Gamma Interferon Impedes the Establishment of Herpes Simplex Virus Type 1 Latent Infection but Has No Impact on Its Maintenance or Reactivation in Mice

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Cell-mediated immunity is a critical component of the host response to herpes simplex virus type 1 (HSV-1) and HSV-2 infections, as is evident from the vast clinical experience and formal studies of the susceptibility of neonates and immunodeficient patients to these viruses (3, 4, 22, 23). Among the pivotal mediators of virus-specific cellular immunity is gamma interferon (IFN-γ) (13, 15, 21). Studies in a variety of mouse models have revealed the effects of IFN-γ on HSV infection (2, 5, 6, 8–11, 13, 27). Overall, these studies demonstrate that IFN-γ protects mice from fatal HSV-1 encephalitis yet has minimal to no effect on viral replication or neuroinvasiveness. Nothing is known, however, of its role in the establishment of HSV neuronal latency or viral reactivation. We characterized the impact of IFN-γ on the ability of HSV-1 to establish latency and to later reactivate in response to UV light, a physiological stimulus, by conducting studies in targeted gene knockout mice.

Evaluation of HSV-1 latency in the IFN-γ knockout (GKO) background required establishing a model of infection that results in equivalent viral loads at the conclusion of the acute disease phase. To achieve this outcome, it was necessary to select among diverse host mouse strains to identify one with a properly balanced T helper 1 (Th1) and Th2-oriented cell-mediated immune response, as they would define the relative sensitivity and resistance of the mouse to HSV (20). Cellular immunity in C57BL/6 mice appears to be Th1 dominated and may confer substantive resistance to infection with selected organisms. BALB/c mice, on the other hand, are more sensitive to infectious agents that require a Th1-dominated host response and are less likely to manifest pathologic processes mediated by IFN-γ-induced necrosis (19).

In accord with these considerations, we observed that ocular infection with HSV-1 (strain 17syn+) in GKO mice in the C57BL/6 background (obtained from Genentech, Inc., San Francisco, Calif.) (7) resulted in all mice surviving an inoculum of 10^5 PFU of HSV-1 per animal. Moreover, although mortality was evident among C57BL/6 mice infected at 10^5 PFU per animal, there was no significant difference in survival between the GKO and control mice (Fig. 1).

GKO mice in the BALB/c background (Charles River, Wilmington, Mass.), however, showed distinctly different responses. Groups of age- and sex-matched GKO and control mice were inoculated via corneal scarification with 10^3, 10^4, or 10^5 PFU of HSV-1 (Fig. 2). As expected, wild-type BALB/c mice proved more susceptible to HSV-1 than the wild-type C57BL/6 mice, at both 10^3 and 10^5 PFU of HSV-1 (P < 0.001, Wilcoxon test). In direct contrast to observations with C57BL/6 mice, significantly reduced survival was observed in GKO mice in the BALB/c background compared with normal controls, even with an HSV-1 dose of 10^5 PFU/mouse (P < 0.008, Wilcoxon test). Significant differences in survival were not detected in the BALB/c background with higher doses of HSV-1, as virtually all of these mice expired.

Despite significantly different mortality rates in the GKO and control mice, Fig. 3 shows similar titers of HSV-1 in all three tissues from the BALB/c mice. Likewise, infected tissues from C57BL/6 GKO and controls showed equivalent levels of virus (Fig. 4). Virus titration was performed at indicated time points following infection by tissue homogenization and dilution on duplicate Vero cell monolayers (17). Thus, in both mouse strains, the ability of the control animals to elaborate IFN-γ appeared to play no role in further attenuating the local infection in the eye or viral neuroinvasiveness. Nonetheless, the residual titers in the acutely infected tissues of the BALB/c mice were nearly 3 logs greater at its maximum than observed in the more resistant C57BL/6 strain, again in accord with the greater morbidity of the infection in the BALB/c background.

This large difference in viral growth, then, cannot be explained by inherent differences in IFN-γ release in each mouse strain. Importantly, though, these results show similar viral replicative kinetics during acute disease despite the presence or absence of IFN-γ, permitting quantitative analysis of viral latency and reactivation in each mouse strain.

Ex vivo reactivation of HSV-1 (18) occurred equally well (100% by days 8 to 9) from latently infected trigeminal ganglia in the absence and presence of IFN-γ (Table 1, explant cocul-
vation), confirming that the presence or absence of IFN-\(\gamma\) confers no impediment to the establishment of latency in either background. The efficient reactivation of HSV-1 ex vivo, however, does not imply that there are equivalent levels of latent HSV-1 genomes in the ganglia. It is possible that in the absence of IFN-\(\gamma\) there were grossly different levels of latent virus than in its presence, but that the virus could still reactivate efficiently as long as some threshold quantity of latent virus genomes had been exceeded. Thus, it was important to quantitate the latent viral genome copy number established in mice in the presence or absence of IFN-\(\gamma\). To do this, we developed a highly sensitive and reproducible quantitative fluorescence PCR assay. This assay proved to have an intra-assay variation of less than 30% and to be linear over a range from \(3 \times 10^3\) to \(3 \times 10^5\) genomes per 100 ng of total mouse trigeminal ganglia DNA (17).

