ANTIVIRAL CYTOTOXIC T CELL RESPONSE INDUCED BY IN VIVO PRIMING WITH A FREE SYNTHETIC PEPTIDE

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Antiviral cytotoxic T lymphocytes (CTL) are specifically induced against viral antigens presented by MHC class I molecules on the surface of infected cells (1). The intracellularly synthesized antigens are processed and associated with class I antigens within cells in a yet poorly defined way before presentation on the cell surface (2). Induced effector CTL also recognize synthetic peptides bound by MHC class I molecules from without on the cell surface in absence of an apparent need for cell internal processing (3, 4).

Virus-specific CTL have been shown to be crucially involved in recovery from and in protection against lymphocytic choriomeningitis virus (LCMV) (5) and many other virus infections. Induction of CTL response is best achieved in vivo by priming with replicating virus. Since vaccination with live virus may cause, although rarely, some disease, means have been sought for many years to induce virus-specific CTL responses in vivo with nonreplicating synthetic virus antigens.

In the past few years in vivo priming of class I-restricted T cells with free proteins or peptides was shown for ovalbumin with whole soluble protein (6) or tryptic digests of the whole protein or relatively large peptides of the relevant T cell epitope of ovalbumin (7, 8). Recently it was shown that short synthetic peptides of influenza virus nucleoprotein can induce specific responses in vivo to the priming peptide as well as to the virus; induction was possible with chemically modified peptides linked to a lipid component but not with free peptides (9).

We evaluated whether the free unmodified 15-mer peptide (RPQASGVYMGN-LTAQ) representing a H-2^d T cell epitope of LCMV nucleoprotein (10, 11) could induce in vivo a specific CTL response to LCMV and we report on the successful demonstration of such a response. Although stringently restricted to the class I MHC allele for the peptide used, these results demonstrate the feasibility of using unmodified T cell epitope peptides as antiviral vaccines in principal.

Materials and Methods

Mice. Inbred BALB/c mice (H-2^d) were purchased from the Institut für Zuchthygiene, University of Zürich, Switzerland.

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Virus. The LCMV-WE strain was originally obtained from Dr. F. Lehmann-Grube, Hamburg, FRG, and was grown in L929 fibroblast cells (12). The vaccinia virus WR strain was grown in BSC-1 cells. Recombinant vaccinia virus expressing amino acids (aa) 1-202 of the LCMV nucleoprotein (vaccNP2) was constructed and grown as described (10).

Synthetic Peptides. Peptides were synthesized manually by the solid-phase method (13, 14) on *p*-alkoxybenzyl alcohol resin (15). Fluorenylmethyloxycarbonyl (Fmoc-) strategy was used throughout the synthesis (Fmoc-N-α and ᵗBU side chain protection) (14). Peptides were cleaved from the resin by 55% trifluoroacetate in dichloromethane, purified by reverse-phase HPLC, ion-exchanged, and lyophilized. The freeze-dried material was analyzed on an Aminoquant HP 1046A amino acid analyzer and by (+)FAB mass spectroscopy.

Monoclonal Antibodies. The mAbs YTS 169.4 (anti-CD8) and YTS 191.1 (anti-CD4) (16) and low tox rabbit complement (Cedarlane Laboratories, Ontario, Canada) were used to deplete lymphocyte subpopulations in vitro.

Priming of Mice and Lymphocyte Cultures. BALB/c (H-2^d) mice were primed subcutaneously at the base of the tail with 100 μg free peptide in IFA three times with 1-wk intervals. 7 d after the last injection lymphocytes from draining lymphnodes were restimulated in vitro (3×10^6/well) with irradiated syngeneic spleen cells (6×10^6/well) in 24-well Costar plates in 10% FCS/Iscove's modified Dulbecco Medium for 6 d. Stimulator cells were sensitized in vitro with 100-200 μg peptide/ml for 3 h and washed before use. Alternatively, mice were injected once intravenously with 4×10^6 PFU recombinant vaccinia virus (vaccNP2) and 6 wk later lymphocytes from spleens were restimulated in vitro as described.

Cytotoxicity Assay. In vitro restimulated lymphocytes from lymphnodes or spleens or primary LCMV immune bulk splenocytes from mice infected intravenously with 200 PFU LCMV-WE 8 d previously were tested on appropriate target cells, B10.D2 (H-2^d) and DBA/1 (H-2^q), as described in detail elsewhere (10).

Results and Discussion

Major T Cell Epitope in H-2^d Mice. The major T cell response in BALB/c (H-2^d) mice is directed against the aa 112-132 region of LCMV nucleoprotein; the peptide aa 118-132 is well recognized by CTL in association with L^d (10, 11) (Fig. 1 *A*). The cytotoxic T cell response of H-2^d mice to LCMV resulted in lysis of target cells infected with LCMV or preincubated with peptide aa 118-132, but not of target cells if preincubated with LCMV nucleoprotein peptide aa 157-171 (Fig. 1 *B*). These two peptides were used in the following studies as relevant/positive (aa 118-132) and irrelevant/negative (aa 157-171) peptide antigens.

