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CUTTING EDGE

Cutting Edge: Recombinant Listeria monocytogenes Expressing a Single Immune-Dominant Peptide Confers Protective Immunity to Herpes Simplex Virus-1 Infection

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The vast majority of the world’s population is infected with HSV. Although antiviral therapy can reduce the incidence of reactivation and asymptomatic viral shedding, and limit morbidity and mortality from active disease, it cannot cure infection. Therefore, the development of an effective vaccine is an important global health priority. In this study, we demonstrate that recombinant Listeria monocytogenes (Lm) expressing the H-2Kb glycoprotein B (gB)498–505 peptide from HSV-1 triggers a robust CD8 T cell response to this Ag resulting in protective immunity to HSV infection. Following challenge with HSV-1, immune-competent mice primed with recombinant Lm-expressing gBAg were protected from HSV-induced paralysis. Protection was associated with dramatic reductions in recoverable virus, and early expansion of HSV-1-specific CD8 T cells in the regional lymph nodes. Thus, recombinant Lm-expressing Ag from HSV represents a promising new class of vaccines against HSV infection. The Journal of Immunology, 2007, 178: 4731–4735.

Herpes simplex virus types 1 and 2 are ubiquitous human pathogens. Depending on the population examined, between 50 and 100% of adults have seroevidence of infection with HSV-1 or HSV-2 (1). After primary infection in immune-competent hosts, the virus establishes lifelong latency within the sensory ganglia that is associated with sporadic recurrences of clinical lesions and asymptomatic virus shedding. However, infection in neonates or other immune-compromised hosts commonly causes disseminated infection resulting in a high rate of morbidity and mortality. Although antiviral therapy can reduce morbidity and mortality in disseminated infection, and long-term suppressive therapy can reduce the frequency and severity of viral reactivation, antivirals cannot not “cure” infection (2, 3). Furthermore, the increasing incidence of HSV resistant to common antiviral medications emphasizes the current need for an effective vaccine to prevent HSV infection (4, 5).

CD8 T cells contribute to protective immunity to HSV infection. In biopsy specimens from humans with recurrent HSV infection, viral clearance is associated with a high concentration of local CD8 T cells with cytolytic activity against infected cells (6). In animal models, depletion of CD8 T cells impairs clearance of virus from the CNS, whereas TCR transgenic CD8 T cells specific for the immune-dominant H-2Kb-restricted peptide in HSV-1 glycoprotein B (gB)498–505 transferred into mice lacking other components of adaptive immunity results in viral clearance (7, 8). These studies demonstrate that HSV-specific CD8 T cells play a protective role in HSV infection.

Infection with the Gram-positive intracellular bacterium Listeria monocytogenes (Lm) is a well-characterized experimental model in which Lm-specific CD8 T cells can confer protective immunity to secondary Lm infection (9, 10). Moreover, recombinant Lm expressing defined Ags from other intracellular viral pathogens such as lymphocytic choriomeningitis virus, influenza, HIV, SIV, or feline immune deficiency virus primes CD8 T cells specific for these heterologous Ags that protect against subsequent viral challenge (11–17). Accordingly, in this study, we examined the ability of recombinant Lm expressing a single immune-dominant Ag from HSV-1 to prime HSV-specific CD8 T cells and confer protective immunity to HSV challenge.

Materials and Methods

Bacteria

Lm ΔactA strain DPL1942 and recombinant Lm secreting the OVA protein behind the Lm bly promoter (Lm-OVA) have been described previously (18, 19). Transformation of Lm was performed by penicillin treatment as described (20). For infections, Lm was grown and subcultured in brain-heart infusion medium containing chloramphenicol (20 μg/ml) to early log-phase (OD600 0.1), washed, and diluted in PBS to a final concentration of 1 × 107 CFUs per 200 μl and inoculated i.v. into mice.

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3 Abbreviations used in this paper: gB, glycoprotein B; Lm, Listeria monocytogenes; HA, hemagglutinin.

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Expression constructs
The DNA fragment encoding OVA, hemagglutinin (HA) tag, Lm hly promoter, and signal sequence was PCR amplified from Lm-OVA (19) using the following primers: 5'-ctctgtaactgtaacagcgag-3' and 5'-pgoctcaaggagaacccagcagcegcggttggacaggg-3'. Underlined DNA sequence indicate restriction enzyme sites. This construct was then cut with PvuI and SmaI, and ligated with the overlapping primer sets for pHSVG (coding strand, 5'-ttacctccctcctcagctgctaaggaggaactcgagggg-3') and noncoding strand, 5'-ctctgtaacagcgacgctcgtgtaggccggcggagggc-3') (Fig. 1A). Underlined DNA sequences were verified by DNA sequencing.

