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Peptide-induced antiviral protection by cytotoxic T cells

(vaccination/lymphocytic choriomeningitis virus/T-cell epitope)

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ABSTRACT **A specific antiviral cytotoxic immune response *in vivo* could be induced by the subcutaneous injection of the T-cell epitope of the lymphocytic choriomeningitis virus (LCMV) nucleoprotein as an unmodified free synthetic peptide (Arg-Pro-Gln-Ala-Ser-Gly-Val-Tyr-Met-Gly-Asn-Leu-Thr-Ala-Gln) emulsified in incomplete Freund's adjuvant. This immunization rendered mice into a LCMV-specific protective state as shown by the inhibition of LCMV replication in spleens of such mice. The protection level of these mice correlated with the ability to respond to the peptide challenge by $CD8^+$ virus-specific cytotoxic T cells. This is a direct demonstration that peptide vaccines can be antivirally protective *in vivo*, thus encouraging further search for appropriate mixtures of stable peptides that may be used as T-cell vaccines.**

The seminal discovery that peptides presented by major histocompatibility gene complexes class I and class II products are recognized as complexes by T cells has triggered a search for potential subunit (peptide) vaccines against infectious agents (1). Induction of cytotoxic T cells by peptides or fragmented protein has recently been documented for ovalbumin, for influenza hemagglutinin coupled to lipids, or for human immunodeficiency virus envelope glycoprotein (GP) mixed with immunostimulating complexes (2–6). In the lymphocytic choriomeningitis virus (LCMV) system the primary immune response to the virus is predominantly mediated by cytotoxic T cells (7) when assessed by virus elimination from organs. We were able to induce a classical antiviral cytotoxic T-cell response *in vivo* by simply injecting subcutaneously (s.c.) a T-cell epitope of LCMV nucleoprotein (NP) (8) as a free synthetic peptide (Arg-Pro-Gln-Ala-Ser-Gly-Val-Tyr-Met-Gly-Asn-Leu-Thr-Ala-Gln) emulsified in a mild adjuvant (incomplete Freund's adjuvant; IFA). We demonstrate here that such peptide vaccines are antivirally protective *in vivo*, proving that mixtures of appropriate peptides may be used as T-cell vaccines.

MATERIAL AND METHODS

Mice. Inbred BALB/c mice ($H\text{-}2^d$), ICR ($H\text{-}2^q$), and C57BL/6 ($H\text{-}2^b$) were purchased from the Institut für Zuchthygiene (University of Zürich, Switzerland).

Virus. The LCMV WE strain was originally obtained from F. Lehmann-Grube (Hamburg, F.R.G.) and was grown on L929 fibroblast cells (9). The vaccinia virus WR was grown on BSC-1 cells. Recombinant vaccinia virus (vaccNP2) expressing part of the LCMV NP (amino acids 1–202) was constructed and grown as described (10).

Synthetic Peptides. Peptides NP-(118–132), GP-(32–42), and GP-(275–288) synthesized by a solid-phase method were obtained from Neosystem Laboratoire (Strasbourg, France). Peptide NP-(157–171) was synthesized manually as described (10).

Monoclonal Antibodies. The monoclonal antibodies YTS 169.4 (anti-CD8) and YTS 191.1 (anti-CD4) were used to deplete lymphocyte subpopulations *in vivo* (11). The *in vivo* treatment of mice with antibodies was on days −6, −3, and −1 before virus challenge with anti-CD4 antibodies, and on days −1 and +1 for anti-CD8 antibodies with respect to virus challenge.

Priming of Mice. Mice were treated once by a s.c. injection of 200 μg of peptide emulsified in 30 μl of IFA (Difco) or with IFA at the base of the tail. Ten days later, mice were challenged by i.v. injection of 2×10^2 plaque-forming units (pfu) of LCMV (WE strain). For comparisons, mice treated by i.v. injection of 3×10^6 pfu of recombinant vaccinia virus (vaccNP2) expressing amino acids 1–202 of LCMV NP (12) were challenged 3 weeks later with LCMV and tested for protection.

Virus Titration. On different days after the challenge with 2×10^2 pfu of LCMV infectious virus, titers were determined from spleen by a plaque assay on L929 fibroblasts (9). For comparisons, virus titers were calculated per g of spleen ± SEM.

Cytotoxicity Assay. Spleen cells from controls, peptide-, or virus-primed mice on different days after the i.v. challenge with 2×10^2 pfu of LCMV were tested on LCMV-infected and uninfected B10.D2 ($H\text{-}2^d$) fibroblasts by a conventional cytotoxicity assay (4 hr) as described in detail elsewhere (8).