Induction In Vivo of LCMV and Peptide-specific CTL by a T Cell Epitope Peptide. The capacity of these peptides to induce a peptide- and virus-specific CTL response in vivo was evaluated in H-2^d mice by subcutaneous injection of unmodified free peptides emulsified in IFA. 7 d after the last injection the lymphocytes from the draining lymphnodes were isolated and restimulated in vitro with irradiated syngeneic spleen cells coated with the relevant or irrelevant peptide. Lymphocytes from mice primed with peptide aa 118-132 and restimulated with this peptide in vitro generated a specific cytotoxic T cell response against LCMV-infected and peptide-coated target cells (Fig. 2 *A*). The response was specific since neither peptide aa 157-171-coated target cells nor vaccinia virus WR-infected target cells were recognized by peptide aa 118-132-primed lymphocytes.

Restimulation of this same effector population was specific since peptide aa 157-171-coated stimulator cells failed to induce a significant response (Fig. 2 *B*).

When the peptide aa 157-171 was used for in vivo priming and peptide aa 118-132 for restimulation in vitro, no specific T cell response was generated neither against

A LCMV Nucleoprotein peptides

aa 118-132 RPQASGVYMGNLTAQ

aa 157-171 VRVWDVKDSSLLNNQ

B

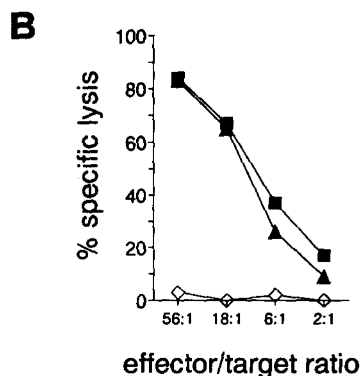

FIGURE 1. LCMV T cell epitope in H-2^d mice. (*A*) Amino acid sequence of the T cell epitope (peptide aa 118-132) and peptide aa 157-171, which was used as a control peptide in all the studies. (*B*) Specific lysis by LCMV immune splenocytes of B10.D2 (H-2^d) fibroblast cells infected with LCMV-WE (■) or sensitized before the assay with peptide aa 118-132 (▲) or with peptide aa 157-171 (◇).

the irrelevant peptide nor the peptide aa 118-132 (Fig. 2 *C*). This result shows that the relevant peptide aa 118-132 cannot readily induce a virus-specific effector population in vitro. Both priming and restimulation with the irrelevant peptide aa 157-171 failed to induce a specific CTL response to any of the described target cells (Fig. 2 *D*).

A comparable CTL response is triggered in mice by infection with a recombinant vaccinia virus expressing the relevant subregion aa 1-202 of the nucleoprotein of LCMV (10). This subunit vaccine expresses all the nucleoprotein sequences necessary for endogenous processing of the antigen (17). T lymphocytes from mice primed with vaccNP2 (aa 1-202) express specific cytotoxic activity only when restimulated in vitro with the relevant peptide aa 118-132 (Fig. 2 *E*) but not with peptide aa 157-171 (Fig. 2 *F*). The specific effector T cell population induced by peptide aa 118-132 in IFA and those induced by recombinant vaccinia virus vaccNP2 and restimulated in vitro were comparably specific for LCMV-infected target cells and for target cells coated with peptide aa 118-132 (Fig. 2 *A*, *E*).

In Vivo Peptide Primed Cytotoxic Activity Is Mediated by $CD8^+$ and MHC Class I-restricted T Cells. The following experiments were performed to exclude that free peptides might induce $CD4^+$ effector lymphocytes and to confirm that effector cells were classical $CD8^+$ CTLs. Effector T cells induced by peptide aa 118-132 in vivo (Fig. 2 *A*) were susceptible to treatment with anti-CD8 plus complement, whereas treatment with anti-CD4 had no effect (Fig. 3 *A*).

In vivo peptide-primed cytotoxic T cells of BALB/c (H-2^d) origin were tested on syngeneic (H-2^d, B10.D2) and allogeneic (H-2^q, DBA/1) target cells infected with LCMV. Both target cells do not express class II MHC antigens. There is a clear restriction specificity for H-2^d target cells (Fig. 3 *B*); this specificity control is particularly convincing since the same T cell epitope defined by peptide aa 118-132 of LCMV nucleoprotein is recognized on H-2 L^d (10, 18) and H-2 L^q (Schulz, M., P. Aichele, R. M. Zinkernagel, and H. Hengartner, manuscript in preparation).

