Protection against Lymphocytic Choriomeningitis Virus Infection Induced by a Reduced Peptide Bond Analogue of the H-2Db-restricted CD8+ T Cell Epitope GP33*

(Received for publication, November 12, 1998, and in revised form, December 18, 1998)

Christine Stemmer‡, Anne Quesnel§, Armelle Prévost-Blondel, Christine Zimmermann, Sylviane Muller§, Jean-Paul Briand§, and Hanspeter Pircher†

From the Institute for Medical Microbiology and Hygiene, Department of Immunology, University of Freiburg, 79104 Freiburg, Germany and the Institut de Biologie Moléculaire et Cellulaire, UPR 9021 CNRS, 67000 Strasbourg, France

Recent investigations have suggested that pseudopeptides containing modified peptide bonds might advantageously replace natural peptides in therapeutic strategies. We have generated eight reduced peptide bond \(\Psi(CH_2-NH)\) analogues corresponding to the H-2Db-restricted CD8+ T cell epitope (called GP33) of the glycoprotein of the lymphocytic choriomeningitis virus. One of these pseudopeptides, containing a reduced peptide bond between residues 6 and 7 (\(\Psi(6-7)\)), displayed very similar properties of binding to major histocompatibility complex (MHC) and recognition by T cell receptor transgenic T cells specific for GP33 when compared with the parent peptide. We assessed in vitro and in vivo the proteolytic resistance of GP33 and \(\Psi(6-7)\) and analyzed its contribution to the priming properties of these peptides. The \(\Psi(6-7)\) analogue exhibited a dramatically increased proteolytic resistance when compared with GP33, and we show for the first time that MHC-peptide complexes formed in vivo with a pseudopeptide display a sustained half-life compared with the complexes formed with the natural peptide. Furthermore, in contrast to immunizations with GP33, three injections of \(\Psi(6-7)\) in saline induced significant antiviral protection in mice. The enhanced ability of \(\Psi(6-7)\) to induce antiviral protection may result from the higher stability of the analogue and/or of the MHC-analogue complexes.

T cells recognize antigenic peptides in association with MHC1 molecules and play a key role in protection against harmful pathogens and in tumor elimination. Over the past years many attempts have been made to use synthetic peptides as potential vaccines and immunoregulatory agents (1, 2). Recent studies have provided evidence that pseudopeptides, in which one or several of the natural amide bonds (CO–NH) are replaced by CO–NH isosters (3), can have enhanced antigenic and immunogenic properties (4–6). Most interestingly, it has been shown that such peptide analogues can bind to class I and II MHC molecules (7–13), and some of them also induce differential effects on T cell responsiveness (14) similar to those described with altered peptide ligands, which contain single amino acid replacements (15). These several recent studies thus demonstrated the potential interest of pseudopeptides as possible therapeutic strategies in T cell-mediated disorders.

The high susceptibility of synthetic peptides to proteases is considered to be a major drawback for their use as vaccines or immunoregulatory molecules (16). We have previously shown that several pseudopeptides with enhanced antigenic activity are more resistant to proteolytic degradation in vitro (5, 6, 14, 17). However, no information on in vivo stability of such peptide analogues and the possible direct contribution of this resistance in their ability to modulate the immune response is available. Increased biological activity of protease-resistant pseudopeptide analogues has already been widely shown in other fields of medical chemistry (for review, see Refs. 18, 19–22). The retro-inverso analogue of the immunostimulatory molecule tuftsin is an outstanding example of a bioactive tetrapeptide for which biological efficiency in vivo has been remarkably increased by introducing one modified peptide bond (22). To examine the possible influence of proteolytic resistance of MHC class I binding peptides on their biological activity, we designed a series of eight reduced peptide bond pseudopeptides of the immunodominant CD8+ T cell epitope of LCMV in C57BL/6 (B6, H-2b) mice. This CTL epitope (called GP33) is located in residues 33–41 of the LCMV glycoprotein (23). We tested the capacity of these eight analogues, each containing one reduced peptide bond \(\Psi(CH_2-NH)\) at successive positions, to bind to H-2Db MHC molecules. The peptides that bound significantly to H-2Db were further analyzed with respect to their recognition by GP33-specific T cells from a Tg mouse that expresses a H-2Db-restricted T cell receptor specific for this epitope and for their ability to induce antiviral protection.

