillation counter (Topcount). Responses for the individual wells were considered positive when CTL activity in the presence of peptide was 15% higher than in the absence of peptide. Frequencies were calculated using χ² analysis according to the method of Taswell (38) on a computer program (kindly provided to us by Dr L. Selin, University of Massachusetts Medical Center, Worcester, MA). CTL precursor frequencies were calculated on the regression curve by interpolating the number of responder cells required to give 37% negative cultures. Only those experiments where data fitted in the single-hit model were considered.

Immunization for cytokine measurement

Groups of three mice were immunized by s.c. injection at the base of the tail and footpads with 60 μg of peptide emulsified in IFA. Ten days after the primary injection, animals were killed and LN cells removed. The lymphocytes were then plated on 96-well plates at 8×10⁵ cells/well with culture medium alone or with serial dilutions of peptide in the same medium to a final volume of 0.25 ml. In selected experiments, 0.1 mg/ml of different antibodies was added during the incubation period. Supernatants (0.5 μl) were removed 24 h later to measure IL-2 content, and 48 h later for IFN-γ and IL-4.

Measurement of cytokine production

IL-2 content in culture supernatants was measured by using a CTLL bioassay as described (39). IFN-γ production was measured by ELISA (Genzyme, Cambridge, MA) according to manufacturer’s instructions. IL-4 content was measured by CT4S bioassay (kindly provided by Drs W. E. Paul and G. J. Watson, National Institutes of Health, Bethesda, MD). Briefly, CT4S cells were re-suspended in culture medium and were plated (5×10³ cells/well) in a 96-well flat-bottomed plate with the supernatant to be assayed (50% v/v). After 48 h of culture, cells were pulsed with 0.5 μCi/well of [³H]thymidine for 18 h, harvested and thymidine incorporation was determined in a
scintillation counter (Topcount). Standard curves using known amounts of rIL-4 were also included to determine the amount of IL-4.

Depletion of CD4⁺ and CD8⁺ cells in vivo
Mice were depleted of CD4⁺ or CD8⁺ cells by i.p. injection of 0.3 mg of anti-CD4 (obtained from rat anti-mouse hybridoma GK1-5) or anti-CD8 (obtained from rat anti-mouse hybridoma H35.17.2) antibodies respectively on days −1, 0, 1, 6, 7, 8, 13, 14 and 15 as previously described (19,40); day 0 being the day of immunization. The efficiency of depletions was assessed by flow cytometry on day 21.

Results

Immunization with peptide E1(121–135) induced a CD8⁺ CTL response
In a first experiment, BALB/c mice were immunized with peptide E1(121–135) from HCV E1 protein in IFA. As shown in Fig. 1, both LN cells (Fig. 1A) and spleen cells (Fig. 1B) from these animals were able to lyse P815 target cells pulsed with peptide E1(121–135). Thus, peptide E1(121–135) can induce CTL in vivo without external help from a TD peptide. Addition of anti-CD8 antibodies plus complement during the ⁵¹Cr-release assay, but not of anti-CD4 antibodies plus complement, was able to abrogate the lysis of P815 target cells pulsed with E1(121–135), showing that the phenotype of the induced CTL was CD8⁺ (Fig. 1C).

In order to identify the region of E1(121–135) recognized by CD8⁺ cells, LN cells from BALB/c mice immunized with E1(121–135) were stimulated in vitro with E1(121–135) for 5 days and their CTL activity measured against P815 cells pulsed with a number of truncated peptides. Since truncated peptides SGHRMAWDMMMNWA and HVSGHRMAWDM were equally active, and share the region SGHRMAWDM, it was concluded that this region is the one recognized by induced CTL. This was confirmed when using nonapeptide SGHRMAWDM in the CTL assay (Fig. 2).

Immunization with peptide E1(121–135) stimulated a CD4⁺ T_{h}1 cytokine profile and concomitantly CTL induction
To characterize the cytokine profile induced after immunization with E1(121–135), LN cells from BALB/c mice immunized with this peptide were stimulated in vitro with 40 µg/ml of peptide. IL-2, IL-4 and IFN-γ were quantified from culture supernatants after 24, 48 and 48 h respectively. As shown in Table 1, the pattern of cytokine production was of the T_{h}1 subtype, i.e. high IL-2 and IFN-γ production, and no detectable IL-4. Experiments carried out in the presence or absence of anti-CD4 or anti-CD8 antibodies showed that only CD4⁺ cells were responsible for cytokine production. To characterize the region of E1(121–135) responsible for T_{h}1 cell activity, LN cells from BALB/c mice immunized with E1(121–135) were stimulated in vitro with 40 µg/ml of wild-type peptide or with a number of truncated peptides (Table 2). Results show that deletion of residues from either the N-terminus or the C-terminus substantially decreases IL-2 production, showing that the entire sequence is required to provide T cell help.

