Recombinant Sindbis Viruses Expressing a Cytotoxic T-Lymphocyte Epitope of a Malaria Parasite or of Influenza Virus Elicit Protection against the Corresponding Pathogen in Mice

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Subcutaneous administration in mice of recombinant Sindbis viruses expressing a class I major histocompatibility complex-restricted 9-mer epitope of the Plasmodium yoelii circumsporozoite protein or the nucleoprotein of influenza virus induces a large epitope-specific CD8+ T-cell response. This immunization also elicits a high degree of protection against infection with malaria or influenza A virus.

Alpha-RNA viruses such as Sindbis, Semliki Forest, and Venezuelan equine encephalitis viruses are attractive vectors for gene therapy and vaccine development because of their capacities to express large amounts of foreign proteins (7, 10, 24). Sindbis virus offers certain additional advantages, such as a broad range of susceptible host cells (23) and the relative ease of engineering and manipulating recombinant (RE) RNA molecules (10). It is also important that, in spite of its small size of approximately 11.8 kb, this virus is capable of expressing foreign inserts of up to 3.2 kb (18).

Earlier data demonstrated the potential of RE Sindbis viruses, expressing foreign antigen, to induce immunity against a variety of intracellular pathogens (10, 14, 18). We have determined the capacity of these RE viruses expressing the corresponding foreign antigen or epitope to generate CD8+ T-cell responses and protection against infection by the malaria parasite Plasmodium yoelii and the influenza A virus, respectively. In both systems, CD8+ T cells were shown to play major roles in mediating protective immunity (2, 8, 13, 19, 22).

RE Sindbis viruses expressing a minigen encoding only for the CD8+ T-cell epitope SYVPSAEQI of the circumsporozoite (CS) protein of P. yoelii (SIN.Mal), recognized by H-2b mice, or TYQRTRALV from the nucleoprotein (NP) of the influenza A virus (SIN.Flu) were generated by using infectious mRNA transcripts, as described elsewhere (12).

Six- to 8-week-old BALB/c mice (Jackson Laboratory, Bar Harbor, Maine) were immunized by various routes, namely, subcutaneously (s.c.), intramuscularly (i.m.), intravenously (i.v.), intraperitoneally (i.p.), and intranasally (i.n.) (17). The magnitudes of the epitope-specific CD8+ T-cell responses were measured 12 days after immunization with an ELISPOT assay. This assay has been described in detail by us (15, 20) and other investigators (4, 5, 11, 16) using various experimental models. In all of these systems it was shown that the results of the ELISPOT correlate closely with the chromium release assay, the ELISPOT being considerably more sensitive and quantitative.

The largest numbers of CS-specific CD8+ T cells, detected 12 days after immunization, were elicited in mice immunized s.c. with 5 × 106 PFU of SIN.Mal (~2 × 103 CS-specific CD8+ T cells/106 spleen cells) (Fig. 1A). The i.m. immunization was only slightly less efficient. Thirty-two days after immunization, the total number of epitope-specific CD8+ T cells had decreased by approximately 50%. As observed at day 12, the magnitude of the CD8+ T-cell response closely depended on the route of immunization, and the greatest response also resulted from s.c. or i.m. inoculation (not shown). It is noteworthy that the magnitude of the anti-SYVPSAEQI CD8+ T-cell response, induced by the RE Sindbis virus, is the greatest we have observed so far, compared to past immunization with other RE vaccinia, influenza, or adenoviruses (17, 19).

The largest numbers of influenza virus NP-specific CD8+ T cells were also observed upon s.c. or i.m. immunization with SIN.Flu (Fig. 1B). The total numbers of NP-specific CD8+ T cells in the spleens of these mice were smaller than those elicited by inoculation of an equal number of PFU of SIN.Mal, but the rankings of results obtained by the different routes of inoculation were the same.

The number of CS-specific CD8+ T cells closely correlated with the viral dose used for immunization. Animals inoculated s.c. with 103 or 106 PFU of SIN.Mal displayed the largest numbers of CS-specific CD8+ T cells (Fig. 2A). Immunization with doses greater than 109 PFU of SIN.Mal failed to increase the frequency of CS-specific T cells (data not shown). The level of the NP-specific CD8+ T-cell response of s.c. immunized mice was also found to correlate closely with the inoculum size of SIN.Flu. Mice immunized with 106 PFU of the RE virus displayed the highest numbers of NP-specific CD8+ T cells in the spleen, while a larger dose of SIN.Flu, 108 PFU, elicited lower numbers of NP-specific CD8+ T cells (Fig. 2B). This close correlation between virus dose and CD8+ T-cell response may reflect a very low replication rate of the Sindbis virus in vivo. Although pTE32J-derived RE double-subgenomic Sindbis viruses are replication competent in vitro, as shown by amplification at a low multiplicity of infection without loss of either infectivity or foreign protein expression, the stability significantly depends on the length and protein product of the foreign gene insert (6, 10, 18). Inoculation with up to 109 PFU of double-subgenomic Sindbis virus recombinants causes independent infections in 6- to 8-week-old mice, independent of the route of immunization. Furthermore, productive replication in vivo may be very low, as virus titers in the serum were dimin-

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ished within the first two days after i.p. inoculation with $5 \times 10^7$ PFU and were undetectable thereafter (2a).