MATERIALS AND METHODS

Peptides—LCMV glycoprotein 33–41 peptide (GP33, KAVYNFATM) (23) and the control Db-restricted adenovirus peptide 234–243 (E1A, SGPSNTPPEI) (24) were purchased from NeoSystem (Strasbourg, France). To prevent dimer formation, the original cysteine residue present at the anchor position 41 of the GP33 peptide was replaced by a methionine residue. The reduced peptide bond analogues were synthesized, purified, and analyzed as described previously (25).

Protease Resistance Analysis in Vitro and in Vivo—To study the resistance of GP33 and \(\Psi(6-7)\) peptides to proteases in vitro, peptides (625 μg/ml) were incubated at 20 °C in fresh mouse serum diluted two times in PBS, pH 7.4. The reaction was stopped at intervals by adding trifluoroacetic acid (10% of the final volume). The suspension was
Reduced Peptide Bond Analogues of a CTL Epitope

TABLE I

| Name          | Sequence          | Rt<sup>a</sup> | Mass<sup>b</sup> |
|---------------|-------------------|----------------|-----------------|
| GP33          | H-Lys<sup>33</sup>-Ala-Val-Tyr-Asn-Phe-Ala-Thr-Met<sup>41</sup>-OH | 11.05          | 1045.3          |
| (1-2)         | H-Lys<sup>33</sup>-Ψ(CH<sub>2</sub>-NH)-Ala-Val-Tyr-Asn-Phe-Ala-Thr-Met<sup>41</sup>-OH | 11.29          | 1030.7          |
| (2-3)         | H-Lys<sup>33</sup>-Ala-Ψ(CH<sub>2</sub>-NH)-Val-Tyr-Asn-Phe-Ala-Thr-Met<sup>41</sup>-OH | 11.35          | 1030.2          |
| (3-4)         | H-Lys<sup>33</sup>-Ala-Val-Ψ(CH<sub>2</sub>-NH)-Tyr-Asn-Phe-Ala-Thr-Met<sup>41</sup>-OH | 10.59          | 1031.3          |
| (4-5)         | H-Lys<sup>33</sup>-Ala-Val-Tyr-Ψ(CH<sub>2</sub>-NH)-Asn-Phe-Ala-Thr-Met<sup>41</sup>-OH | 10.98          | 1031.5          |
| (5-6)         | H-Lys<sup>33</sup>-Ala-Val-Tyr-Asn-Ψ(CH<sub>2</sub>-NH)-Phe-Ala-Thr-Met<sup>41</sup>-OH | 10.31          | 1029.7          |
| (6-7)         | H-Lys<sup>33</sup>-Ala-Val-Tyr-Asn-Phe-Ψ(CH<sub>2</sub>-NH)-Ala-Thr-Met<sup>41</sup>-OH | 10.56          | 1030.5          |
| (7-8)         | H-Lys<sup>33</sup>-Ala-Val-Tyr-Asn-Phe-Ψ(CH<sub>2</sub>-NH)-Thr-Met<sup>41</sup>-OH | 11.25          | 1029.9          |
| (8-9)         | H-Lys<sup>33</sup>-Ala-Val-Tyr-Asn-Phe-Ψ(CH<sub>2</sub>-NH)-Met<sup>41</sup>-OH | 10.77          | 1030.9          |

<sup>a</sup> Linear gradient of 0.1% trifluoroacetic acid and acetonitrile containing 0.08% trifluoroacetic acid, 5–65% acetonitrile in 30 min.

<sup>b</sup> Determined by matrix-assisted laser desorption and ionization mass spectrometry.

RESULTS

Binding of GP33 Analogues to H-2D<sup>b</sup> MHC Molecules—Eight reduced peptide bond pseudopeptides corresponding to the H-2D<sup>b</sup>-restricted CDS<sup>b</sup> T cell GP33 LCMV epitope were used in this study. These analogues were obtained by replacing one natural peptide bond at a time by a reduced peptide bond Ψ(CH<sub>2</sub>-NH). The amino acid sequence and characteristics of these peptides are shown in Table I. The eight analogues were first tested for their ability to bind to MHC D<sup>b</sup> class I molecules using transporter associated with antigen processing-deficient RNA-S cells. The stabilized D<sup>b</sup>-peptide complexes were quantified by flow cytometry using a D<sup>b</sup>-specific monoclonal antibody. Two of the eight pseudopeptides, namely those containing a reduced peptide bond between residues Asn<sup>5</sup> and Phe<sup>6</sup>, analogue Ψ(5–6), and between Phe<sup>6</sup> and Ala<sup>7</sup>, analogue Ψ(6–7), bound to D<sup>b</sup> molecules as efficiently as the parent GP33 peptide (Fig. 1). The six other analogues were unable to stabilize D<sup>b</sup> molecules at a physiological range of peptide concentration.