In another group of experiments, we studied the induction of CTL by immunization with peptide E1(121–135) in the absence of adjuvant. Mice were immunized by s.c. injection with 60 µg of peptide emulsified in IFA at days 0 and 15. Ten days after the last immunization LN (A) and spleen cells (B) were stimulated in vitro for 5 days in the presence of 5 µg/ml of peptide. CTL activity was measured using P815 target cells previously incubated with 5 µg/ml of E1(121–135) or with medium alone. The phenotype of the induced CTL was characterized using P815 target cells incubated with E1(121–135) in the presence or in the absence of 0.1 µg/ml of anti-CD4 or anti-CD8 antibodies plus complement (C).
Thi determinants for CTL induction

Fig. 2. Region of E1(121–135) recognized by CTL. LN cells from BALB/c mice immunized with peptide E1(121–135) were stimulated in vitro for 5 days. CTL activity was measured against P815 target cells incubated with 5 µg/ml of E1(121–135) truncated peptides shown. An E:T ratio of 25:1 was used in all experiments.

Table 1. Cytokine production after in vitro stimulation with peptide E1(121–135)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-2 (mU/ml)</th>
<th>IFN-γ (pg/ml)</th>
<th>IL-4 (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>94.1</td>
<td>333.1</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Anti-CD4</td>
<td>2.01</td>
<td>&lt;38</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Anti-CD8</td>
<td>88.0</td>
<td>257.4</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

LN cells from BALB/c mice immunized with E1(121–135) were stimulated with 40 µg/ml of peptide. Supernatants were harvested 24 h later and production of IL-2 was determined using the CTLL and CT4S bioassay, respectively, and IFN-γ content was determined by ELISA.

Fig. 3. Effect of in vivo depletion of CD4⁺ or CD8⁺ cells on CTL induction. Mice were immunized by i.p. injection with 60 µg of E1(121–135) in 0.5 ml of saline at days 0, 7 and 14. They were depleted of CD4⁺ or CD8⁺ cells in vivo or left untreated before and during immunization. Animals were killed 10 days after the last immunization and CTL activity was measured after culturing spleen cells for 5 days in the presence of peptide E1(121–135). CTL activity was measured against P815 target cells pulsed with or without peptide. Results are expressed as the difference between percentage of lysis against peptide pulsed and unpulsed target cells. Specific lysis against unpulsed P815 target cells was always <10%.

Table 2. Characterization of the region within E1(121–135) responsible for Th cell activity

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IL-2 (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVSGHRMAWDMMNWNA</td>
<td>14.9</td>
</tr>
<tr>
<td>VSGHRMAWDMMNWNA</td>
<td>2.0</td>
</tr>
<tr>
<td>SCRMAWDMMNWNA</td>
<td>3.2</td>
</tr>
<tr>
<td>GHRMAWDMMNWNA</td>
<td>1.9</td>
</tr>
<tr>
<td>HRMAWDMMNWNA</td>
<td>1.6</td>
</tr>
<tr>
<td>RMAMDMMNWNA</td>
<td>1.8</td>
</tr>
<tr>
<td>MMAWDMMNWNA</td>
<td>1.2</td>
</tr>
<tr>
<td>AMDMMNWNA</td>
<td>1.7</td>
</tr>
<tr>
<td>HVSGHRMAWDMMN</td>
<td>3.2</td>
</tr>
<tr>
<td>HVSGHRMAWD</td>
<td>1.6</td>
</tr>
<tr>
<td>HVSGHRMAWM</td>
<td>2.4</td>
</tr>
<tr>
<td>HVSGHRMAWD</td>
<td>2.2</td>
</tr>
<tr>
<td>HVSGHRMAW</td>
<td>1.3</td>
</tr>
</tbody>
</table>

LN cells from BALB/c mice immunized with E1(121–135) were completely abrogated IL-2 production and IFN-γ production stimulated with 40 µg/ml of E1(121–135) truncated peptides. Culture supernatants were harvested 24 h later and production of IL-2 was determined using a CTLL bioassay. IL-2 production is expressed as stimulation index (SI) (ratio between the c.p.m. of CTLL in the presence of peptide and in the absence of peptide).