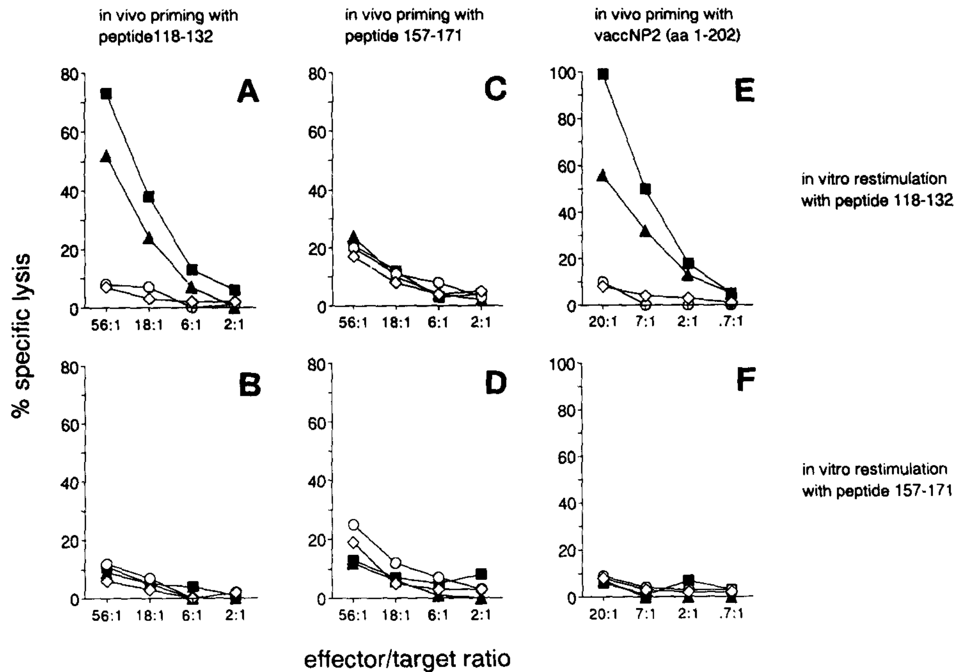

FIGURE 2. Peptide- and virus-specific CTL priming in vivo. BALB/c (H-2^d) mice were primed either by 3× injection s.c. with peptide aa 118–132 (*A*, *B*) or peptide aa 157–171 (*C*, *D*), or once by i.v. injection of recombinant vaccinia virus vaccNP2 (aa 1–202) (*E*, *F*). Draining lymph node cells (*A–D*) or spleen cells (*E*, *F*) were prepared and restimulated in vitro with irradiated spleen cells, which were incubated before either with peptide aa 118–132 (*A*, *C*, *E*) or with peptide aa 157–171 (*B*, *D*, *F*). After 6 d in culture, cytotoxic activity was tested on syngeneic target cells infected with LCMV-WE (■) or vaccinia virus WR (○), or target cells sensitized before with peptide aa 118–132 (▲) or peptide aa 157–171 (◇).

The present results show that unmodified viral peptides mixed with a mild adjuvant (not containing BCG) are able to induce MHC-dependent, virus-specific cytotoxic T cells in vivo. Together with the recent demonstration that peptides covalently linked to a lipid component (9) can readily induce a CTL response, the present evidence suggests that cocktails of viral peptides may be used as antiviral T cell vaccines.

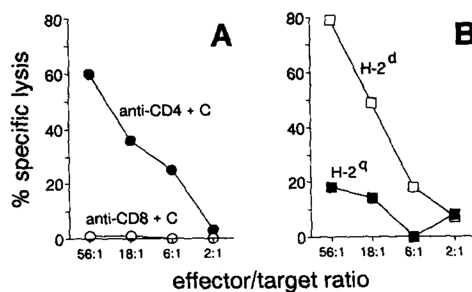

FIGURE 3. Peptide-primed effector cells are CD8⁺ and MHC class I restricted. CTL of BALB/c (H-2^d) were primed in vivo and restimulated in vitro with the relevant peptide aa 118–132. (*A*) Effector populations were depleted of $CD4^+$ (●) or $CD8^+$ (○) cells by specific antibody plus complement treatment and remaining specific lysis was tested on LCMV-WE infected target cells. (*B*) Lysis of B10.D2 (H-2^d) (□) or DBA/1 (H-2^q) (■) fibroblasts infected with LCMV-WE by in vivo peptide-primed CTL; these fibroblast cell lines are MHC class II antigens-negative.

Summary

Induction in vivo of antiviral cytotoxic T cell response was achieved in a MHC class I-dependent fashion by immunizing mice three times with a free unmodified 15-mer peptide derived from the nucleoprotein of lymphocytic choriomeningitis virus in IFA. The effector T cells are $CD8^+$, restricted to the class I L^d allele of the analyzed mouse strain, and are specific both at the level of secondary restimulation in vitro and at the effector T cell level. These results suggest that cocktails of viral peptides may be used as antiviral T cell vaccines.

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