The Ψ(6–7) Analogue Is Recognized by T Cells from LCMV TCR<sup>+</sup> Mice as Efficiently as the GP33 Parent Peptide—Proliferation assays with CDS<sup>b</sup> T cells from LCMV TCR<sup>+</sup> mice were used to examine T cell recognition of the two D<sup>b</sup> binding analogues Ψ(5–6) and Ψ(6–7). As shown in Fig. 2A, the Ψ(5–6) analogue did not stimulate LCMV TCR<sup>+</sup> T cells. In contrast, the Ψ(6–7) analogue induced proliferation of Tg T cells as efficiently as the GP33 peptide. Peptide recognition by LCMV TCR<sup>+</sup> effector T cells was further examined in 3<sup>11</sup>Cr release assays using EL-4 target cells loaded with different concentrations of GP33 Ψ(5–6) and Ψ(6–7) peptides and the control D<sup>b</sup>-restricted adenovirus E1A peptide. The Tg effector T cells, generated in vitro in the presence of GP33-loaded APC, lysed target cells presenting GP33 and Ψ(6–7) peptides to a similar extent but failed to recognize target cells loaded with the Ψ(5–6) peptide and control E1A peptide (Fig. 2B). Taken together, these data clearly show that the Ψ(6–7) analogue is recognized as efficiently as the parent GP33 peptide by LCMV TCR<sup>+</sup> T cells. For further analysis, only the Ψ(6–7) analogue was used.

Stability to Proteases of the GP33 Parent and Ψ(6–7) Peptides in Vitro—The proteolytic degradation of the GP33 and Ψ(6–7) peptides by proteases present in mouse serum was examined by HPLC. The half-life of the Ψ(6–7) peptide in serum was superior to 1 h, whereas the parent peptide GP33 was almost completely degraded within a few minutes (Fig. 3A). When the GP33 peptide was analyzed by HPLC shortly after digestion (1 min), two main peaks were observed (Fig. 3B). Mass spectrometry analysis revealed that the complete GP33 peptide of sequence KAVYNFATM was present in peak 1, whereas a peptide fragment corresponding to the 6 N-termin...
different concentrations (10−4 to 10−10 m) to stabilize MHC class I H-2Db molecules present on the surface of RMA-S cells. Stabilization of molecules was determined by flow cytometry using the Dba-specific monoclonal antibody B-22-243.

The (6–7) Analogue Exhibits a Longer Half-life in Vivo—To measure the half-life of the GP33 and (6–7) peptides in vivo, B6 mice were injected intravenously with peptides, and the peptide remaining over the time in the serum of these animals was determined using antigen-induced proliferation of LCMV TCR+ T cells as an experimental readout. Using the mouse sera diluted 1:100 in this very sensitive functional test, the presence of GP33 peptide could not be detected in blood 5 min after peptide injection (Fig. 4A, A and B). In contrast, (6–7) analogue was readily detectable after 5 min using 1:3000-fold diluted sera from mice injected with the analogue (Fig. 4A). Using the sera diluted 1:100, the presence of the analogue was still clearly measurable 1 h after injection of mice (Fig. 4B).

In Vivo Half-life of the Peptide-MHC Complexes—The in vivo half-life of the peptide-MHC complexes was determined on spleen cells from mice injected with either the parent GP33 peptide or the (6–7) analogue. In these experiments, spleen cells from peptide-injected B6 mice were directly used as APC for LCMV TCR+ T cells in a proliferation assay. As shown in Fig. 4C, the spleen cells were rapidly loaded with both GP33 and (6–7) peptides (~10 min after injection), and the stimulatory capacities of spleen cells remained comparable for both peptides until ~15 h. However, 24–30 h after peptide injection, only spleen cells from (6–7) peptide-injected mice were found to be stimulatory for LCMV TCR+ T cells. Thus, the half-life of the (6–7) analogue in vivo on spleen cells was ~2-fold increased when compared with the parent GP33 peptide (30–40 versus 15–24 h).