Table 3. Peptide E1(121–135) induced a long-lasting response

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IL-2 (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVSGHRMAWDMMNWNA</td>
<td>14.9</td>
</tr>
<tr>
<td>VSGHRMAWDMMNWNA</td>
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<td>GHRMAWDMMNWNA</td>
<td>1.9</td>
</tr>
<tr>
<td>HRMAWDMMNWNA</td>
<td>1.6</td>
</tr>
<tr>
<td>RMAMDMMNWNA</td>
<td>1.8</td>
</tr>
<tr>
<td>MMAWDMMNWNA</td>
<td>1.2</td>
</tr>
<tr>
<td>AMDMMNWNA</td>
<td>1.7</td>
</tr>
<tr>
<td>HVSGHRMAWDMMN</td>
<td>3.2</td>
</tr>
<tr>
<td>HVSGHRMAWD</td>
<td>1.6</td>
</tr>
<tr>
<td>HVSGHRMAWM</td>
<td>2.4</td>
</tr>
<tr>
<td>HVSGHRMAWD</td>
<td>2.2</td>
</tr>
<tr>
<td>HVSGHRMAW</td>
<td>1.3</td>
</tr>
</tbody>
</table>

LN cells from BALB/c mice immunized with E1(121–135) were stimulated with 40 µg/ml of E1(121–135) truncated peptides. Culture supernatants were harvested 24 h later and production of IL-2 was determined using a CTLL bioassay. IL-2 production is expressed as stimulation index (SI) (ratio between the c.p.m. of CTLL in the presence of peptide and in the absence of peptide).

Replacement of Val122 by Ala abrogated CD4⁺ and concomitantly CD8⁺ responses: restoration of CTL induction by co-immunization with an external Th1 peptide

Since CD4⁺ Th cells were necessary to induce a CTL response with E1(121–135), we decided to modify this peptide outside the CD8 epitope with the aim of abrogating the CD4⁺ response. Thus, we replaced Val122 by Ala and studied the effect of this replacement on the CD4⁺ and CD8⁺ responses. Replacement of Val122 in peptide E1(121–135) by Ala completely abrogated IL-2 production and IFN-γ production (Table 3). Moreover, no CTL were induced after immunization with E1(121–135, Ala122) (Fig. 5). As expected, because position 122 is outside the CTL epitope, replacement of Val122 by Ala did not change the capacity of the peptide to be
Table 3. Patterns of cytokine production after immunization with different peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IL-2 (mU/ml)</th>
<th>IFN-γ (µg/ml)</th>
<th>IL-4 (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1(121–135)</td>
<td>25.4</td>
<td>499</td>
<td>&lt;25</td>
</tr>
<tr>
<td>E1(121–135, Ala122)</td>
<td>&lt;0.5</td>
<td>&lt;38</td>
<td>&lt;25</td>
</tr>
<tr>
<td>OVA(323–339)</td>
<td>33.5</td>
<td>504</td>
<td>&lt;25</td>
</tr>
<tr>
<td>FIS³</td>
<td>50.2</td>
<td>886</td>
<td>318</td>
</tr>
</tbody>
</table>

LN cells from BALB/c mice immunized with different peptides were stimulated with 40 µg/ml of peptide. Supernatants were harvested after 24, 48 and 48 h to quantify IL-2, IL-4 and IFN-γ respectively. IL-2 and IL-4 content was determined using the CTLL and CT4S bioassay respectively, and IFN-γ content was determined by ELISA.³Peptide FIS corresponds to amino acid sequence 106–118 from sperm whale myoglobin.

Fig. 4. Duration of the CTL response induced after immunization with peptide E1(121–135). Mice were immunized at days 0 and 15 with peptide E1(121–135) emulsified in IFA. They were sacrificed at days 30, 60 or 90 after the first immunization. Spleen cells were stimulated in vitro for 5 days and CTL activity measured against P815 target cells pulsed with or without E1(121–135). E:T ratio was 50:1 in all cases.

Fig. 5. Effect of internal and external T cell help in CTL induction. Mice were immunized with peptide E1(121–135), with E1(121–135, 122Ala), with E1(121–135, 122Ala) in conjunction with the Th1 peptide OVA(323–339) or with E1(121–135, 122Ala) in conjunction with the Th0 peptide FIS emulsified in IFA. LN cells were stimulated in vitro for 5 days in the presence of the corresponding CTL peptide used for immunization and CTL activity measured. In all cases an E:T ratio of 50 was used. Results shown are representative of three different experiments.

