Long-Term Presence of Virus-Specific Plasma Cells in Sensory Ganglia and Spinal Cord following Intravaginal Inoculation of Herpes Simplex Virus Type 2

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The tissue sites of long-term herpes simplex virus type 2 (HSV-2)-specific antibody production in mice and guinea pigs were identified. In addition to secondary lymphoid tissue and bone marrow, HSV-specific plasma cells were detected in spinal cords of mice up to 10 months after intravaginal inoculation with a thymidine kinase-deficient HSV-2 strain and in lumbosacral ganglia and spinal cords of guinea pigs inoculated with HSV-2 strain MS. The long-term retention of virus-specific plasma cells in the peripheral and central nervous systems following HSV infection may be important for resistance to reinfection of neuronal tissues or may play a role in modulation of reactivation from latency.

Herpes simplex virus type 2 (HSV-2) infects epithelial cells at mucosal surfaces and establishes a lifelong latent infection of sensory neurons in the sensory ganglia. The virus periodicallyreactivates, resulting in either symptomatic or asymptomatic virus shedding near the site of original infection. Recent studies have suggested that HSV is not transcriptionally silent during latency. Viral gene transcripts and viral proteins have been detected in latently infected ganglia (3, 5) which have been correlated with the presence of immune cell infiltrates and persistent cytokine expression in mice (2, 6, 12, 21) and monkeys (27). HSV-specific CD8+ T cells have been demonstrated in the trigeminal ganglia of mice following ocular inoculation of HSV-1 (8), suggesting that virus-specific lymphocytes may be maintained by presentation of viral antigen by HSV-infected neurons. In the current study, we extended these observations by demonstrating the long-term presence of HSV-specific, immunoglobulin G (IgG)-secreting plasma cells in the peripheral and central nervous systems following intravaginal HSV-2 inoculation.

We previously showed that mice inoculated intravaginally with a thymidine kinase-deficient HSV-2 strain (HSV-2 333 tk−) developed vigorous serum IgG antibody responses (16). To determine the durability of the response, Swiss Webster mice (Harlan Sprague Dawley, Indianapolis, IN) were treated with 2.0 mg medroxyprogesterone and inoculated intravaginally with HSV-2 strain MS. The long-term retention of virus-specific plasma cells in the peripheral and central nervous systems following HSV infection may be important for resistance to reinfection of neuronal tissues or may play a role in modulation of reactivation from latency.

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Previously, HSV-specific plasma cells were detected in spinal cords of mice up to 10 months after intravaginal inoculation with a thymidine kinase-deficient HSV-2 strain and in lumbosacral ganglia and spinal cords of guinea pigs inoculated with HSV-2 strain MS. The long-term retention of virus-specific plasma cells in the peripheral and central nervous systems following HSV infection may be important for resistance to reinfection of neuronal tissues or may play a role in modulation of reactivation from latency. We identified the tissue sites responsible for long-term production of HSV-specific IgG antibody using HSV-specific enzyme-linked immunospot (ELISPOT) assays, as described previously (16), to test for the presence of HSV-specific plasma cells in mice inoculated 7 to 10 months previously with HSV-2 333 tk−. Lymphocytes were obtained from spinal cords and vaginal tissue by digestion with dispase-collagenase (1.0 mg/ml in Mg2+-Ca2+-free phosphate-buffered saline; Roche Diagnostics, Mannheim, Germany). Tissue digests were resuspended in 30% Percoll (Sigma-Aldrich, Inc., St. Louis, MO) and centrifuged on a 70% Percoll cushion, and cells at the interface were collected for analysis. Bone marrow cells were obtained by flushing femurs with Hanks’ balanced salt solution, and centrifuged on a 70% Percoll cushion, and cells at the interface were collected for analysis. Bone marrow cells were obtained by flushing femurs with Hanks’ balanced salt solution (Sigma-Aldrich). In agreement with studies with other viral systems (23), the majority of HSV-specific plasma cells were detected in the bone marrow, with lower frequencies in the spleen and iliac lymph nodes (Table 1, experiment 1). Interestingly, although HSV-2 333 tk− has been shown to replicate poorly in neuronal tissue (14), we routinely detected HSV-specific IgG-secreting plasma cells in the lumbar region of spinal cords from intravaginally inoculated mice. Because no HSV-specific plasma cells were detected in peripheral blood, these plasma cells most likely represented resident tissue cells rather than blood contamination. Interestingly, HSV-specific plasma cells were not detected in the vaginal epithelium (Table 1, experiment 2). Together, these results suggested that the microenvironment of the infected spinal cord supported the long-term retention of HSV-specific, IgG-secreting plasma cells. It remains to be determined if these cells represent long-lived plasma cells (23) or the continuous differentiation of short-lived plasma cells from central nervous system-infiltrating, virus-specific memory B cells (18). It is possible that the differentiation and maintenance of this population may be orchestrated by cytokines secreted by local inflammatory cells.
and the continued production of viral proteins during HSV latency (3, 5).