The (6–7) Analogue Efficiently Induces Antiviral Protection—The data described above show that as efficiently as the parent peptide GP33, the (6–7) analogue is recognized by the Tg LCMV TCR and that the half-life of the (6–7) analogue is significantly increased compared with that of GP33 peptide-Dβ complex. To further examine the potential advantage of using the (6–7) analogue in vivo, we tested the ability of the analogue to induce antiviral protection.

Because of the high frequency of GP33-specific T cells in LCMV TCR+ mice, antiviral protection induced by peptides cannot be directly examined in these mice, because inoculated virus is rapidly cleared (23). Therefore, we examined antiviral protection in B6 mice in which a small number (105/mouse) of LCMV Tg LCMV TCR+ T cells were adoptively transferred. After peptide immunization using subcutaneous injection of either the GP33 parent peptide or (6–7) analogue in IFA induced a similar extent of antiviral protection. On the other hand, no significant protection was observed when mice received a single subcutaneous injection of 100 μg of GP33 or (6–7) peptides in the absence of IFA (Fig. 5B). However, when mice received three successive subcutaneous injections of 50 μg of peptides in the absence of IFA (Fig. 5B), a significant decrease (10–100-fold) of virus titer was observed in mice im-
Reduced Peptide Bond Analogues of a CTL Epitope

Fig. 3. The \(\Psi(6-7)\) analogue is highly resistant to in vitro protease digestion, and the major proteolytic cleavage site is located between residues 6 and 7. A, proteolytic degradation of GP33 and \(\Psi(6-7)\) peptides in mouse serum. At the time points indicated, the digestion was stopped, and the remaining peptide fragments were analyzed by HPLC as described under "Materials and Methods." B, determination of the cleavage site in the GP33 parent peptide after digestion by proteolytic enzymes. At time points 0 and 1 min of incubation of the GP33 parent peptide with mouse serum, the main peaks eluted from the HPLC column were analyzed by mass spectrometry. The cleavage site was deduced from the mass of the main fragment appearing after 1 min of digestion (Peak 2). Peak 1 corresponds to undigested GP33 peptide.

The use of peptides corresponding to MHC class I epitopes to induce a protective CTL response against viruses or tumors is of particular interest in the development of peptide-based vaccines. Recent investigations have suggested that antigenic pseudopeptides containing one or several peptide bond isosters might advantageously replace natural peptides in therapeutic strategies because they can bind to MHC class I molecules and generate an efficient T cell response (7, 13, 14). In this study, we have examined the antigenic and immunogenic properties of pseudopeptide analogues derived from the LCMV glycoprotein peptide 33–41 (GP33) in which one CO–NH amide bond at a time was replaced by a reduced peptide bond \(\Psi(CH,N)\) in the native sequence. GP33 is presented by H-2D\(^b\) MHC molecules and recognized in this context by specific CD8\(^+\) T cells. This model was particularly interesting for at least three reasons. First, although infection with cytopathic viruses (e.g., vaccina, vesicular stomatitis, Semliki Forest, or influenza virus) is controlled by soluble mediators such as antibodies and cytokines, T cell-mediated cytotoxicity is crucial for the resolution of infections with noncytopathic viruses such as LCMV (31). LCMV is thus an excellent model to study in vivo the efficacy of the CTL response induced by modified peptides. Second, it is known that although the parent peptide GP33 is particularly efficient to induce protection against a viral challenge when it is injected in the presence of IFA, this peptide is unable to generate protection when used in saline solution. Third, a transgenic model containing within the CD8\(^+\) T cell population 40–60% transgenic TCR\(^+\) (V\(_{\alpha}2/V\(_{\beta}8\)) T cells specific for peptide GP33 presented in the H-2D\(^b\) MHC context is available (27), thus allowing in vivo study of the recognition of the peptide (or pseudopeptide)-MHC complexes by this TCR.

Two of eight analogues studied, namely \(\Psi(5-6)\) and \(\Psi(6-7)\), were able to bind to H-2D\(^b\) molecules with an apparent affinity similar to that of the parent GP33 peptide. Guichard et al. (7) previously found that five of eight reduced peptide bond analogues derived from a Plasmodium berghei MHC class I epitope could bind to soluble recombinant H-2K\(^d\) molecules. However, the relative affinity of these pseudopeptides to MHC molecules was 5–10-fold lower than that of the parent peptide. The present finding that most of the reduced peptide bond analogues of GP33 exhibited a decreased or no MHC binding capacity correlates with crystallographic data indicating that the peptide backbone plays an important role for binding of peptide to MHC class I molecules (32). It also suggests that in this case, the carbonyl oxygens of the residues in positions 5 and 6 are not essential for peptide-MHC binding.