Fig. 6. Effect of single amino acid changes inside and outside the CTL epitope on CTL recognition. Mice were immunized by s.c. injection with 60 µg of peptide emulsified in IFA at days 0 and 15. Ten days after the last immunization LN cells were stimulated in vitro for 5 days in the presence of 5 µg/ml of peptide E1(121–135). CTL activity was measured using P815 target cells previously incubated with 5 µg/ml of E1(121–135), E1(121–135, 122Ala), E1(121–135, 129Ala) or with medium alone. An E:T ratio of 50 was used.

recognized by CTL induced with E1(121–135). However, when Trp129 (which is part of the CTL epitope) was replaced by Ala, the peptide was not recognized (Fig. 6).

We then carried out experiments to restore the CTL activity of this peptide by providing external T cell help and characterize the type of T cell help required. As shown in Table 3, immunization with Tn peptides OVA(323–339) (encompassing residues 323–339 from ovalbumin) and FIS (encompassing residues 106–118 from sperm whale myoglobin) elicited Tn1 and Tn0 cytokine profiles respectively. Thus, we decided to use these peptides to provide external T cell help to E1(121–135, Ala122). Four groups of mice were immunized with single peptides or peptide mixtures emulsified in IFA: (i) E1(121–135), (ii) E1(121–135, Ala122), (iii) E1(121–135, Ala122) in conjunction with OVA(323–339) and (iv) E1(121–135, Ala122) in conjunction with FIS. LN cells were stimulated in vitro for 5 days with either E1(121–135) (group (i) or E1(121–135, Ala122) (groups (ii), (iii) and (iv)) and CTL activity measured against P815 cells pulsed with the same peptide used in vitro stimulation. As shown in Fig. 5, immunization with peptide E1(121–135, Ala122) by itself is unable to induce CTL in vivo. However, co-immunization of OVA(323–339) and E1(121–135, Ala122) was able to induce a CTL activity of the same level obtained when immunizing with the unmodified E1(121–135). By contrast, co-immunization of FIS and E1(121–135, Ala122) was unable to restore CTL activity to the level attained with E1(121–135). Results from Fig. 5 are representative of experiments carried out 3 times. In order to get a further insight on the magnitude of the CTL responses induced after these immunizations, the T cell precursor frequency was measured by limiting dilution assay. As shown in Table 4, the T cell precursor frequency is in good agreement with the percentage of specific lysis induced (Fig. 5). Thus, co-immunization of E1(121–135, Ala122) with OVA(323–339) affords a frequency of CTL similar to the one attained with E1(121–135), whereas co-immunization with FIS affords intermediate CTL precursor levels.

**Discussion**

It has been reported that CTL induction can be achieved after immunization with short synthetic peptides (18–20,22,41).
or immunogenic constructs containing combinations of CD4 and CD8 epitopes (23–26). In most of these cases, it was reported that a TDh should be present in the peptide. Also, as known from in vivo models, when a pathogen enters the body, CTL induction is helped by activation of CD4+ cells of the Th1 subtype. Thus, we hypothesized that short synthetic peptides that are able to induce a CTL response should contain two types of determinants: those recognized by CD4+ Th1 cells and those recognized by CD8+ cells. To address this hypothesis we selected peptide E1(121–135) from hepatitis C virus E1 protein which we had found was able to induce a CTL response in BALB/c mice. Characterization of the cytokine profile induced by this peptide showed a Th1 cytokine profile mediated by CD4+ cells. Also, depletion of CD4+ or CD8+ cells in mice before immunization with peptide E1(121–135) completely abrogated the ability of this peptide to induce CTL in vivo, demonstrating that both types of cells are required for CTL induction. Moreover, using truncated peptides from E1(121–135) we showed that the region recognized by CTL is encompassed by amino acids 123–131 and that the whole peptide E1(121–135) was necessary for providing efficient T cell help for CTL induction.

To study the role of the TDh encompassed by E1(121–135) we replaced Val122 by Ala to give peptide E1(121–135, Ala122). This was done because Val122 might be an anchor residue for binding to MHC class II molecules, as described for other peptides having TDh character (42). Replacement of Val122 completely abrogated IL-2 and IFN-γ production (Table 3) as well as CTL induction, showing that Val122 is essential for providing efficient T cell help. Since only the region encompassed by amino acids 123–131 is the one recognized by CTL, replacement of Val122 did not alter the CD8 epitope in peptide E1(121–135, Ala122). Thus, changes in CTL induction can only be attributed to abrogation of the CD4+ Th1 response. Moreover, target cells pulsed with peptide E1(121–135, Ala122) were efficiently recognized by E1(121–135)-specific CTL, showing that the modified peptide had a competent CD8 epitope, and retained the ability to be processed and presented by antigen-presenting cell class I molecules (Fig. 6). Since E1(121–135, Ala122) was not immunogenic per se but contained a TDh, it opened the possibility of studying the effect of an external TDh peptide to induce CTL in vivo, in a similar manner to the study carried out by Shirai et al. (43) where they tested the use of intrinsic and extrinsic helper epitopes for CTL induction. However, these authors did not study the cytokine profiles induced by the helper peptides. In our work we wished to study the type of cytokines required for efficient CTL induction. Thus, we selected two TDh peptides, OVA(323–339) and FIS, that are able to induce a Th1 and a Th0 cytokine profile respectively.