RESULTS AND DISCUSSION

To evaluate the antiviral protective effect of peptide vaccines, mice were primed once s.c. with the 15-mer peptide NP-(118–132) (Arg-Pro-Gln-Ala-Ser-Gly-Val-Tyr-Met-Gly-Asn-Leu-Thr-Ala-Gln) emulsified in IFA; they were able to eliminate i.v.-injected virus much faster than mice treated with an inappropriate peptide or IFA alone (Fig. 1). Only 4 days after virus injection, no virus could be detected either in spleen (Fig. 1) or in liver (data not shown). In control animals, the maximal titer of virus was reached around that time in the spleen and fell below detectable levels by day 8. A similarly enhanced virus clearance was seen in mice treated once i.v. with a recombinant vaccinia virus expressing the relevant T-cell epitope NP-(118–132) within the first 202 amino acids of the LCMV NP (Fig. 1), confirming earlier results (12).

To quantify effector cells that enhance virus elimination, spleen cells from controls and mice primed 10 days before with peptide were harvested at various time points after an i.v. challenge with virus. Specific antiviral cytotoxic activity was maximal at about day 8 after infection with low doses of virus (2×10^2 pfu) in BALB/c mice (Fig. 2*A*)—i.e., 3–4 days after virus had reached maximal titers (Fig. 1). In peptide-primed mice, only a weak cytotoxic activity was measurable, which reached a relative maximum on day 4 (Fig. 2*A*). However, in animals infected with high doses of virus (2×10^5

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Abbreviations: LCMV, lymphocytic choriomeningitis virus; pfu, plaque-forming unit(s); NP, nucleoprotein; GP, glycoprotein; IFA, incomplete Freund's adjuvant.

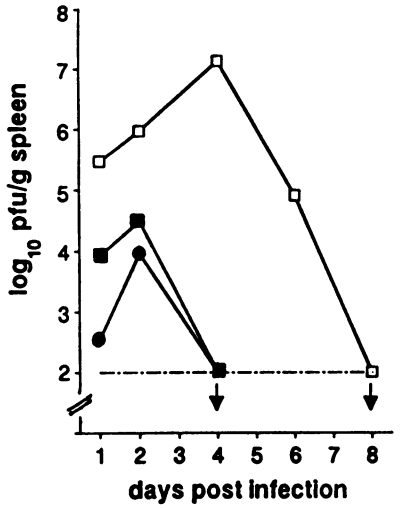

FIG. 1. Inhibition of virus replication in peptide-primed mice. Virus titers were determined in groups of four or five mice primed with peptide in IFA (■), injected with IFA alone (□), or injected with recombinant vaccinia virus expressing the appropriate sequence of the LCMV NP-(1–202) (10) (●).

pfu) increased effector cell frequencies in peptide-primed mice were well-documented by the early and rapid increase

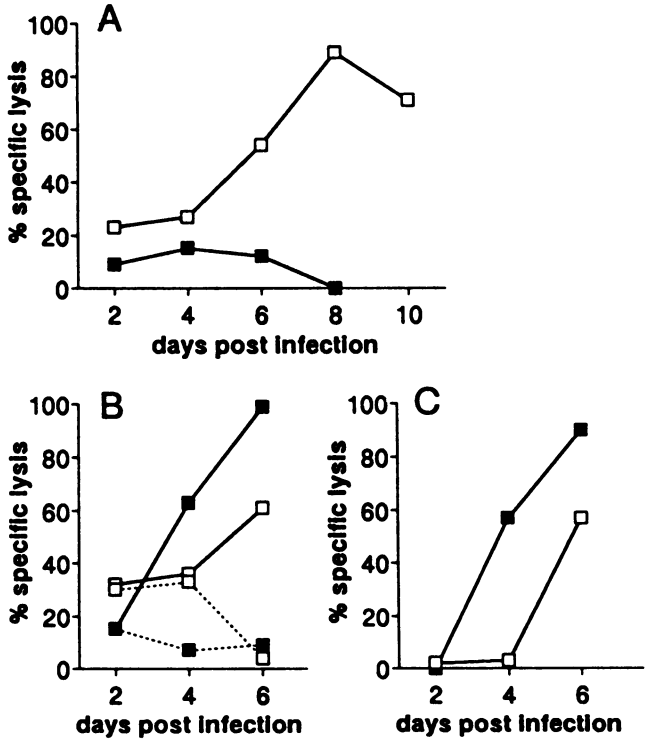

FIG. 2. In peptide-primed mice, high levels of virus-specific effector cells preexist when compared to IFA-injected control mice. (*A*) Cytotoxic T-cell activity of NP-(118–132) (Arg-Pro-Gln-Ala-Ser-Gly-Val-Tyr-Met-Gly-Asn-Leu-Thr-Ala-Gln)-primed mice (■) challenged with low doses of virus (2×10^2 pfu) compared to control mice (□). (*B*) Cytotoxic T-cell activity in mice injected with high doses of virus (2×10^5 pfu) in peptide-primed mice (■) compared to control mice (□) tested on LCMV WE strain-infected B10.D2 (H-2^d) target cells (—) and on uninfected control target cells (···). Mice were treated as described in the legend to Fig. 1. On different days after infection, splenocytes were tested on LCMV-infected and uninfected B10.D2 (H-2^d) fibroblast cells in a conventional cytotoxicity assay (4 hr) as described in detail (10). (*C*) Values of specific antiviral cytotoxicity were calculated from percentage ^{51}Cr release from infected cells minus percentage release from uninfected target cells (*B*). Spontaneous chromium release was between 20% and 24%.