CD8\(^+\) T cells from LCMV TCR\(^+\) mice recognized equally well the parent GP33 peptide and the \(\Psi(6-7)\) analogue. In contrast, they did not recognize the \(\Psi(5-6)\) analogue presented in the H-2D\(^b\) context. This result suggests either that the carbonyl oxygen of the residue in position 5 is directly involved in the interaction with the TCR or that this oxygen atom influences the orientation of the Phe\(^6\) side chain that has been shown to be crucial for TCR recognition (23, 33, 34).

Because the parent and \(\Psi(6-7)\) peptides share similar antigenic properties, the role of their respective susceptibility to proteases in relation to their biological activity could be investigated. We found that the level of GP33 resistance to mouse proteases drastically increased when a single peptide bond located between residues 6 and 7 was replaced by a reduced peptide bond in analogue \(\Psi(6-7)\). This result fits well with the observation that a highly protease-sensitive cleavage site is located between positions 6 and 7 in GP33. The detailed molecular mechanisms of GP33 degradation have not yet been elucidated. Cleavage by an endopeptidase remains the most likely possibility, although intervention of carboxypeptidases cannot be excluded. When examined in vivo, the stability of the \(\Psi(6-7)\) analogue was also significantly increased. Its half-life in the serum of injected mice was increased by >10 times compared with GP33. The rapid disappearance of GP33 from serum (by renal clearance or most probably as a result of proteolytic degradation), however, may be balanced by the very fast loading of GP33 to MHC molecules from APC. Ten minutes after peptide injection, GP33 (as well as the \(\Psi(6-7)\) peptide) was present on splenocytes. This rapid loading of exogenously provided peptides suggests a direct binding to MHC molecules without internalization.

In good agreement with previous results (35, 36), the estimated functional half-life of complexes formed in vivo by H-2D\(^b\) and GP33 was ~10–15 h. In the same test, the half-life of the \(\Psi(6-7)\)-MHC complexes on APC was increased by a factor of two. Several possibilities may account for this increased stabil-
ity of $\Psi(6-7)$-MHC complexes. As shown in a stabilization assay with RMA-S cells, the binding of $\Psi(6-7)$ and GP33 peptides to MHC molecules was similar. Nevertheless, we cannot rule out the possibility that the affinity equilibrium constant of the parent peptide and the $\Psi(6-7)$ analogue to $\text{D}^b$ molecules are slightly different. A second possibility is that reloading on the cell surface after dissociation from the MHC groove is increased in the case of the $\Psi(6-7)$ analogue because this analogue probably also exhibits an enhanced resistance to proteases present in the extracellular matrix. Finally, it is possible that peptide-MHC complexes are internalized after a few hours, and that because of increased proteolytic resistance to cytoplasmic proteases, the $\Psi(6-7)$ analogue can be reloaded on MHC molecules and represented at the cell surface.

Because of the fact that GP33 and $\Psi(6-7)$ are loaded to MHC molecules with a similar initial efficacy, when we assessed the immunogenic activity of the $\Psi(6-7)$ analogue in antiviral protection experiments (peptides injected in IFA), we did not find an improved T cell response to the more stable peptide $\Psi(6-7)$. The pseudopeptide effectively showed antiviral protection properties, which is a novel observation, but these were not significantly different from those observed with GP33. This

