Vaccination with a Synthetic Peptide Modulates Lymphocytic Choriomeningitis Virus-Mediated Immunopathology

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Vaccination with a nucleopeptide (NP 118; amino acids 118 to 132) representing a cytotoxic T-cell epitope of lymphocytic choriomeningitis virus (LCMV) can modulate immunopathology. Immunization with NP 118 protected H-2b mice against intracerebral infection with the LCMV-ARMSTRONG isolate. However, when NP 118-primed H-2b mice were challenged intracerebralex with an intermediate dose (5 x 10^6 PFU) of the LCMV-DOCILE strain, all mice primed with NP 118 emulsified in incomplete Freund’s adjuvant died, whereas unprimed mice survived. Correspondingly, peptide vaccination enhanced specifically the cytotoxic T-cell response, influencing the critical balance between T-cell response and virus spread.

Protection against an acute lymphocytic choriomeningitis virus (LCMV) infection is apparently mediated almost exclusively by cytotoxic T lymphocytes (CTLs) (6, 26); the same CTLs may also cause immunopathology (8, 9, 12, 17, 26). The kinetics of viral spread and the T-cell response are important parameters influencing the outcome of disease (6, 12, 19). After intracerebral (i.c.) infection, the T-cell immune response is only protective when a great number of T cells are recruited early while relatively few choriomeningial cells are infected. When virus spreads rapidly and too many choriomeningial cells are infected, immune T cells cause extensive and therefore lethal immunopathological disease (18). Because LCMV may cause immune suppression also of the CTL response against itself (so-called high-dose immune paralysis [12]), mice may survive high doses of LCMV-WE or high doses of LCMV-DOCILE injected i.c. (6, 16, 19).

Immunization with recombinant vaccinia virus expressing the LCMV glycoprotein (vacc-LCMV-GP) or nucleocapsid (vacc-LCMV-NP) has been shown to protect mice from LCM disease (10, 11, 15) by induction of a protective CTL response. However, under certain circumstances, immunization with vacc-LCMV-GP or vacc-LCMV-NP has been found to aggravate disease, e.g., BALB/c mice infected with a high dose of the LCMV-DOCILE isolate survived except for those immunized with vacc-LCMV-GP or vacc-LCMV-NP (18).

To evaluate whether priming with a synthetic peptide similarly influences the immune balance in vivo, we used the nucleoprotein peptide 118 (NP 118) of LCMV that has been defined to be a major immunogenic epitope for CTLs in mice of the H-2d haplotype (20–22). The experiments presented document that NP 118, able to stimulate CTL responses, may enhance or inhibit immunopathology depending on various factors such as the virus strain, the infectious dose, and the mouse strain studied.

Role of immunization in survival. Groups of BALB/c mice (H-2b) were primed with one subcutaneous injection of 400 μg of NP 118 emulsified in 30 μl of incomplete Freund’s adjuvant (IFA) (Difco) or with NP 118 without IFA; control mice were injected with balanced salt solution (BSS) emulsified in IFA. NP 118 (amino acids 118 to 132) was synthesized by a solid-phase method (purchased from Neosystem Laboratoire, Strasbourg, France). Ten or 30 days later, mice were challenged i.c. with various doses of LCMV-DOCILE (provided by C. Pfau, Renesselaer Polytechnic Institute, Troy, N.Y.) (13, 19). When given a subsequent i.c. injection on day 10 after priming, mice survived (as did unprimed controls) a high dose of LCMV-DOCILE (5 x 10^6 PFU) because of high-dose immune paralysis (Fig. 1A). On the other hand, mice infected with lower doses of virus (5 x 10^3 PFU or less) died, regardless of the status of immunization. Vaccination did not protect the mice from a mild to a lethal choriomeningitis. When BALB/c mice were challenged with 5 x 10^4 PFU of LCMV-DOCILE (Fig. 1B), all survived except for those immunized with NP 118/IFA, which all died 8 days after i.c. infection. Also, 75% of the mice primed with NP 118/IFA 30 days prior to infection died. DBA/2 (H-2b) mice, which have lower concentrations of CD8+ T cells than most other strains (24), survived high- and intermediate-dose LCMV-DOCILE infections regardless of whether they were primed or left unprimed (Fig. 1C). However, 50% of the DBA/2 mice infected with LCMV-DOCILE (50 PFU) died when primed with NP 118/IFA. Again, control mice and mice primed with NP 118 alone (data not shown) survived a subsequent i.c. infection.

Unprimed BALB/c mice (BSS/IFA) injected i.c. with a low dose (100 PFU) of LCMV-ARMSTRONG CA 1371 (6), a neurotropic virus strain, lived longer than 10 days later. Thus, disease is influenced by immunization with NP 118 depending on the infectious dose and the challenging virus strain injected.