With this assay, we detected increased quantities of HSV-1 DNA in latently infected trigeminal ganglia from BALB/c GKO mice compared with control mice (Fig. 5). Specifically, ganglia from seven infected BALB/c GKO mice contained \(3.5 \pm 0.1\) viral genome copies (mean \pm standard error in log_{10}) per 100 ng of total DNA, compared with \(2.3 \pm 0.2\) viral genome copies per 100 ng of total DNA from six control mice (\(P = 0.01\), Wilcoxon test). These results suggest that IFN-\(\gamma\) expression affects the ultimate latent viral load in BALB/c mouse strains, despite the equivalent viral loads in GKO and wild-type mice during acute infection (Fig. 1). In contrast, latently infected ganglia from C57BL/6 GKO and control mice contained similar amounts of viral DNA. Specifically, eight C57BL/6 GKO mice contained \(3.6 \pm 0.1\) viral genome copies, compared with ganglia containing \(3.8 \pm 0.1\) viral genome copies in eight control mice (\(P = 0.27\), Wilcoxon test). Thus, the expression of IFN-\(\gamma\) had no effect on latent viral load in the C57BL/6 background.

Removal of the trigeminal ganglion and its cocultivation ex vivo revealed comparable rates of reactivation in the presence or absence of IFN-\(\gamma\) in each background; however, explant cocultivation is a maximal and nonphysiologic stimulus to HSV reactivation. Physiologic reactivation processes would entail either spontaneous or stress-induced recurrence of infectious virus, as in humans with recurrent herpetic keratoconjunctivitis. Since HSV-1 does not reactivate spontaneously in mouse eyes with any appreciable frequency, the role of IFN-\(\gamma\) in HSV-1 reactivation, and the impact of differential viral loads in the BALB/c background, were explored by exposure of latently
infected mice to UV light (360 mJ) and subsequent analysis of ganglia for replicating virus (16, 17).

The results in Table 1 show no significant differences between GKO and control animals in the C57BL/6 background in the proportion of ganglia with UV-induced HSV-1 reactivation or in the time to its reactivation. In the BALB/c background, equivalent proportions of ganglia demonstrated viral reactivation; however, the mean time to reactivation were significantly shorter in the GKO mice compared with the wild-type controls, in accord with the significantly higher latent viral load in the BALB/c GKO mice ($P < 0.01$, Wilcoxon test). Latently infected ganglia of GKO and control mice receiving no UV stimulation showed no spontaneous reactivation of HSV-1, indicating that IFN-γ expression has no apparent influence on maintaining latency or preventing ganglionic reactivation.

Our data show that IFN-γ contributes to the initial restraint of HSV-1 infection and reduces the establishment of HSV-1 latency, at least in the BALB/c background, as determined by the diminished quantity of latent virion DNA per ganglion in wild-type mice compared with knockout mice. These findings support previous and circumstantial evidence of IFN-γ involvement in HSV-1 latency. Upon resolution of acute disease, HSV-1 establishes a latent state within sensory neurons in trigeminal and dorsal root ganglia. Lymphocyte-mediated mechanisms, including IFN-γ expression, constitute a system of immunologic surveillance of latently infected tissues that continues beyond the initial symptomatic period, as evidenced by numerous clinical observations and laboratory studies. Peripheral blood mononuclear cells from HSV-1 latently infected patients produce higher levels of IFN-γ and interleukin-2 (IL-2) compared with cells from seronegative control subjects (14, 25). Additionally, individuals with relatively frequent HSV-1 recurrences may produce higher levels of IFN-γ compared with patients with few recurrences (25). In mice, latently infected trigeminal ganglia express increased levels of IL-2, IL-10, and IFN-γ up to 135 days following HSV-1 infection (12). Persistent infiltrates in ganglia of CD4$^+$ and CD8$^+$ lymphocytes also have been detected up to 6 months following infection, with concomitant increases in local IFN-γ secretion (5). Most telling of all is the predictable increase in rates of symptomatic and asymptomatic reactivation of HSV in individuals with inborn or acquired defects of cellular immunity (23). Our results show IFN-γ has a direct role in reducing the establishment of HSV-1 neuronal latency.