---

**Fig. 4.** The $\Psi(6-7)$ analogue persists longer than the parent peptide both in the serum and on the spleen cells of peptide-injected B6 mice. For experiments described in A–C, mice were injected one time intravenously in the tail vein with 200 $\mu$g of GP33 or $\Psi(6-7)$ peptides. A, 5 min after peptide injection, serum was taken from the retroorbital venous plexus and incubated with LCMV TCR $^+$ cells in a proliferation assay. At dilutions <1:100, mouse serum was toxic for the cultures. B, pharmacokinetics of the $\Psi(6-7)$ analogue and the GP33 peptide in the serum. At the indicated time points after peptide injection, serum was taken and incubated at a 1:100 dilution with LCMV TCR $^+$ cells in a proliferation assay. C, sustained presence of the $\Psi(6-7)$ analogue on spleen cells. At the indicated time points after peptide injection, mice were killed, and splenocytes were used as stimulators in a proliferation assay with LCMV TCR $^+$ T cells. The data in A–C correspond to the mean obtained from experimental groups of at least three mice.
result suggests that possibly because of the presence of oil in the adjuvant, the advantage of increased proteolytic resistance and prolonged 
\textit{in vivo} persistence did not improve the apparent biological activity of the analogue. Furthermore, once peptides are bound to MHC molecules they are apparently protected from digestion (37). It is known that injection of GP33 inoculated without adjuvant is not able to protect mice from LCMV after challenge infection (Ref. 38 and Fig. 5B). An important observation shown in this study is that three subcutaneous injections of the free analogue C (6–7) in saline were able to reduce the virus titer in the spleen of immunized mice. This result suggests that C (6–7) but not GP33 is able to prime T cells in the absence of IFA. It remains to be analyzed whether this result is attributable to the higher stability of the C (6–7) analogue in the circulation or to the prolonged half-life of the C (6–7) peptide-MHC complexes. However, the reduction of virus titers obtained with C (6–7) in PBS was not as impressive as observed with peptides in IFA, suggesting the need of an inflammatory process to reach complete antiviral protection. Because our conclusions are of immediate relevance to vaccination, it would be interesting to test the presence and reactivity of memory CD8 T cells generated after pseudopeptide vaccination. Finally, to better understand the possible mechanisms involved in the clearance of virus by peptide-activated T cells, it will be important to examine the immunological properties of modified peptide ligands containing different types of amide bond isosteric replacements in different viral systems.

\textit{Acknowledgments}—We thank S. Batsford and G. Guichard for comments on the manuscript, M. Rawiel for excellent technical assistance, and S. Denkler and T. Imhof for animal husbandry.

\textbf{REFERENCES}

1. Liu, M. A. (1998) \textit{Nat. Med.} 4, Suppl. 5, 503
2. Stemmer, C., and Guichard, G. (1998) Exp. Opin. Ther. Patents 8, 819–830
3. Gante, J. (1994) Angew. Chem. Int. Ed. Engl. 33, 1699–1720
4. Guichard, G., Benkirane, N., Graff, R., Muller, S., and Briand, J.-P. (1994) \textit{Pept. Res.} 7, 309–321
5. Benkirane, N., Guichard, G., Briand, J.-P., and Muller, S. (1996) \textit{J. Biol. Chem.} 271, 33218–33224
6. Briand, J.-P., Benkirane, N., Guichard, G., Newman, J. F. E., Van Regenmortel, M. H. V., Brown, F., and Muller, S. (1997) \textit{Proc. Natl. Acad. Sci. U. S. A.} 94, 12545–12550
7. Guichard, G., Calbo, S., Muller, S., Kourilsky, P., Briand, J.-P., and Abastado, J. P. (1995) \textit{J. Biol. Chem.} 270, 26057–26059
8. Guichard, G., Connan, F., Graff, R., Ostankovitch, M., Muller, S., Gaillet, J.-G., Choppin, J., and Briand, J.-P. (1996) \textit{J. Med. Chem.} 39, 2030–2039
9. Hill, C. M., Liu, A., Marshall, K. W., Meyer, J., Jorgensen, B., Yuan, B., Cubben, R. M., Nichols, E. A., Wicker, L. S., and Rothbard, J. B. (1994) \textit{J. Immunol.} 152, 2890–2898
10. Ettouati, L., Salvi, J.-P., Trescol-Biemont, M. C., Walchshofer, N., Gerlier, D., Rabourdin-Combe, C., and Paris, J. (1996) \textit{Pept. Res.} 9, 245–253

\textbf{FIG. 5. Antiviral protection induced by GP33 and C (6–7) peptides.} One day before peptide immunization, LCMV TCR naive T cells (10\textsuperscript{5} cells/mouse in 500 \textmu l of PBS) were transferred into non-transgenic B6 mice. Ten days after immunizations, mice were challenged with LCMV, and 4 days later, virus titers in the spleens were determined. \textit{Dots} represent individual mice (at least three per group). The \textit{full lines} are the mean of all values within each experimental group. The \textit{dotted line} corresponds to the detection limit of the virus plaque assay. \textit{A}, peptides injected subcutaneously at the indicated doses in the presence of IFA. \textit{B}, peptides injected in PBS using the indicated routes and frequency of injection.
Reduced Peptide Bond Analogues of a CTL Epitope