Western blotting
Supernatant protein was prepared by filtering (0.2-μm syringe filter) Lm culture medium (brain-heart infusion) with bacteria in log-phase growth (2–4 h after 1/100 back-dilution of stationary phase cultures to OD600 0.4–0.6), trichloroacetic acid precipitation (10%), and separation by SDS gel electrophoresis. Supernatant protein was prepared by filtering (0.2-μm syringe filter) Lm culture medium (brain-heart infusion) with bacteria in log-phase growth (2–4 h after 1/100 back-dilution of stationary phase cultures to OD600 0.4–0.6), trichloroacetic acid precipitation (10%), and separation by SDS gel electrophoresis. Proteins were transferred to nitrocellulose and probed with rabbit anti-HA (clone HA-11; Covance Research Products).

Herpes simplex virus
HSV-1 (KOS strain) viral stocks were prepared for hind footpad infection (2.5 × 10^6 PFU per footpad) following dermal abrasion, as described previously (22). After infection, mice were monitored twice daily for 14 days for HSV CNS disease manifested as ataxia and/or hind limb paralysis. Our previous studies have demonstrated that >80% of mice that develop these symptoms later succumb to infection, and accordingly paralyzed mice were euthanized in accordance with our Institutional Animal Care and Use Committee protocol. For determining tissue HSV titers, the hind footpads and spinal cord were harvested, snap frozen, homogenized, and titered on Vero cells (22).

Mice
Female C57BL/6 (H-2b) and IFN-γ-deficient mice on the C57BL/6 background were purchased from The Jackson Laboratory. MyD88-deficient mice were harvested, snap frozen, homogenized, and titered on Vero cells (22).

FIGURE 1.
Generation of Lm ΔactA pHSVG or Lm ΔactA pCONTROL. A, Construct map for creating recombinant HA-tagged fusion proteins containing HSV-1 gB498-505 and mTB ESAT61-20 peptides that are expressed and secreted under the Lm hly promoter and signal sequence within the pAM401 vector (cat, chloramphenicol acetyltransferase). B, Western blot of supernatant protein from Lm ΔactA pHSVG (lane 1), Lm ΔactA pCONTROL (lane 2), and Lm-OVA (lane 3) (19) with anti-HA Ab.
To further evaluate the HSV-specific CD8 T cell response triggered by Lm ΔactA pHSVgB infection, we examined the Ag-specific response in splenocytes at the peak of the T cell response (day 8). At this time point, CD8+ splenocytes from Lm ΔactA pHSVgB-infected mice readily produced IFN-γ in response to stimulation with gB498–505 peptide as determined by both intracellular cytokine staining and ELISA, whereas splenocytes from Lm ΔactA pCONTROL-infected mice produced only background amounts of cytokine (Fig. 2, B–D). Thus, infection with Lm ΔactA pHSVgB primes a robust CD8 T cell response to gB498–505 in B6 mice. To confirm that mice infected with Lm ΔactA pHSVgB and Lm ΔactA pCONTROL only differed by the CD8 T cell response to gB498–505, we examined the ability of the endogenous listeriolysin-O peptide, LLO189–201, to induce cytokine production (Fig. 2, E). Similar frequencies of IFN-γ-producing CD4 T cells and total IFN-γ production were found in both infection groups after stimulation with this peptide.