of virus-specific cytotoxicity compared to control mice (Fig. 2 *B* and *C*); this earlier and greater cytotoxic T-cell response in peptide-primed mice was also reflected by the low natural killer (NK) activity compared with the prominent NK activity in control IFA-infected mice up to day 4 after infection with high doses of virus (Fig. 2*B*) (13, 14). These findings fit the notion that 10 days after priming increased effector cell frequencies were present in peptide-primed animals, which were quickly activated to control viral replication rapidly. This early control of virus growth limited further antigen-driven expansion of cytotoxic T cells in mice infected with low doses of virus. Antiviral protection induced by the 15-mer in BALB/c mice lasted for at least 7 weeks; it was best when tested 10 days after vaccination (>10^5-fold protection; e.g., <2.0 $\log_{10}$ pfu per g of spleen vs. 7.1 $\log_{10}$ pfu per g of spleen in controls) (Table 1) and was diminished to ≈10^4-fold protection 3 weeks (3.2 $\log_{10}$ pfu per g of spleen in immunized mice) and 10-fold protection (6.1 $\log_{10}$ pfu per g of spleen) 7 weeks after vaccination measured on day 4 after challenge infection with 2×10^2 pfu of LCMV (data not shown).

As expected from experiments showing that L^d and L^q mice respond to the same LCMV peptide NP-(118–132), the antiviral protection by this peptide was induced in both H-2^d and H-2^q mice (Table 1). Even a nonamer peptide was sufficient to inhibit virus replication. Protection was mediated by $CD8^+$ but not by $CD4^+$ T cells, since depletion *in vivo* of $CD8^+$ T cells but not of $CD4^+$ T cells by monoclonal antibodies prevented protection (Table 1).

We also evaluated 2 of the 3 different T-cell epitopes defined for H-2^b mice—i.e., LCMV GP-(32–42) (15) and GP-(275–288) (16) with respect to their vaccine potential. Vaccination with GP-(32–42) caused an enhanced elimination of virus from spleens on day 4 postinfection, whereas GP-(275–288) showed no effect. These experiments also showed formally that peptide vaccines are specific—i.e., they are restricted to the relevant H-2 type (Table 1).

The data presented here document that treatment of mice with peptide emulsified in a mild adjuvant induces antiviral protection by $CD8^+$ cytotoxic T cells *in vivo*. As shown earlier with recombinant vaccinia virus vaccines (10, 12), the experiments with viral peptides show again that antiviral

Table 1. Inhibition of LCMV replication by treatment with various T-cell epitope peptides

Immunization	Virus titers in different mouse strains: BALB/c (H-2^d)	ICR (H-2^q)	C57BL/6 (H-2^b)
	Experiment 1		
No peptide	7.1 ± 0.1	7.2 ± 0.2	7.3 ± 0.1
NP-(118–132)*	<2.0	<2.0	ND
NP-(118–126)	3.4 ± 0.4	ND	ND
NP-(118–132), αCD4	<2.0	<2.0	ND
NP-(118–132), αCD8	7.9 ± 0.0	7.2 ± 0.1	ND
NP-(157–171)†	7.5 ± 0.1	6.9 ± 0.1	ND
NP-(157–171), αCD4	7.5 ± 0.1	7.2 ± 0.1	ND
GP-(32–42)‡	ND	ND	3.1 ± 0.4
	Experiment 2		
No peptide	6.2 ± 0.1	6.2 ± 0.2	6.3 ± 0.1
NP-(118–132)	<2.0	<2.0	5.3 ± 0.4
GP-(32–42)‡	5.3 ± 0.2	5.9 ± 0.1	ND
GP-(275–288)‡	5.8 ± 0.3	6.0 ± 0.2	6.1 ± 0.2

Mice were immunized with different T-cell epitope peptides in various mouse strains. Ten days later, mice were challenged with 2×10^2 pfu (Experiment 1) or 1×10^2 pfu (Experiment 2) of LCMV with or without *in vivo* treatment of mice with anti-CD4 or anti-CD8 antibodies. ND, not determined.

*H-2^d-specific LCMV T-cell epitope.

†Nonimmunogenic peptide for BALB/c mice, negative control (10).

‡H-2^b-specific LCMV T-cell epitopes (8).

protection by vaccines containing only one or a few epitopes are restricted to some but not all major histocompatibility complex (H-2 or HLA) types (1, 17). This and possibly varying stabilities of peptides obviously limit the general use of one peptide as a vaccine, but this disadvantage may be readily corrected by vaccines made of mixtures of relevant and more stable peptides.

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