11. Mezière, C., Viguier, M., Dumortier, H., Lo-Man, M. R., Leclere, C., Guillet, J.-G., Briand, J.-P., and Muller, S. (1997) J. Immunol. 159, 3230–3237
12. Cotton, J., Hervé, M., Pouvelle, S., Maillère, B., and Ménez, A. (1998) Int. Immunol. 10, 159–166
13. Bianco, A., Brock, C., Zabel, C., Walk, T., Walden, P., and Jung, G. (1998) J. Biol. Chem. 273, 28759–28765
14. Ostankovitch, M., Guichard, G., Connon, F., Muller, S., Chaboissier, A., Hoebeke, J., Choppin, J., Briand, J.-P., and Guillet, J.-G. (1998) J. Immunol. 161, 200–208
15. Sloan-Lancaster, L. J., and Allen, P. M. (1995) Curr. Opin. Immunol. 7, 103–109
16. Muller, S., and Briand, J.-P. (1998) Res. Immunol. 149, 43–45
17. Guichard, G., Benkirane, N., Zeder-Lutz, G., Van Regenmortel, M. H. V., Briand, J.-P., and Muller, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9765–9769
18. Fauche`re, J.-L., and Thurieau, C. (1992) Adv. Drug. Res. 23, 127–159
19. Buss, J. E., and Marethers, J. C. J. (1995) Chem. Biol. 2, 787–791
20. Teal, P. E., and Nachman, R. J. (1997) Regul. Pept. 72, 161–167
21. Azay, J., Nagain, C., Llinares, M., Devir, C., Fehrentz, J. A., Bernad, N., Roze, C., and Martinez, J. (1998) Peptides 19, 57–63
22. Verdini, A. S., Silvestri, S., Beccherucci, C., Longobardi, M. G., Parente, L., Peppoloni, S., Perretti, M., Pileri, P., Pinori, M., Viscomi, G. C., and Nencioni, L. (1991) J. Med. Chem. 34, 3372–3379
23. Pircher, H., Moskophidou, D., Rohrer, U., Burk, H., Hengartner, H., and Zinkernagel, R. M. (1990) Nature 346, 629–633
24. Luetscher, I. F., Loex, J. A., Malissen, B., and Cerottini, J. C. (1992) J. Immunol. 148, 1003–1011
25. Quesnel, A., and Briand, J.-P. (1998) J Pept Res 52, 107–111
26. Pircher, H., Burk, K., Lang, R., Hengartner, H., and Zinkernagel, R. M. (1989) Nature 342, 559–561
27. Battegay, M., Cooper, S., Althage, A., Baenziger, J., Hengartner, H., Zinkernagel, R., and Pircher, H. (1991) J. Virol. Methods 33, 191–198
28. Zinkernagel, R. M., Leist, T. P., Hengartner, H., and Althage, A. (1985) J. Exp. Med. 162, 2125–2141
29. Townsend, A., Ohlen, C., Bastin, J., Ljunggren, H. G., Foster, L., and Karre, K. (1989) Nature 340, 443–448
30. Hammerling, G. J., Hammerling, U., and Lemke, H. (1979) Immunogenetics 8, 433–445
31. Kagi, D., and Hengartner, H. (1996) Curr. Opin. Immunol. 8, 472–477
32. Young, A. C., Zhang, W., Sacchettini, J. C., and Nathenson, S. G. (1994) Cell 76, 39–50
33. Seheda, E., Kundig, T. M., Thomson, C. T., Akki, K., Mak, S. Y., Mayer, J. P., Zamborelli, T., Nathenson, S. G., and Ohashi, P. S. (1996) J. Exp. Med. 183, 1093–1104
34. Hufrisier, D., Oldstone, M. B., and Gairin, J.-E. (1997) Virology 234, 62–73
35. Romero, P., Corradin, G., Luetscher, I. F., and Maryanski, J. L. (1991) J. Exp. Med. 174, 603–612
36. Eberl, G., Widmann, C., and Corradin, G. (1996) Eur. J. Immunol. 26, 1993–1999
37. Mouritsen, S., Meldal, M., Werdelin, O., Hansen, A. S., and Buus, S. (1992) J. Immunol. 149, 1897–1993
38. Aichele, P., Brduscha, R. K., Zinkernagel, R. M., Hengartner, H., and Pircher, H. (1995) J. Exp. Med. 182, 261–266