Infection with Lm ΔactA pHSVgB confers protective immunity to HSV-1 infection

To examine whether the gB-specific CD8 T cell response triggered by Lm ΔactA pHSVgB infection confers protection to HSV-1 infection, groups of mice infected with either Lm ΔactA pHSVgB or Lm ΔactA pCONTROL were infected in the hind footpads 28 days later with an inoculum of HSV-1 that normally causes ataxia and/or hind limb paralysis in naive mice. Many features of disease pathogenesis in human infection are represented in this acute infection model. After infection, virus travels anterogradely through the enervating sciatic nerve to the dorsal root ganglia, replicates in the ganglia, and then returns to the site of infection via retrograde axonal transport resulting in a primary lesion of the footpad (22). Virus in the dorsal root ganglia can also cross the synapse, enter the spinal cord, and ascend to the brain causing paralysis. In the first 7–9 days after HSV infection, 85% (17 of 20) of mice primed with Lm ΔactA pCONTROL developed hind limb paralysis compared with only 25% (5 of 20) of mice primed with Lm ΔactA pHSVgB (p = 0.0002) (Fig. 3A). These mice were monitored for up to 14 days after infection, and no additional paralysis developed for any mice beyond day 9 after HSV infection.
To determine whether protection from paralysis was directly related to reductions in viral burden, we quantified the amount of recoverable virus just before mice develop paralysis (day 6). Mice primed with Lm ΔactA pHSVgB, when compared with mice primed with Lm ΔactA pCONTROL, gB peptide in alum, or naive mice, had ~10-fold and ~200-fold reductions in HSV-1 titers in the footpad and spinal cord, respectively (Fig. 3F). Additionally, these protective effects of prior Lm ΔactA pHSVgB infection were associated with a rapid and robust expansion of gB-specific CD8 T cells in the draining popliteal lymph nodes in response to HSV-1 challenge. By day 3 after HSV-1 infection, 1.2% of CD8 T cells in the lymph nodes from Lm ΔactA pHSVgB-primed mice produced IFN-γ in response to gB<sub>498-505</sub> peptide stimulation, whereas lymph node cells from Lm ΔactA pCONTROL-primed or naive mice had no detectable Ag-specific response (Fig. 3C). By 6 days postinfection, the gB-specific response in popliteal lymph node cells reached ~15% of total CD8 T cells in Lm ΔactA pHSVgB-primed mice, compared with a ~7% response in Lm ΔactA pCONTROL or naive mice (Fig. 3D). The delayed and dampened response in control mice represented the primary CD8 T cell response to HSV, which was not sufficient to protect them; the majority of these mice developed HSV-induced paralysis and axatia and had markedly increased amounts of virus in both the CNS and peripheral tissues.

Finally, we examined the ability of Lm ΔactA pHSVgB to trigger protective immunity in immune-deficient mice that are more susceptible to HSV-1 infection. In both IFN-γ-deficient and MyD88-deficient mice, a gB-specific immune response was readily detected by dimer staining after Lm ΔactA pHSVgB inoculation (data not shown); however, no significant protection from HSV-1 challenge could be detected for these mice (Fig. 3D). Taken together, these data demonstrate that recombinant Lm expressing a single peptide from HSV-1 confers protection to HSV-1 infection in immune-competent mice.

Discussion

Numerous properties make recombinant Lm an attractive vaccine vector candidate for priming Ag-specific CD8 T cells. First and foremost, Lm infection is a strong adjuvant, and the result of Ag-specific CD8 T cell response to Lm or recombinant Ag is protective and long-lasting (13, 25). These properties are directly related to the ability of the bacterium to gain access to the cytoplasmic compartment of infected cells delivering Ags to the MHC class I Ag presentation pathway. Second, preexisting immunity to Lm does not diminish the therapeutic efficacy of recombinant Lm strains (26, 27). Third, genetic manipulation allowing for expression of heterologous Ag and targeted disruption of virulence factors is readily accomplished. Accordingly, numerous attenuated Lm strains that cause minimal disease yet maintain immunogenicity have been described previously (28, 29). Together, these qualities make attenuated Lm expressing recombinant Ag promising vaccine candidates for priming Ag-specific T cells.

The widespread prevalence of HSV infection combined with the lack of “curative” therapy and increasing resistance to standard antiviral therapy emphasizes the need for developing a vaccine that can prevent HSV infection (30, 31). In this study, we examined the potential for recombinant Lm expressing a single immune-dominant MHC class I-restricted peptide from HSV-1 to prime HSV-specific CD8 T cells to reduce disease. We demonstrate that Lm ΔactA pHsvgB induces a robust CD8 T cell response to HSV gB with ~2% of peripheral CD8 T cells specific for this heterologous Ag at the peak of primary expansion in immune-competent and IFN-γ-deficient and MyD88-deficient mice. In response to HSV-1 challenge, immune-competent mice were protected from HSV-1-induced disease, had marked reductions in the amount of recoverable virus, and a more rapid and robust expansion of Ag-specific CD8 T cells. Although recombinant Lm ΔactA pHSVgB triggered a robust gB-specific CD8 T cell response in IFN-γ-deficient or MyD88-deficient mice, these responses were associated with little or protection against subsequent HSV-1 infection. These results are inconsistent with the readily achievable protective immunity to subsequent Lm infection in these mice after priming with Lm ΔactA (23, 24), and may reflect differences in CD8 T cell effectors, or other MyD88- or IFN-γ-dependent immune mechanisms required for adaptive immunity to HSV-1 compared with Lm.

To our knowledge, this is the first study demonstrating protection from infection-associated disease in addition to reduction in viral burden conferred by recombinant Lm. Ideally, a HSV vaccine would both prevent new infection and be curative for established latent infection thereby preventing recurrent disease. However, HSV rarely reactivates from latency in mice, preventing assessment of this aspect of the vaccine. Nevertheless, the data presented here represent an important first step in the development of recombinant Listeria as a novel class of vaccine vectors against HSV infection.

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Disclosures

The authors have no financial conflict of interest.

References