As shown in Fig. 5 and Table 4, the external T cell help provided by the Th1 peptide was able to efficiently restore CTL induction, whereas the use of Th0 peptide under our experimental conditions restored CTL activity only partially. This result indicates that Th peptides that are able to induce Th1 cytokine profiles are more efficient (if not essential) to induce CTL in vivo than peptides that elicit Th0 profiles. In vitro studies with CD4+ and CD8+ subpopulations stimulated with synthetic peptides have demonstrated the importance of different cytokines in CTL induction. Thus, IL-2 and IFN-γ are important in early stages of induction, whereas IL-4 is utilized in later stages of CTL maturation (44). It has been reported that IL-12 secretion (one of the most powerful Th1 skewing cytokines) by DC is dependent upon interaction with CD4+ Th1 cells, whereas CD4+ Th1 cells inhibit IL-12 secretion (45). Thus ‘good’ levels of IL-2 and IFN-γ and low production of IL-4 might be important for CTL induction. However, as we and others have recently demonstrated (46–48), very high levels of IL-12 and IFN-γ may have a suppressive effect on the induction of Th1 and CTL responses. Thus, ‘adequate’ levels of these cytokines are essential for an efficient CTL induction.

Table 4. CTL precursor frequencies induced after immunization of mice with different peptides or peptide mixtures

<table>
<thead>
<tr>
<th>Immunized with</th>
<th>CTL precursors</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1(121–135)</td>
<td>1/356,419</td>
<td>1/282,846–1/481,724</td>
</tr>
<tr>
<td>E1(121–135,122A)</td>
<td>1/2,459,329</td>
<td>1/1,536,882–1/6,151,504</td>
</tr>
<tr>
<td>E1(121–135, 122A) + OVA(323–339)</td>
<td>1/326,984</td>
<td>1/254,428–1/457,447</td>
</tr>
<tr>
<td>E1(121–135, 122A) + FIS</td>
<td>1/1,097,642</td>
<td>1/760,032–1/1,974,902</td>
</tr>
</tbody>
</table>

Mice were immunized with peptide E1(121–135), with E1(121–135, 122Ala), with E1(121–135, 122Ala) in conjunction with the Th1 peptide OVA(323–339) or with E1(121–135, 122Ala) in conjunction with the Th0 peptide FIS emulsified in IFA. LN cells were stimulated in vitro in the presence of E1(121–135) or E1(121–135, 122Ala) peptides and the CTL precursor frequency measured after 5 days as described in Methods.
vaccination against tumors as well as viruses. External help from a CD4+ T cell peptide in peptide mixtures has been a very important tool for the characterization of CTL epitopes and their properties (53–56). However, since induction of CTL responses with constructs containing covalently linked epitopes seems to be stronger than the one obtained when using peptide mixtures (24,25,57), these constructs would be more convenient for vaccination as well as for treatment of chronic infections or tumors. Another strategy to induce CTL is the use of naked DNA (58,59). Thus, we carried out preliminary experiments using gene gun-based DNA immunization with a plasmid expressing E1(121–135). Among three immunizations with the gene gun, CTL were induced with an efficiency similar to the one attained after immunization with peptide E1(121–135) in IFA (data not shown). We are planning to explore this alternative in more depth in future experiments.

Because of the diversity of MHC class I and class II molecules in humans, peptides used in vaccination or treatment of chronic infections should contain several TDh and TDc determinants, to attempt to cover the widest possible proportion of class I and class II restrictions. This implies that the joint effect of several TDh peptides on the cytokine profile should be studied in great detail, because some TDh peptides with Th1 character for a given MHC restriction might have Th0 or Th2 character in other restrictions. If this was the case, some combinations of TDh peptides might be deleterious for efficient CTL induction. Research is being carried out to address these questions.

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Abbreviations

- CTL: cytotoxic T lymphocytes
- DC: dendritic cells
- HCV: hepatitis C virus
- IFA: incomplete Freund’s adjuvant
- LN: lymph node
- OVA: ovalbumin
- TDh: T cell determinant recognized by CTL
- TDc: T cell determinant recognized by Th lymphocytes

References