Measurement of CTL responses and virus titer. To further characterize the change in immune response, we determined CTLs after i.c. infection in NP 118-primed and control mice. Five and 7 days after i.c. challenge with 5 x 10^5 PFU of LCMV-DOCILE, spleen cells from control animals or peptide-primed mice were tested on LCMV-infected or peptide-loaded B10.D2 (H-2b) fibroblasts by a conventional cytotoxicity assay (4 to 5 h) (10, 27). The same protocol was used to test T-cell-mediated cytotoxicity of lymphocytes obtained from inflammatory infiltrates from brains. LCMV-specific cytotoxic T-cell response is shown as cytolytic units (7) per milliliter of cerebrospinal fluid or per spleen (Fig. 3). One

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cytolytic unit is defined as the number of lymphocytes causing 30% specific lysis.

Cytolytic units measured in the spleen (days 5 and 7) and in the cerebrospinal fluid (day 7) after i.c. infection with 5 × 10^4 PFU of LCMV-DOCILE were highest in mice primed with NP 118/IFA. Cytolytic units determined in the spleen on day 5 after infection were more than 200-fold higher in mice primed with NP 118/IFA compared with unprimed mice or mice primed with NP 118 alone; in the latter case, the cytolytic units were only slightly above background.

On day 7 after i.c. challenge with LCMV-DOCILE (5 × 10^4 PFU), virus titers (4) in the brains were about 10-fold lower in NP 118/IFA-primed mice ([2.35 ± 0.65] × 10^5) than in NP 118/BSS-primed mice ([2.54 ± 0.55] × 10^6) or control animals ([2.5 ± 0.89] × 10^6). However, because of the variation (see standard error of the mean), the values are not statistically significantly different. This result indicated that LCMV had replicated extensively and indicated that the relatively earlier and greater CTL response correlated best with lethal LCM in primed mice (8, 9, 16).

The data presented here confirm that vaccination of mice with a peptide emulsified in a relatively mild adjuvant can induce a CTL response (3, 14) and thereby modulates the response to LCMV in a beneficial or exceptionally in a detrimental fashion. These findings are comparable to those found with vaccinia virus recombinant vaccines (18). To understand the paradoxical effect of vaccination, it has to be stated clearly that LCMV is immunosuppressive in certain circumstances. Early and high i.c. virus titers achieved either by infection with a high virus dose or by injection of a rapidly replicating virus isolate, such as LCMV-DOCILE, overwhelmingly infect choriomeningeal cells and the cells of the immune system; but in addition, they may also cause severe immune suppression that includes the T-cell response against LCMV (5, 12, 17). Under these circumstances, the antiviral T-cell response is suppressed and cannot

FIG. 2. Protection of NP 118-primed mice against i.c. LCMV-ARMSTRONG infection. BALB/c mice were primed with NP 118 with ( ) or without (△) IFA; control mice were injected with BSS/IFA (●). Mice were all challenged 10 days later with 100 PFU of LCMV-ARMSTRONG i.c.

FIG. 3. CTL response determined in the spleen and cerebrospinal fluid (CSF). (A) Cytolytic units determined in the spleen 5 days after challenge with LCMV-DOCILE (5 × 10^4 PFU). BALB/c mice were primed with NP 118/IFA, NP 118/BSS, or BSS/IFA (controls) 10 days prior to infection. (B) Cytolytic units in the spleen and CSF 7 days after infection with LCMV-DOCILE. Mice and priming as in panel A.
control virus spread nor cause lethal immunopathological damage (27). The experiments presented here demonstrated that vaccination with NP 118 modulated the secondary immune response in such a way that virus spread of the neurotropic LCMV-ARMSTRONG was controlled early enough to prevent fatal choriomeningitis. After i.c. infection with an intermediate dose of LCMV-DOCILE (5 × 10⁴ PFU), the balance between kinetics of virus spread and that of immune response is of critical importance with respect to LCM disease. Under these circumstances, immunization with NP 118 caused lethal LCM, probably because it prevented the induction of high-dose immune paralysis. The accelerated secondary T-cell response resulted in enhanced T-cell-mediated fatal choriomeningitis. This interpretation is also supported by the findings observed similarly in DBA/2 mice with reduced numbers of CD8⁺ CTLs (23, 24). Apparently, in this case the reduced secondary responses failed to prevent establishment of high-dose immune paralysis. Susceptibility was enhanced when BALB/c mice were challenged with an intermediate (but not a higher or a lower) dose of LCMV-DOCILE, indicating that the kinetics of virus spread is also an important factor influencing immunopathology in this model situation.

The experiments presented demonstrate both the potential and limitations of some subunit vaccines. Protection may be induced in a major histocompatibility complex-restricted fashion, calling for vaccines consisting of a cocktail of relevant peptides. Sometimes such vaccines may not fulfill all the necessary parameters of ideal vaccines (1, 2). In the example presented here, immunization with a peptide representing a single CTL epitope was able to increase susceptibility to a T-cell-mediated immunopathology induced by one LCMV isolate. Although this finding is an exception rather than the rule, such potentially unwanted effects of vaccines on susceptibility against immunopathological disease may signal the limitations of such vaccines. These aspects will have to be taken into account when pre- or postexposure vaccinations of T cells are being planned against chronic infectious diseases that trigger T-cell-mediated immunopathology.

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