Intravaginal inoculation of guinea pigs with fully virulent HSV-2 results in a genital infection closely resembling that of humans, including limited acute replication in the genital epithelia, establishment of latency in sensory ganglia, and natural reactivation of latent HSV-2, including development of recurrent genital lesions (25, 26). Intravaginal inoculation of guinea pigs with HSV-2 also resulted in a durable HSV-specific serum IgG response. Serum collected on days 106, 194, and 292 after inoculation was plated on HSV-2 glycoprotein-coated plates and developed with rabbit anti-guinea pig IgG and horseradish peroxidase-conjugated antirabbit IgG (Bethyl Laboratories, Inc., Montgomery, TX). The endpoint titer was determined as described previously (16). As shown in Fig. 1B, a vigorous HSV-specific IgG response developed and was maintained through day 292 post-HSV-2 inoculation.

We utilized this model to confirm the long-term retention of HSV-specific plasma cells in neuronal tissues. Hartley guinea pigs (Charles River Breeding Laboratory, Wilmington, MA) were inoculated by rupture of the vaginal closure membrane with a moistened calcium alginate-tipped swab (Calgiswab #3; Spectrum Laboratories) and instillation of 10^6 PFU of HSV-2 strain MS into the vaginal vault. Guinea pigs were evaluated daily for primary genital skin disease. After recovery from primary infection, guinea pigs were examined daily during days 22 to 63 after inoculation for evidence of spontaneous recurrent herpetic lesions (25, 26). Consistent with our observations with mice, HSV-specific, IgG-secreting plasma cells were detected by ELISPOT assay in the spleen, bone marrow, and spinal cords of guinea pigs on day 40 postinoculation, while no virus-specific plasma cells were detected in tissues from uninoculated animals.

We determined the time course for establishment of responses in neuronal tissues and determined if virus-specific plasma cells were detected by ELISPOT assay in tissues of HSV-2 tk^-inoculated mice. Lymphocytes isolated from the indicated tissues on days 40 or 138 postinoculation were plated on HSV glycoprotein-coated plates and developed with rabbit anti-guinea pig IgG and horseradish peroxidase-conjugated antirabbit IgG (Bethyl Laboratories, Inc., Montgomery, TX). The endpoint titer was determined as described previously (16). As shown in Fig. 1B, a vigorous HSV-specific IgG response developed and was maintained through day 292 post-HSV-2 inoculation.

We utilized this model to confirm the long-term retention of HSV-specific plasma cells in neuronal tissues. Hartley guinea pigs (Charles River Breeding Laboratory, Wilmington, MA) were inoculated by rupture of the vaginal closure membrane with a moistened calcium alginate-tipped swab (Calgiswab #3; Spectrum Laboratories) and instillation of 10^6 PFU of HSV-2 strain MS into the vaginal vault. Guinea pigs were evaluated daily for primary genital skin disease. After recovery from primary infection, guinea pigs were examined daily during days 22 to 63 after inoculation for evidence of spontaneous recurrent herpetic lesions (25, 26). Consistent with our observations with mice, HSV-specific, IgG-secreting plasma cells were detected by ELISPOT assay in the spleen, bone marrow, and spinal cords of guinea pigs on day 40 postinoculation, while no virus-specific plasma cells were detected in any of these tissues from uninoculated animals (Fig. 2). Greater numbers of virus-specific plasma cells were detected in bone marrow and spinal cords 138 days postinoculation.

We determined the time course for establishment of responses in neuronal tissues and determined if virus-specific
plasma cells were also retained in vaginal tissue in animals that had experienced recurrent genital lesions. Guinea pigs were inoculated intravaginally with HSV-2 strain MS, and all demonstrated primary disease symptoms. Lymphocytes from the blood, spleen, sensory ganglia, spinal cord, and vaginal tissue were obtained on days 8, 15, 28, and 83 after inoculation. As shown in Table 2, HSV-specific IgG-secreting cells were rarely detected on any day in peripheral blood; however, they were detected at high numbers in the spleens on day 8 after virus inoculation. The response waned at this site following resolution of the primary infection (day 15), although low numbers of HSV-specific IgG-secreting cells were detectable on day 83 postinoculation. Virus-specific plasma cells were detected in both the lumbarosacral ganglia and spinal cords on day 8 postinoculation, and these responses were maintained in these tissues through day 83 following virus inoculation. HSV-specific IgG-secreting cells were detected in the vaginas of 3/5 and 5/5 animals, respectively, on days 8 and 15 postinoculation. Interestingly, virus-specific plasma cells were detected in the vaginas of only 2/5 and 1/4 animals, respectively, on days 28 and 83 postinoculation, although 8/9 of these animals had experienced recurrent genital lesions.

The ability of antibody to protect neuronal tissues against HSV has been demonstrated (1, 9, 19, 22) and may involve interfering with axonal spread of virus or interfering with virus replication within the neuron (17, 20). Intravaginal inoculation of mice or guinea pigs with an attenuated strain of HSV-2 results in a high level of resistance to reactivation of the sensory ganglia (14, 15, 24). The presence of HSV-specific, IgG-secreting plasma cells within the peripheral and central nervous systems may be an important component of this resistance. Additionally, since antibody has been shown to interfere with viral replication in neurons by HSV and other viruses (17, 10, 7), the current data suggest that the long-term presence of HSV-specific plasma cells in neuronal tissue may also play a role in modulation of HSV reactivation, perhaps in conjunction with HSV-specific T lymphocytes (13), by inhibiting replication of reactivating virus without damaging infected neurons.

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REFERENCES


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