In order to assess the in vivo antiparasite activity of the specific CD8$^+$ T-cell epitope, (A) Groups of three BALB/c mice were immunized by inoculation with $5 \times 10^7$ PFU of SIN.Mal administered s.c., i.m., i.v., i.p., or i.n. (B) Groups of three BALB/c mice were immunized by inoculation with $5 \times 10^6$ PFU of SIN.Flu administered by various routes. The numbers of CS- and NP-specific CD8$^+$ T cells in the spleens of the immunized mice were established 12 days after RE virus inoculation by an ELISPOT assay, which detects epitope-specific gamma interferon (IFN-$\gamma$) secretion by single cells. The corresponding results are expressed as averages ± standard errors of triplicate cultures.

FIG. 1. Results of ELISPOT performed on spleen cells of mice immunized by different routes with RE Sindbis viruses expressing a CS-specific or an NP-specific CD8$^+$ T-cell epitope. (A) Groups of three BALB/c mice were immunized by inoculation with $5 \times 10^6$ PFU of SIN.Mal administered s.c., i.m., i.v., i.p., or i.n. (B) Groups of three BALB/c mice were immunized by inoculation with $5 \times 10^6$ PFU of SIN.Flu administered by various routes. The numbers of CS- and NP-specific CD8$^+$ T cells in the spleens of the immunized mice were established 12 days after RE virus inoculation by an ELISPOT assay, which detects epitope-specific gamma interferon (IFN-$\gamma$) secretion by single cells. The corresponding results are expressed as averages ± standard errors of triplicate cultures.

FIG. 2. Effect of inoculum size on the numbers of specific splenic CD8$^+$ precursor T cells resulting from immunization. (A) Numbers of gamma interferon (IFN-$\gamma$)-secreting CS-specific CD8$^+$ T cells detected in the spleens of groups of three mice immunized by s.c. inoculation with different doses of SIN.Mal ($10^4$ to $10^8$ PFU). (B) Numbers of NP-specific CD8$^+$ T cells in the spleens of groups of five mice immunized by s.c. inoculation with different doses of SIN.Flu ($10^4$ to $10^9$ PFU). The numbers of IFN-$\gamma$-secreting CS- and NP-specific CD8$^+$ T cells in the spleens of these mice were detected by an ELISPOT assay 12 days after immunization. The results reflect two identical experiments and are expressed as averages ± standard errors of triplicate cultures.

To determine whether immunization with SIN.Flu expressing the influenza NP epitope could elicit protection against influenza virus infection, mice were inoculated s.c. with $10^6$ PFU of SIN.Flu. Other mice were given a comparable dose of SIN.Mal for use as a specificity control. Two weeks after immunization, these and nonimmunized mice were challenged i.n. with $10^6$ PFU of influenza A/WSN/33 virus (subtype H1N1) (13). Viral titers in the lungs of these mice were measured either on day 4 or on day 7 after challenge by using a standard plaque assay to determine the numbers of PFU on Madin-Darby canine kidney cell monolayers (21). Mice immunized with SIN.Flu and challenged with the influenza virus developed much lower virus titers in their lungs than did naive mice or mice immunized with SIN.Mal (Fig. 3B). On day 4, and particularly on day 7, after challenge, the influenza virus titers in the lungs of SIN.Flu-immunized mice were nearly $2 \log_{10}$
T-cell responses and protective immunity against these infections. Previous immunization studies with RE vaccinia viruses expressing the entire malaria CS antigen (13) or the influenza virus NP (1, 9) report the induction of apparently similar or less-efficient protective immunity against these infectious pathogens.

Although Sindbis virus infects humans, this infection is mostly limited to the local skin site, causing a minor rash. Moreover, recent advances in genetic engineering make it feasible to generate replication-defective Sindbis viruses which appear to be nonpathogenic (12). If the immunogenicity of these defective Sindbis viruses remains unaltered, they might be very attractive candidates for the development of safer vaccines against several infectious diseases.

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REFERENCES