Reactivation of HSV-1 from latency may be closely tied to the immune response to systemic stress. UV irradiation, a common inducer of HSV-1 reactivation, alters Th1 and Th2 cytokine production in affected tissues. It reduces production

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**TABLE 1. In vitro and in vivo reactivation of latent HSV-1**

<table>
<thead>
<tr>
<th>Study group $^a$</th>
<th>No. of ganglion pairs reactivating/total tested</th>
<th>$P$ value $^b$</th>
<th>Mean days to reactivation$^c$ $^d$ ± SEM</th>
<th>$P$ value $^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Explant cocultivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB WT</td>
<td>6/6</td>
<td>8.5 ± 0.3</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>BALB GKO</td>
<td>4/4</td>
<td>8.5 ± 0.4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>B6 WT</td>
<td>10/10</td>
<td>8.5 ± 0.4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>B6 GKO</td>
<td>10/10</td>
<td>8.2 ± 0.4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td><strong>UV irradiation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB WT</td>
<td>7/15</td>
<td>8.9 ± 0.9</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>BALB GKO</td>
<td>9/18</td>
<td>5.4 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6 WT</td>
<td>3/10</td>
<td>6.0 ± 1.0</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>B6 GKO</td>
<td>12/20</td>
<td>4.3 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB WT, no UV</td>
<td>0/5</td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>BALB GKO, no UV</td>
<td>1/5</td>
<td>6.0 ± 0.0</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>B6 WT, no UV</td>
<td>0/5</td>
<td>NA</td>
<td></td>
<td>NA</td>
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<tr>
<td>B6 GKO, no UV</td>
<td>0/5</td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$ BALB, BALB/c, WT, wild type; B6, C57BL/6.

$^b$ For comparison between wild-type and GKO per mouse strain.

$^c$ NA, not applicable.

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**FIG. 4.** HSV-1 replicates in and propagates to the central nervous system equally well in C57BL/6 GKO and control mice. Mice were inoculated on day 0 with $10^5$ PFU of HSV-1. Each time point represents the geometric mean of the viral titers of indicated tissues taken from five animals. Error bars represent standard error.

**FIG. 5.** The contents of latent HSV DNA in trigeminal ganglia were similar in GKO and control mice of both C57BL/6 and BALB/c backgrounds. Genome copies of HSV-1 were determined by a quantitative fluorescent PCR assay. Significant differences in geometric means (log$_{10}$) between ganglia of BALB/c GKO (3.5 ± 0.1) and BALB/c control mice (2.3 ± 0.2) were detected. Geometric means of HSV-1 genome copy numbers in ganglia of C57BL/6 GKO mice (3.6 ± 0.1) were not statistically different from those of control mice (3.8 ± 0.1).
of IL-2 and IFN-γ and augments levels of the Th2 cytokines IL-4 and IL-5 (1, 26). Augmented expression of tumor necrosis factor alpha and IL-6 is also seen in reactivating ganglia (24).

These observations suggest that IFN-γ may be capable of suppressing HSV-1 reactivation. Our results, though, revealed no difference in the overall in vivo reactivation rates between GKO and wild-type littermates, both in the BALB/c and C57BL/6 backgrounds, arguing against a role for IFN-γ in modulating HSV-1 reactivation. Time to reactivation, however, was significantly shortened in the absence of IFN-γ, in the BALB/c background. This shorter time to reactivation likely reflects the higher latent viral load permitted by the loss of IFN-γ activity during primary infection. Finally, reactivation rates following UV stimulation were slightly lower in C57BL/6 mice than in BALB/c mice, despite equivalent or higher levels of latent viral DNA in C57BL/6 mice (Fig. 5). C57BL/6 mice are more resistant to infectious agents that induce Th1-mediated immune responses. The lower reactivation rates measured in C57BL/6 mice suggest a Th-1 related, IFN-γ-independent mechanism of suppressing reactivation in the C57BL/6 strain.

We and others (2) show that BALB/c GKO mice have increased susceptibility to HSV-1 encephalitis compared with wild-type littermates. Why greater morbidity and mortality occur is unclear. Viral titers in acutely infected tissues were equivalent in both GKO and wild-type mice. Thus, the reduction in mortality when IFN-γ is expressed must not be related to its effects on virus replication per se. Additionally, IFN-γ has no effect on HSV-1's neuroinvasiveness, as shown by equivalent virus titers in the brains of both GKO and wild-type mice during acute infection. Other studies of IFN-γ-deficient animals also demonstrate this phenomenon. Rho-gamma mice that express ectopic intraocular IFN-γ (8, 9) show increased survival from HSV-1, as well as protection from infection of the contralateral eye, compared with wild-type mice (8, 9). However, as in our model, no differences in viral titer or neuroinvasiveness were detected between groups of animals (8, 9).

Our results and those of others (2, 6, 8, 9, 11) demonstrate that IFN-γ significantly improves survival from HSV-1 encephalitis. Furthermore, IFN-γ reduces the levels of latent viral DNA independent of the viral load during acute disease. Our data also show an associated reduction in time to reactivation following a physiological stimulus, suggesting a correlation between the quantity of latent virus and reactivation rate. The cumulative data and clinical observations suggest that IFN-γ plays two roles in HSV-1 infection, both of which are exerted in the periphery. First, it suppresses acute disease and limits the quantity of virus amenable to ganglionic latency; second, it limits the spread of virus once reactivated so that the recurrent infection will be less evident clinically or less severe.

REFERENCES


